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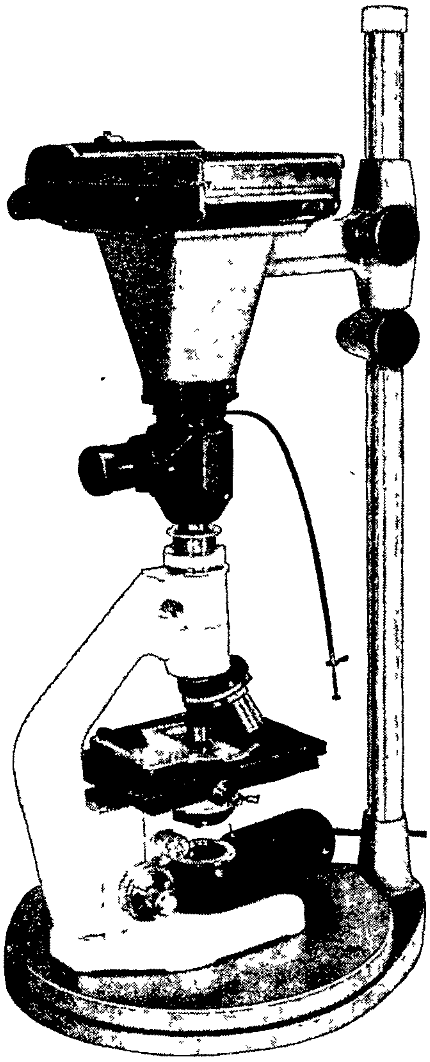

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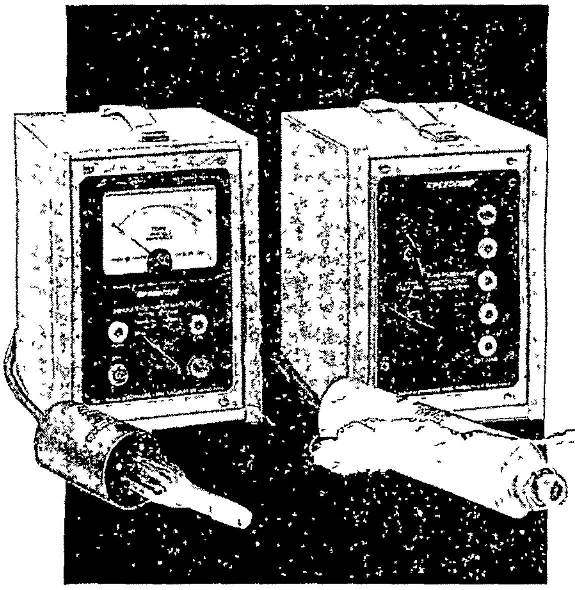

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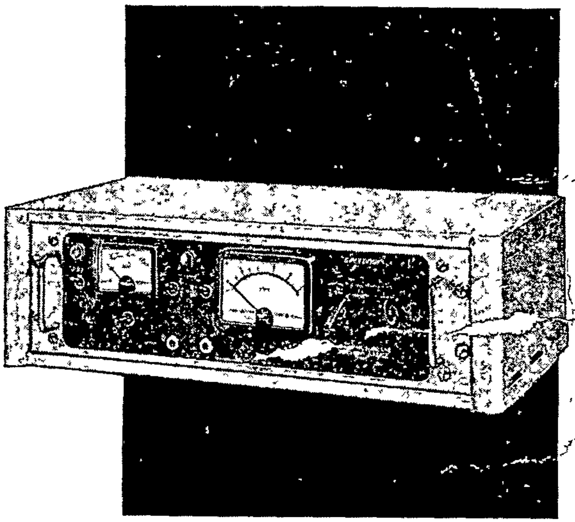

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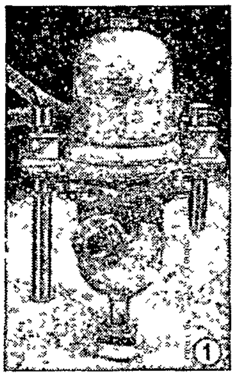

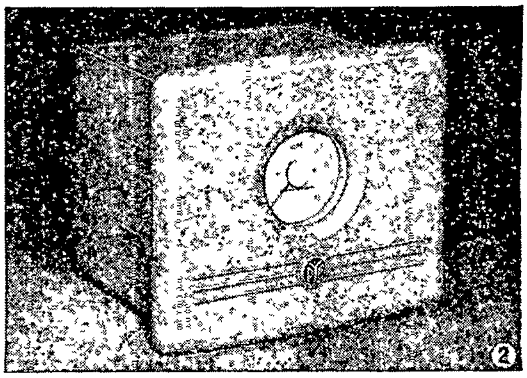

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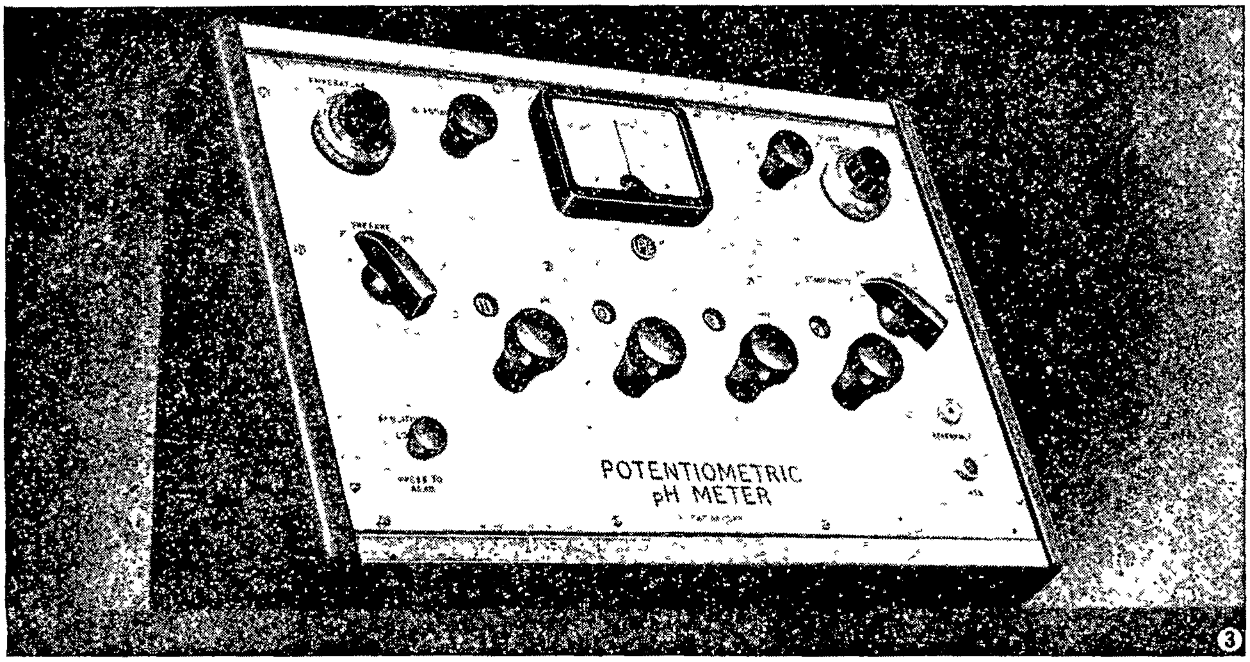

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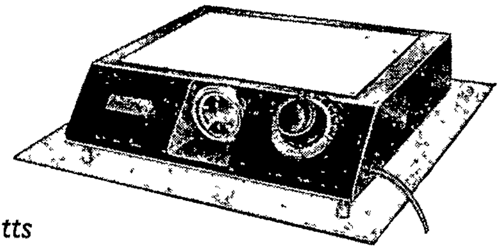

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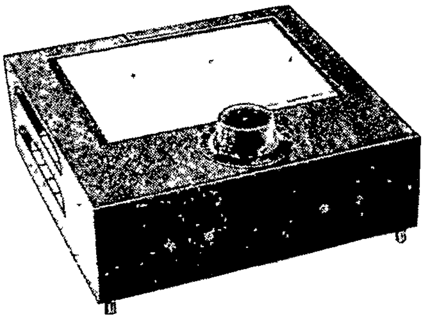

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THE PRICE OF GROWTH*

PHYSICS LI [illegible] RY

THE growth of output is now receiving more emphasis than ever before This is so in the rich countries as well as in the poor, it is true of the Communist world even more than of the West Paradoxically the affluent society has been much criticized in the West Growth and affluence are both emotive words, one good and the other bad

There are several reasons for the increased emphasis on growth Competition has been stimulated by international league tables prepared by economic statisticians, who may be the most subfusc of agitators, but are not the least powerful The needs of national security afford another reason Another is the reluctant but growing recognition that little can be achieved by the redistribution of income, the new emphasis therefore reflects a new realism which may imply a less apocalyptic view of social improvement Moreover, the high degree of success in preventing unemployment since the War has diverted attention to the importance of raising productivity

Growth often involves a price. How much should be sacrificed in other directions for the sake of how much more growth? A more rapid growth of output must not be described as an economic objective while personal freedom or defence or the preservation of local communities are held to be non-economic objectives This is a misuse of terms Economics deals with means not ends Formal welfare analysis has been cautious but modern applied economics is less so It must be recognized that genuine conflicts involving value judgements will be encountered If they do nothing else, economists can at least help to disentangle the issues, and to indicate where possible the orders of magnitude, and to point out where the ultimate value judgements must be made

It has been held that growth would be fostered if the Trade Unions were to be emasculated, as they have been in the U S S R But we would hesitate in the West before overthrowing one of the most effective forms of countervailing power confronting the State Of course, the factual assumptions need to be examined in detail and the possibility of a third alternative investigated. But sacrifices may sometimes have to be accepted Cost-inflation, 'stop - start' and a slower rate of growth may result from a refusal to enforce an incomes policy if such a policy is not accepted voluntarily This should be borne in mind in making international comparisons

If growth were to be given the emphasis in Great Britain which it receives in Communist countries, the best managers might not be greatly affected but the weaker managers would be and the inquisitions and the punishments which would accompany such a policy would represent, in a broader sense, a loss of social efficiency Another question is how far equality should be sacrificed for the sake of growth Even if rapid growth does not mean that there are losers as well as gainers, the gap between rich and poor may sometimes widen domestically or internationally Would more equality be preferred even if everyone were poor? Regional policy affords another example rapid growth is not consistent with bolstering up industry in every location whether suitable or otherwise

The needs of growth will often conflict with other needs, but these conflicts must not be exaggerated Rapid growth will often be the best way of solving other problems as well as raising the average level of income For example, growth in the West has clearly drawn much of the sting from inequality, or again, within the less-prosperous areas. there may be potential zones of growth and the national objective of higher output may coincide with local needs Sometimes an apparent conflict reflects a failure to appreciate the facts It would be foolish to direct labour into particular occupations merely for the sake of growth, direction is inefficient, and individual freedom would often be sacrificed for no purpose Moreover, the quantities must be examined Thus the fashionable claim that for the sake of growth Britain must spend less abroad on aid and defence ignores the orders of magnitude and implies a disproportionate sacrifice for a modest gain

Finally, the role of the economist in these matters was reviewed by Prof Wilson He showed how the economist can break up complex propositions into their consistent parts measurable factual considerations, non-measurable factual considerations; considerations of a kind not professionally studied by economists such as public health or strategy, ultimate value judgements. The value judgements will often be present, but it is suggested that they will give rise to less disagreement than is frequently supposed provided the rest of the analysis has been adequately performed

---

* Substance of presidential address delivered by Prof T Wilson, Professor of Political Economy, University of Glasgow, on September 2 before Section F (Economics) of the British Association meeting in Aberdeen

THE SUN AND THE EARTH'S ENVIRONMENT

Geophysics

The Earth's Environment—Lectures delivered at Les Houches during the 1962 Session of the Summer School of Theoretical Physics, University of Grenoble. Edited by C DeWitt, J. Hieblot and A. Lebeau. Pp xiv + 624 (New York and London. Gordon and Breach, 1963) 10 50 dollars, cloth, 8 50 dollars, paper.

Physique Solaire et Géophysique

Par Prof A Dauvillier. Pp 362 + 1 planche (Paris. Masson et Cie, 1962) 72 NF

DURING the past few years there has been intensive study of the Earth's upper atmosphere and of that part of the Sun's outer envelope which lies beyond it The fascination of this field of research, and the availability of new facts resulting from experiments in rockets and satellites, have led to a voluminous outpouring of publications, some important and some trivial The writings are so numerous that even those closely interested in the subject find it difficult to follow the advance of their own little speciality, those studying the subject for the first time find it unusually confusing Any writings which aim at summarizing the results either for the expert or for those starting research on the subject are particularly welcome, and deserve close examination to decide just how useful they are

Geophysics The Earth's Environment contains chapters based on a series of lectures delivered at Les Houches during the 1962 session of the summer school of theoretical physics, the University of Grenoble

The subjects and authors are as follows "Introduction à l'Electrodynamique des Plasmas" (J F Denisse), "Lectures on Magnetoionic Theory" (K G Budden), "Motion of Charged Particles in Earth's Magnetic Field" (J W Chamberlain) "La Diffusion des Ondes Electromagnétiques par les Plasmas" (R Benoit), "La Constitution et la Composition de l'Atmosphère Supérieure" (Marcel Nicolet), "The Structure of the Ionosphere" (G J F MacDonald), "Introduction à l'étude de la Luminescence Atmosphérique et de l'aurore Polaire" (D Barbier), "Solar Plasma, Geomagnetism and Aurora" (Sydney Chapman), "The Structure of the Exosphere or Adventures in Velocity Space" (J W Dungey), "Whistlers" (R Gallet), "Motions in the High Atmosphere" (G. J. F MacDonald)

The first part of the book, devoted to basic theory, will perhaps prove the most useful to many readers, since it collects, in an admirably succinct way, the important theoretical considerations from a wide field of knowledge Denisse deals with such topics as the diamagnetism of a plasma, the diffusion of magnetic field into a plasma, Alfvén waves, magneto-sound waves, Landau damping. Budden discusses, among other topics, the travel of wave-packets and the concept of rays, the ionospheric dispersion of pulses and short wave-trains, the concept of wave admittance applied to the reflexion of waves from the ionosphere, and the phase-integral method of calculation Chamberlain's treatment of the orbits of magnetically trapped particles, in which he shows the relation between the approaches of Stormer, Alfvén, and the hydromagnetic theory, is particularly helpful Dungey's article might well have been included in Part 1, it contains a useful section on hydromagnetic waves

From Part 2, dealing with the composition and constitution of the Earth's environment, it is natural to pick out the articles by Nicolet and Chapman for particular comment Among other topics, Nicolet deals with the constitution and temperature of the neutral atmosphere, the effect on it of solar radiation, and the diffusion of the resulting ions and electrons Chapman discusses geomagnetism, the solar plasma on its way to the Earth, the mutual influence of the solar gas and the geomagnetic field, magnetic disturbance and aurora, and the ring current and its effects Both these chapters take the form of concise summarized progress reports from workers active in their own fields They are most valuable

The foregoing list of authors and subjects guarantees the scholarly nature and the reliability of the treatment, what it does not indicate is the admirably concise and clear way in which the articles are written A considerable effort has clearly been put into the selection of the material and its presentation in a straightforward and logical manner The highly successful outcome results probably from the fact that these were lectures in which emphasis had to be placed on essentials and fundamentals The authors have suceeded in dealing with the more important and newer parts of their subjects without becoming involved in detail, or losing touch with fundamental principles

The book is up to date, authoritative, selective, and unusually well composed It will be an essential part of the library of all serious workers concerned with the Earth's environment Beginners will find in it a succinct introduction to their chosen subject of research and to related fields

Physique Solaire et Géophysique, by A Dauvillier, might be expected, from its title, to be equally valuable Surprisingly, however, it contains little dealing with research work of recent years, and seems to be largely an account of the subject as known in about 1950 The sub-title lists the contents of the book "L'Héliomagnetisme—le vent solaire l'émission d'électrons solaires rélativistes, la théorie oscillatoire de l'activité solaire, rélations entre les phenomènes solaires et terrestres" The portions on solar-terrestrial relations are so inadequate that the inclusion of *Géophysique* in the title is misleading For example, the section on "Geomagnetism, Variations and Origin" lists 46 references all dated prior to 1952, and occupies only 16 pages, that on the ionosphere lists 23 references previous to 1952 and 6 after that date and occupies 8 pages

The book from Les Houches is so outstandingly good that it is perhaps a little unfair to review the other with it, but even if it had been considered alone, the book *Physique Solaire et Géophysique* could scarcely be described as an up-to-date text-book for any purpose

J A RATCLIFFE

MASS TRANSFER SIMPLIFIED

Convective Mass Transfer

An Introduction. By Prof D B Spalding Pp xv + 448 (London Edward Arnold (Publishers), Ltd, 1963) 70*s* net

PROF SPALDING is known for his idiosyncratic approach to heat and mass transfer This introductory volume sees his method displayed to full advantage and may well win converts to his cause His enthusiasm and determination are obviously inexhaustible, for there is not a sloppy paragraph in the whole book

After a model preface which explains his present purpose, he surveys the field of his endeavour in a short introductory chapter, this gracefully encompasses such diverse topics as evaporation from the ocean, the formation of smoke in a flame, the ablation of a meteorite and the Bessemer converter, besides the many separation and extraction processes of chemical engineering With admirable precision, he then enunciates his "Provisional Theory of Mass Transfer" (some might prefer the word interpretation to the word theory, but this is to be petty), and describes the two concepts on which it is based Reynolds flow and driving force By choosing a graded sequence of real phenomena, the author shows how these 'fundamental' quantities are derived in progressively more complex examples of mass and heat transfer across an interface, and finally shows how transfer rates can be calculated in a comprehensive range of practical circumstances, most of them in the field of chemical engineering and fuel technology

Simplicity and directness, ever desirable qualities in an engineer, are the mainspring of his approach An engineer seeks to calculate mass-transfer rates, not necessarily to understand the physical and chemical processes leading to them Prof Spalding evidently realizes this, and has made a great effort in this volume to provide a consistent and comprehensive elementary text-book for the practising engineer It proves equally suited to undergraduate teaching This utilitarian attitude is not the present trend in pedagogy, and for this reason such a book deserves careful consideration The author succeeds magnificently in his task of providing a simple framework that will admit the results of modern theory and experiment through progressive elaboration of the description of the 'fundamental' quantities Constants become functions, new dimensionless groups appear, boundary conditions are refined

All this is done without reference to anything more elaborate than derivatives and integrals The results of more exact theories relying on the solution of differential equations are quoted and used, but their derivation is relegated to later volumes This 'layman's' approach uses engineering science as a means to an end, and no more A refreshing verdict and one that should commend itself to those who are attempting to foster technological progress in Britain As the numbers undergoing higher education increase, teachers will need more and more text-books of this type, in which the complicated results of modern science are digested, formalized, simplified and shown to be useful in well-defined contexts Intending authors could do little better than study Prof Spalding's exemplary model Starting from a wealth of scholarship and experience, he has provided a lucid and logically developed exposition of a topic that can now almost be regarded as a discipline in its own right A generous number of worked examples and a profusion of diagrams further enhance the value of this work, which is singularly free from the errors, omissions, ambiguities and inelegancies that characterize most text-books published to-day

J R. A Pearson

PETROLOGICAL MINERALOGY

Rock-forming Minerals

By Prof W A Deer, Dr R A Howie and Dr J Zussman Vol 2 Chain Silicates Pp x+379 Vol 3 Sheet Silicates Pp x+270 Vol 4 Framework Silicates Pp x+435 Vol 5 Non-Silicates Pp ix+371 (London Longmans, Green and Co , Ltd , 1962–63.) 95*s* each volume

THE first volume of this series was reviewed in *Nature* of July 7, 1962, p 4 The work is now complete in four further volumes of similar average length of about 350 pages Variations of chemical composition within a group, discussed on a structural basis, are treated throughout as fully as in Volume 1 The descriptions of the pyroxene group and of the amphibole group, for example, each occupy a little less than one-half of volume 2, and each account is accompanied by more than 200 selected analyses (there are more than 1,600 in the whole work), with optical data where available and the appropriate structural recalculations of numbers of ions The account of each species concludes with a valuable discussion of the paragenesis

It is difficult to find fault with the selections of references to recent work appended to the accounts of each species References are stated to be comprehensive to 1959 (in Volume 4 to 1960), but there are many later additions—from Volume 4 one can trace stishovite, and recent work on melanophlogite, through references dated 1962 References are also given to selected classical papers, and here one sometimes misses a favourite, there seems to be no reference, for example, to Wahl's work on pyroxenes A similar problem of selection has faced the authors in the matter of obsolescent mineral names In Volume 2 there are indexed references to names such as magnophorite, osannite, and imerinite, though they are discarded as unnecessary, one cannot, however, trace damourite, gilbertite, or margarodite through Volume 3 Among preferred spellings are piemontite, katophorite, and felspar

I have noticed even fewer printer's errors in these volumes than in Volume 1 This strengthens confidence in the accuracy of reproduction of the whole mass of numerical data, which can, of course, only be checked in detail by use The index 110 is misplaced in Fig 24 *b* of Volume 4, and the indexing of the figure of barytes is not in accordance with the structural description It is not clear what is implied by the assertion, in the tabular statement of properties, that the *orthorhombic* pyroxenes show 'simple and lamellar' twinning on {100}, though there is, of course, a discussion of the significance of the lamellar structures later in the text Fassaite is characterized, surely, by the strong development of the form {110}, and not of the zone [110] Structurally it is improbable that periclase shows an octahedral cleavage though the statement is repeated in many text-books

Inevitably, the speed with which mineralogical research is progressing means that some passages in these volumes already call for modification—the phase diagrams of Figs 15 and 17 of Volume 2, for example, in the light of work published since 1960 One hopes that the make-up in five reasonably sized volumes (which are a delight to handle) will enable the authors more easily to keep pace with the revised editions which will surely be demanded This sub-division will also enable individuals to avoid the expense of the whole series, variously described by reviewers of Volume 1 in terms ranging from "rather expensive" to "lamentable", by acquiring only the volumes with which they are directly concerned It seems likely that Volumes 2 and 4 will be most consulted, closely followed by Volume 1 Specialized literature already exists in the field of the clay-minerals and their allies, covered in the last third of Volume 3, and also for a number of the groups of non-silicates in Volume 5, such as the opaque ores In the remainder of the series, however, covering major mineral groups for which no comprehensive literature has hitherto been available, the authors have gone far beyond mere compilation and provide accounts which are informative and critical There is no general index to the five volumes, this is little drawback to tracing mineral descriptions in view of the clear-cut division of the first four volumes on the basis of structural types, but it does sometimes make it less easy to locate more general matter such as references to a particular system

Reviewing the work as a whole, one is left with a feeling of gratitude to the three authors for undertaking this very necessary but highly onerous task, and with nothing but admiration of the successful way in which they have carried it out Just as one has automatically turned to 'Dana' for crystallographic data, and to 'Winchell' for optical data over the whole field of minerals, so 'Deer, Howie, and Zussman' will certainly establish itself as the standard source of information on the chemical, structural, and optical properties of the important rock-forming minerals

F C Phillips

DIAMONDS IN INDUSTRY

Proceedings of the First International Congress on Diamonds in Industry

Held at the Centre des Conférences Internationales, Paris, 28 May–2 June, 1962 Pp xi+407 (London Industrial Diamond Information Bureau, 1963) n p

THIS handsome volume of 400 quarto pages provides a valuable permanent record of papers read and discussed at the first International Congress on Diamonds in Industry, which was opened in Paris on Monday, May 28, 1962, and closed on the following Saturday No fewer than 38 quite important papers were presented at the Congress, and in most cases were followed by a lively discussion The inclusion of a report of these discussions in the symposium adds considerably not only to its scientific and technological but also to its human interest

Diamond is, of course, of outstanding importance as a gemstone and (because of its extreme hardness) as an industrial material, but it is also of very special interest in solid-state physics on account of its unique behaviour and the (largely unpredictable) variations in its behaviour Although its elementary composition has been realized for centuries and its crystal structure was one of the first (1913) to be established by the methods of X-ray crystal analysis, this was far from meaning that our knowledge of the mineral was complete The discovery that, based on differences in their absorptive properties, diamonds can be classified into two 'types' was described by Robertson, Fox and Martin (1931) in a famous paper and served as a signal for further research In succeeding decades the interrelation of absorption and fluorescence, the alterations brought about by various forms of irradiation, the 'counting' and semi-conductive properties of certain specimens, and the first repeatable and large-scale production of diamond by artificial means, have all acted as further stimuli for investigation There is now scarcely any aspect of diamond behaviour which has not been closely studied by physicists, and the pace shows no signs of slackening

In recent years, credit for encouraging such intense activity in this field must be given to the de Beers group of diamond companies, which has not only established three well-equipped and well-staffed diamond laboratories in South Africa, but has also sponsored fundamental research in several universities in Britain, and provided specimens for research in all parts of the world Annual conferences have also been arranged at Oxford, Reading, Bristol, etc , where workers on diamond have given research reports and exchanged views

Although the Paris Congress was the first to be held on the industrial diamond, it had much in common with the previous discussions on diamond in general—particularly in the last 15 papers, which are grouped under the heading "Scientific Session" In fact it is symptomatic of modern trends in industry that only some half-dozen papers even in the "Technological Session" could be remotely understood by the non-scientist Sir Gordon Sutherland acted as general chairman of the technological session, and Prof Jean Wiart of the scientific session

The technological papers for the most part follow expected lines, as suggested by some of the titles, for example, "Determination of the Shape of Diamond Grit Particles"; "A Comparison of Different Methods of Particle Size Analysis", "Evaluation of Diamond Grit in Resin Bonded Diamond Wheels", "Theory of Grinding", etc

The purely scientific papers opened with a review of the semiconductivity of diamonds by Prof E W J Mitchell and of optical properties of electron- and neutron-irradiated diamond by Dr C D Clark, both of the University of Reading, where so much of the original work on these topics was carried out The Clarendon Laboratory, Oxford, another important centre for diamond research, was represented by Dr Eileen M Wilks on the hardness of diamond and by Dr R Berman on the graphite-diamond equilibrium diagram

From the Cavendish Laboratory, Cambridge, came a valuable contribution on fracture propagation in diamond, in which the main 'fracture' considered was the octahedral cleavage By means of high-speed photography, a maximum velocity of 7,200 metres per second was recorded for this cleavage Marked differences between the cleavage surfaces of Type I and Type II diamonds have been observed

No diamond conference would be complete without a contribution from Prof Tolansky, whose applications of multiple-beam interferometry have so enriched our knowledge of the detailed nature of diamond surfaces Tolansky gave an account of the surface topography of natural and synthetic diamonds, including crystals made by the General Electric Co of America, the de Beers laboratories, and the Allmanna Svenska Elektriska Aktiebolaget· notable differences were observed between crystals produced in each of these centres, and between all synthetic crystals and those found in Nature

An unusual contribution was one from Michael Seal (New Jersey) in which the experiments of Hannay and Moissan on the synthesis of diamond are re-examined Plastic replicas of the surfaces of the tiny fragments left by Hannay in the British Museum (Natural History) were prepared to enable them to be examined by electron microscopy and compared with the corresponding surfaces in natural and modern synthetic diamonds, and the experiments of Moissan were substantially repeated The residual fragments, after lengthy separation processes, had much in common with those described and figured by Moissan, and in some cases closely resembled diamond, though most were found to be silicon carbide or corundum Mr Seal kept a commendably open mind on the claims of these early pioneers

Space does not permit even the mention of many other valuable papers in this symposium Anyone interested in the properties of diamond will certainly wish to possess this volume The ample references at the end of each paper enable the reader to explore each subject in depth if required Mr Patrick Greene is to be congratulated on his success in assembling, editing, and where necessary translating this mass of information

B W ANDERSON

MUTATION AND GENETICS

Mutation

An Introduction to Research on Mutagenesis By Dr. Charlotte Auerbach Part 1 Methods Pp xii+176+4 plates (Edinburgh and London Oliver and Boyd, Ltd , 1962) 12*s* 6*d* net

The Science of Genetics

By Dr Charlotte Auerbach (Hutchinson Science Library) Pp x+273 (London Hutchinson and Co (Publishers), Ltd , 1962) 21*s* net

DR CHARLOTTE AUERBACH has contributed two more books to the literature of genetics They are written with the economy and lucidity of style for which the author is already renowned, and each is admirably planned and executed for its purpose In *Mutation An Introduction to Research on Mutagenesis Part 1 Methods*, Auerbach introduces her subject with two short general chapters covering types of mutation, methods of distinguishing environmentally induced variations from truly genetic ones, mechanisms of mutation and the concept of mutation rate, and five basic rules for mutation research Methods and procedures for various organisms are then outlined under four main headings *Drosophila*, animals other than *Drosophila*, flowering plants and micro-organisms The last category occupies one-third of the book and includes explicit accounts of screening tests in *Neurospora* and bacteria, the detection of biochemical and other physiological mutations in fungi, algae and bacteria, and the genetical analysis of mutants in these types and in viruses A final chapter deals with new systems such as transforming principle and mammalian cells The book is illustrated by diagrams and tables which are helpful in supplementing the text and providing condensed material for reference Each group of chapters is followed by a few references to books and papers, with titles, and sometimes with a note about contents, making it easy for the reader to pursue particular topics This small book will be valuable not only to students and investigators in the field of mutation research. but also to many non-geneticists who need a means of understanding experiments in mutagenesis

The second book, *The Science of Genetics*, is an elementary text-book, taking the reader all the way from a definition of the subject to virus and bacterial genetics, transforming principle, and a description of the DNA double helix and its genetic code The stages in this journey are easy, the chapters are short and each follows smoothly from the one before, consolidating in unobtrusive but reassuring ways the progress already made No previous biological training is assumed The book is admirably suited for school use at the same time its range and substance are such as to make it rewarding also to the educated biologist, and few adult readers will fail to wish that an equally brief, lucid and informative text had been available in their younger days Here are all the fundamentals of the science, as well as a view into its more advanced chapters and its wider implications Mendel's laws, the chromosomal basis, linkage and crossing-over, sex-linked and lethal genes, quantitative characters, gene-controlled metabolic pathways, interaction between genes and environment, cytoplasmic inheritance, the genetic basis of evolution, eugenics, and much more

Illustrations are chosen primarily for their ability to make the point clear, but when possible from human genetics, or from familiar plants and animals. Throughout the book, the author's own sense of the fascination and excitement of her subject shows through the easy personal style of the writing Occasionally the reader is explicitly restrained from incorrectly extending genetical conclusions to human racial questions In this way the author shows her awareness of the readiness with which the human mind finds material to justify its prejudices.

More than eighty refreshingly informal drawings and diagrams by G. Auerbach Linker not only add charm and lightness to the book, but also play an essential part in supplementing the text There are a very short list of titles for further reading, and an index ALMA HOWARD

THE DAIRY COW

The Dairy Cow

Reproduction, Genetics, Nutrition, Habitat, Principal Diseases By C Craplet, translated by Catherine T M Herriott Pp xv+452. (London Edward Arnold (Publishers) Ltd, 1963) 130*s*

THIS volume consists of six parts, each dealing with one of the following subjects, reproduction and lactation, artificial insemination, genetics, nutrition, housing and disease The author is head of the Animal Husbandry Department in the Grignon School in France and the book was written first in French It would have been helpful if the author had supplied even a short foreword to explain his object in writing the book and to state clearly for whom he intended it, but he has not done so, and one cannot help wondering if the book originated as notes on which was based a series of lectures to students taking a general but fairly elementary course in animal husbandry, the notes in some parts applying, quite naturally, more to France than to Britain, and in some parts being now somewhat out of date

If, for example, one seeks information on iodinated protein, two page numbers are given in the index On p 258 the author explains that "unfortunately" experiments with iodinated protein are still in the laboratory stage, and yet on p 71 he states that the stimulating property of thyroxine on milk secretion "has been recommended to the breeder in the form of thyroxine or iodized protein administration" Neither statement is true for Britain, where work with iodinated protein reached the applied stage some 15 years ago or more, but where the use of thyroxine or iodinated protein is not recommended to breeders

On p 306, guidance is given on the seeding of pastures, but under the heading of "Density" (presumably amount of seed to use per acre) the author simply states, "see modern agricultural text-books" One might feel that in a book on the dairy cow, there would be little need to deal with reseeding of pastures since pasture management, like so many subjects to-day, is becoming more and more a matter for the expert, but if pasture management is to be discussed, surely the paragraph on density could have been a little more informative

Even the relative costs of barbed wire and electric fences are dealt with in this book on the dairy cow, but they are based on prices that prevailed, presumably in France, in 1948, fifteen years before the book was published, a period in which great changes in prices have occurred and in which the development of the electric fence has made much progress In calculating costs the wire fence taken by the author as an example consists of five strands of barbed wire, in Britain it is usually considered sufficient if the top strand only is barbed

Writing about the watering of cattle the author states, "In hot weather cows may drink 80 per cent more than usual, in cold weather their intake decreases slightly, so that heating the water is a good precaution to take" It is to be hoped that any British farmer who may see this statement will not feel that he must add the heating of drinking water for cows to his numerous duties unless the water happens to be undrinkable because it is frozen

In the section that deals with the utilization of the volatile fatty acids this curious sentence occurs, "Experimental work has revealed the facility with which tissues utilize acetic acid and this is the reason for the perfused heart of a rabbit using up more acetate per hour than glucose" But why is that *the reason* ?

In discussing eradication of mastitis on p 391, the author recommends disinfecting the udder with water sterilized with potassium chloride This would be quite ineffective; perhaps he means sodium hypochlorite Two pages later in dealing with staphylococcal mastitis the statement is made that "infected cows should be milked in the dry period for this will give excellent results"

Unfamiliar terms are used in discussing the chemical composition of milk, for example, "de-fatted dry extract" which presumably means solids-not-fat and "lime combined with casein", terms that must seem strange to a modern dairy chemist There are also some irritating little mistakes On p 246, for example, the phrase "permanent water" is used where a piped supply of water is meant, and nutrient supply is said to equal "nutritive value of food X quality consumed"—surely a mistake for "quantity consumed" In the index the name Voisin is given as Viosin Many workers are cited in the text but the book contains no list of references of any sort, so that the reader, unless he knows already, does not know where he can find the work cited or when it was done

This is all most disappointing, since the dairy cow is an important subject and the publishers of this English translation have certainly produced it in a most attractive form The whole lay-out, the type, the figures and the binding are of exceptionally high quality, so much so that a cursory glance might well suggest that the book ought to be well worth the money to a reader in Britain Alas, such a claim would be difficult to justify Indeed research and its application in veterinary and farm practice have advanced so far in recent years that the time may well have come when each separate part of a book like this, which ranges from genetics and physiology to housing and disease, should be written by an expert of its own It is scarcely fair nowadays to expect one man to deal adequately with such a wide field, and in matters concerning animal husbandry what applies in one country may not necessarily apply in another

J A B SMITH

MEDICAL BIOMETEOROLOGY

Medical Biometeorology

Weather, Climate and the Living Organisms By Dr S W Tromp, in co-operation with 26 Contributors Pp xxvii +991 (Amsterdam and London Elsevier Publishing Company, 1963) 120 D fl, 240*s*, 134 D M

THE influence, real or imaginary, of weather on the behaviour of man and animals has been the subject of controversy and consideration by many authorities and individuals ever since the human race began to think The scientific development of the problems, hampered though it was by contributions more conspicuous for their enthusiasm than their logic, has made rapid progress during the past decade, and the author of this volume, together with his twenty-six collaborators, have made a very welcome attempt to summarize what is now known of a very complex subject

Dr S W Tromp is head of the Bioclimatological Research Centre at Leyden in The Netherlands, and his contributors are specialists of high scientific standing in the United States, Germany, Great Britain, Canada, Peru, Australia, The Netherlands and Israel. The book, of nearly a thousand pages, commences with a review of the physical, chemical, air pollution and extra-terrestrial factors of the atmosphere and the methods used in assessing their effects on the living organism Part 4, comprising nearly half the book, entitled "The Effect of Weather, Climate and Season on Man", deals in considerable detail with the meteorological influences on the human being and his semi-artificial living conditions in health and disease Shorter chapters follow discussing effects on animals and insects, and Part 6 deals with the microclimate of space vehicles

The final contribution is of the highest importance, written by Prof F Sargent of the University of Illinois, it discusses the necessity and the organization of graduate training in human biometeorology The urgent need for rapid advances in scientific fields involving two or more disciplines is such that the basic principles stated herein deserve wide recognition The young scientist must be given sound training in one main science followed by a firm working knowledge in the interdisciplinary régime In addition he must be able to work as a member of a team so that specialized training and ability can be combined to solve a problem which is of a multiple scientific nature Most important of all, a student must learn to be able to understand and interpret complex interrelated facts. It must clearly be understood that advances in sciences are not made by the simple collection of endless data, but by the brains of the men who are capable of perceiving the new relationships which can justifiably be deduced from such facts

The last part of the book consists of a very helpful bibliography of more than 4,000 entries The very volume of diverse contributions scattered throughout the scientific journals of the world explains why a book of this nature meets a real need As the author says in his "Retrospect", biometeorologists still have a long way to go before they can solve all the important problems and fully explain the various observations made Nevertheless, the publication of this volume can truly be regarded as a significant step forward on the road towards increased reliable, useful knowledge

The printing and presentation are good, the author deserves our thanks for his work and our admiration for his energy and devotion to his aims His last sentence gives us the clue as to how this can come about—"It is this deeper understanding of the continuous interaction between the living and non-living worlds, which makes biometeorology one of the most fascinating and promising border sciences of the twentieth century"

L P SMITH

THE PRIMARY WORKING GROUP

Organizational Choice

Capabilities of Groups at the Coal Face Under Changing Techniques—The Loss, Re-Discovery and Transformation of a Work Tradition By E L Trist G W Higgin, H Murray and A B Pollock Pp xv+332 (London Tavistock Publications (1959), Ltd, 1963) 48*s* net

IN most low-seam coal-mining areas three main types of mining co-exist the traditional system of single-place working, a conventional system involving the cutting or hewing of longwalls and hand-filling the coal on conveyors, and the attack on longwalls by a composite group with a much higher level of mechanization

In the single-place system the key-figure is the multi-skilled self-supervising face-worker to whom the deputy or foreman stands more in the role of service agent than that of supervisor The face-worker graduates to this position after years of working on supporting jobs and when he has established his right to the commanding position. Under the conventional system a distinct division of labour emerges where specialized tasks are carried out by a number of groups of varying size Each group has separate pay-notes and its own mores, while inter-group rivalry and some hostility are not infrequent Co-ordination and control come directly from management

With the composite longwall system of working the difficulties arising from over-specialized work roles, segregated task groups and lack of group cohesion are successfully overcome There is a common pay-note with an equally shared bonus The working group itself allocates men to shifts and jobs and the result is a multi-skilled commando-like force where all suffer financially if any work is neglected

Organizational Choice is a long-term study by members of the Tavistock Institute of Human Relations, which describes, *inter alia*, investigations into the effects on productivity of the single place, conventional longwall and composite longwall systems of working The conclusions are not unexpected, and, in a truly fascinating way, Trist and his fellow-authors show that where the activities of a primary working group can be planned and controlled by its own members, the only barriers to productivity are those set by physical limitations in man and the environment Over a period of two years, the composite group showed itself not only capable of functioning as a "self-regulating, self-developing social organism", but also able to maintain itself at a high level of productivity throughout the whole period Even more important was that, at the end of the time, the groups were still growing in their capacity to adapt to changes in their task environment and to satisfy the needs of their members What is more significant, however, is that the composite method of working under a fully mechanized system is but a return to the traditional 'butty' system common in coal-mines before the advent of mechanization The wheel has turned almost a full cycle and these investigations show that the ruthless breaking down of jobs without regard to human need and dignity leads only to destructive frustration and a low level of productivity, which is the accompaniment of poor individual and group morale

Apart from a spontaneously generated jargon which characterizes so many Tavistock Institute publications, *Organizational Choice* is a well-written description of a not undramatic story The text unfolds to show a clear design, and this is supported by some illuminating tables and diagrams General conclusions of importance are developed from the specific findings, and all National Coal Board officials should see that the authors' plea for a machine-centred work culture to replace the existing manual work culture could represent the birth of a new attitude to work and workers in the coal-mines The idea of group training of work-groups rather than individuals also has much to commend it It is perhaps unfortunate that details of the present-day pay and incentive schemes have not been included if only to enable responsible management and Trade Union officials to persuade more recalcitrant colleagues that the benefits to the men are not only intangible

So valuable is the book, however, that one wonders whether the authors are doing justice to themselves and their collaborators Their conclusions should be within the reach of all supervisors and workers in the coal-fields of Britain and elsewhere Could their account be turned into film? They might also have indicated other industries and organizations where their findings might have validity. Metal-making rolling and forging? Oil and chemicals? Retail stores? The approach described in this book and the conclusions arrived at by Trist and his colleagues should also form the basis for case-histories wherever managers and supervisors are being trained

T H HAWKINS

SCIENCE AND THE STATE*

PHYSICS - ART

By Prof R. V JONES, C B, C.B.E.

Department of Natural Philosophy, University of Aberdeen

The 1859 British Association Meeting

THE British Association first met in Aberdeen in 1859, and the report of the meeting shows what a great part the Association at that time played both in the world of science and in the life of the nation The Prince Consort was president, and his address was a beacon in the advancement of the status of science in Britain The literature for the meeting included a long list of distinguished visitors from Great Britain and the British Empire, there was also a shorter list of equally distinguished men from other countries, formally but bluntly headed, with Victorian confidence "Foreigners" This confidence was at its peak, still glowing in the triumphs of the Great Exhibition of 1851, and it was a symptom of the energy of the Victorians, and of the enthusiasm of Aberdeen, that when in 1857 the citizens of the city found that they had no building fit to welcome the great assembly of the Association, they floated a private company and subscribed enough capital to build the Music Hall in which Sir Eric Ashby delivered his presidential address to this year's meeting The money was found and the Hall built, all within two years Could we beat that performance to-day ?

The multitude of papers presented at the meeting contained many original results Here again, comparison with our own time shows the 1859 meeting in a most favourable light in Section A, for example, 79 papers were presented, compared with 25 this year There are, of course, reasons why the number has diminished progress and competition in science now preclude the saving-up of major results for an annual meeting But by any standards, the content of the 1859 meeting was remarkable James Clerk Maxwell was a professor of natural philosophy in Aberdeen, and at the meeting he announced his derivation of the law of distribution of molecular velocities in a gas, with Faraday, Kelvin and Joule among his audience

Maxwell, incidentally, was one of the original subscribers to the Music Hall Company, and from time to time small dividends were paid He left Aberdeen in 1860, and died prematurely in 1879, but for long afterwards attempts were made, with Aberdonian honesty, to pay him his dividends In the end, some time in the present century, a desperate advertisement appeared in the Aberdeen local newspaper to the effect "Will anyone who knows the present whereabouts of Mr James Clerk Maxwell please communicate with the undersigned" The advertisement came from the lawyers responsible for the payment of shareholders, because for years they had been sending the dividends to Maxwell at Marischal College, only to have them repeatedly returned with the legend "Not known"

Aberdeen's tendency to forget Maxwell persisted into the 1930's In the brochure produced for the 1934 meeting of the British Association in Aberdeen, his name will not be found among the list of physicists who have been connected with the University of Aberdeen This forgetfulness, since happily corrected, stood in contrast to the attitude towards Kelvin in Glasgow, where Prof Dee found on his arrival in the Department of Natural Philosophy there in 1945 all kinds of Kelvin relics, including a museum display case full of broken glass reverently labelled "Glass broken by Lord Kelvin in an attempt to make a vacuum"

Lyon Playfair

Our attitude towards the great figures of the British Association of the nineteenth century should properly lie between these extremes We do not need to remark every triviality of their everyday lives, but we have every reason to remember with pride and admiration the greatness of their achievements I wish to recall that among the most interesting of all the distinguished men at the 1859 meeting was Lyon Playfair who, at the age of forty-one, was then for the second time the president of the Chemistry Section, and who was to be president of the Association when it next visited Aberdeen in 1885 In his presidential address, Playfair spoke on "Science and the State"[1], and what I propose to do is to see what progress we have since made along the far-seeing lines that he laid down, and to look at some of the new problems that have in the meantime appeared

At the time of his address, Playfair had given nearly fifty years of service both to science and the State, and had thus acquired experience that even now is without parallel He had been a friend and adviser of the Prince Consort since the time of the 1851 Exhibition, and it was natural that he should turn for his theme to the Prince's own address, from which he took as text "We may be justified in hoping that the Legislature and the State will more and more recognize the claims of Science to their attention, so that it may no longer require the begging-box, but speak to the State like a favoured child to its parent, sure of his paternal solicitude for its welfare; that the State will recognize in Science one of its elements of strength and prosperity, to foster which the clearest dictates of self-interest demand".

Science and Education

Playfair started by pointing, as he had so often done before, and as so many of us have done since, to the fact that other countries appeared to value science and education more than Britain did Especially he mentioned the United States, which had reserved "large tracts of its national lands for the promotion of scientific education" This was the origin of the 'land-grant colleges', less-respectfully called the 'cow colleges', which developed into the present State universities that are an important feature of the American educational system Playfair cited with approbation the wisdom of Washington's farewell address to his countrymen "Promote as an object of primary importance institutions for the general diffusion of knowledge In proportion as the structure of a government gives force to public opinion, it is essential that public opinion should be enlightened"

Playfair devoted much of his address to the part that science should play in education, underlining the conclusion[2] of the Duke of Devonshire's Commission on Scientific Instruction in 1873 "Considering the increasing importance of science to the material interests of this country, we cannot but regard its almost total exclusion from the training of the upper and middle classes as little less than a national misfortune".

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* Substance of an Evening Discourse delivered on September 2 at the meeting of the British Association for the Advancement of Science in Aberdeen

The setting-up of that particular commission had, incidentally, been very largely due to Playfair and a body of like-minded men in the British Association and in the Society of Arts. Most of these men are more familiar to us to-day than Playfair is: Huxley, Kelvin, Ruskin, Herbert Spencer, and Matthew Arnold. But there was one even more forgotten than Playfair, and so perhaps I may be permitted to mention him especially. He was Colonel Alexander Strange, a survey officer of the Royal Engineers, who had read a paper before the 1868 meeting of the British Association, "On the Necessity for State Intervention to secure the Progress of Physical Science". Strange developed his ideas further in a lecture to the Royal United Services Institution in 1871. I quote his far-seeing words[3], as a tribute to an almost forgotten officer who saw far more clearly what his country needed than did the lobbies of the Houses of Parliament or the common-rooms of Oxford and Cambridge:

"The duty of the government with respect to science is one of the questions of the day. No question of equal importance has, perhaps, been more carelessly considered and more heedlessly postponed than this. And, now that a hearing has been obtained for it, neither the governing class nor the masses are qualified to discuss it intelligently; the governing class, because it is for the most part composed of men in whose education, as even the highest education was conducted 30 to 50 years ago, science occupied an insignificant place; and the masses, because they may be taken to be virtually destitute of scientific knowledge. Those who wield, and those who confer, the powers of government being alike incapable of dealing with this question, it devolves on another section of the community to urge its claims to attention.

I must guard myself against the supposition that the proposal I have here advocated comprises all that is necessary for the efficient administration of scientific State affairs. It is only one part of a great system that has to be created. Other parts of the system will, no doubt, receive due attention from the Royal Commission now considering them. But there is one part so important that I feel called on to name it—I mean the appointment of a Minister of Science. He need not necessarily be exclusively devoted to science; he might perhaps, with advantage, have charge of education and fine arts also; but some one in Parliament, directly representing the scientific branches of the national services, has become absolutely indispensable. Another urgent want which, as its scientific character is not purely physical, will probably not be dealt with by the Royal Commission on Science, is that of a high war council—a council of naval and military officers of the greatest professional attainments and distinction, constituted for the purpose of advising the government on the highest problems of strategical science. At present we have not a vestige of anything of the kind, and are consequently, as a military nation, almost destitute of the basis of the military art.

"When we have all scientific national institutions under one Minister of State, advised by a permanent, independent, and highly qualified consultative body, when we have a similar body to advise the Ministers of War and Marine in strategical science, then the fact that, in accordance with our marvellous constitution, these ministers must almost necessarily be men without pretension to a knowledge of the affairs which they administer need cause us no alarm. When these combinations have been, as they assuredly will be, sooner or later, effected, the wealth, resources, and intelligence of the nation, having due scope, will render us unapproachable in the arts of peace, and unconquerable in war, but not till then."

Extraordinarily far-sighted though this was, and even though the Royal Commission of 1871 fully supported the statements of Playfair and Strange, insufficient notice was still taken by the educational public. Another Royal Commission, this time on Technical Instruction, was instituted in 1881, and reported in 1884. It warned that although Britain was still in advance as an industrial nation, other countries would soon pass it unless there was increased attention to science in public education. In the first part of his address, Playfair elaborated on this prognosis, particularly in respect of secondary education. The working classes, he said, were now receiving better instruction in science than the middle classes. This curious result may have been due in part to a working-class demand to know something of the principles behind the machines that they were called on to operate; this demand had already led to the establishment of more than five hundred Mechanics' Institutes in the first half of the century. The other main factor was very probably an unfortunate legacy of Thomas Arnold, the great headmaster of Rugby.

Science and the Schools

Physical science was, in Arnold's opinion: "too great a subject to be studied 'ἐν παρέργῳ' and it must either occupy the chief place in the school curriculum or be left out altogether"[4]. "Rather than have physical science the principal thing in my son's mind", he said, "I would gladly have him think that the sun went round the earth, and that the stars were so many spangles set in the bright blue firmament. Surely the one thing needful for a Christian and an Englishman to study is Christian and moral and political philosophy." And as regards what his curriculum should be: "The study of language seems to me as if it was given for the very purpose of forming the human mind in youth; and the Greek and Latin languages seem to be the very instruments by which this is to be effected". At that point English education took an unfortunate turning; but I cannot help wondering whether Thomas Arnold would have been as rigid as many of his followers in resisting the change to a curriculum in which science was fairly represented.

Playfair and his allies, notably Huxley, fought for a complete revision of the secondary-school curriculum. Especially he attacked in his address the large proportion of the time devoted to classical studies; typically, 12–16 hours a week were given to the classics, and 2–3 hours to science. He cited Milton, Montaigne, Cowley, and Locke as earlier partakers in the assault on classical education although, he said, he recommended milder measures than that of the Emperor of China, Chi Hwangti, who, knowing that his country was held back by undue devotion to the classics of Confucius and Mencius, invited five hundred teachers to bring their copies of these authors to a banquet in Peking, and then buried the teachers alive with their manuscripts in a great pit.

In answer to the critics who had claimed that the boys who had been through a classical education were better than those who had studied science, Playfair retorted by pointing to a selection factor that is still familiar to us to-day: "The comparative failure of the modern side of school education arises from constituting it out of boys who are looked on as classical asses". He blamed such influences as the Civil Service examinations, with their biased marking in favour of the classics, for the reluctance of the schools to change the weighting of their curricula.

Much of the bias has now, of course, been swept away. Physics has, I believe, been for some years the commonest subject taken at the Advanced Level of the Certificate of Education. But the bias persisted for nearly a century after it should have disappeared, despite the points so tellingly made against it by Playfair; and we still see vestiges of its survival—there is no paper on science in the Common Entrance Examination to Public Schools, although there is a compulsory paper in Latin and an optional one in Latin verse. We can still feelingly echo Playfair's apothegm: "The flowers of literature should be cultivated and gathered, though it is not wise to send men into our fields of industry to gather the harvest when they have been taught only to cull the poppies and to push aside the wheat"

It would be both tempting and profitable to examine in more detail the progress of the teaching of science in the schools since Playfair's day Genuine efforts were made in the nineteenth century, even in the primary schools some of my earliest awakening in science came from my mother, who had learnt of such things as the properties of mercury and the characteristics of nitrogenous foods in a London elementary school before 1900 To-day, we are trying more than ever to teach science attractively in the schools, and we should remember especially the support being given for this development by the Nuffield Foundation and by industry

Science and the Universities

Playfair was scarcely less critical of the universities than he had been of the schools, although he commended them for not being so slow in changing their traditions, and for encouraging physical science That he knew them well is obvious from the shrewdness of his observation[5]

"You must not judge of other universities by Oxford and Cambridge for they are exceptional The old English universities have not the same function as the Scotch and Irish universities The former teach men how to spend a thousand a year, while the latter aim at showing men how to make a thousand a year "

He compared the paucity of Government support for universities in Britain with the liberality of the French, German, Swiss and Dutch Governments towards their universities Indeed, he said, by a special clause in the Scottish Universities Bill, the Government had asked Parliament to declare that in no circumstances should the Parliamentary grant ever exceed £40,000 "According to the views of the British Treasury," he said, "there is a finality in science and in expanding knowledge "

Things have, of course, changed a good deal since, and we are now being almost saturated with discussions of the extent to which Government support for higher education should go, and of the proportion of the nation's youth which should be given a university education We are awaiting the report of Lord Robbins's Committee, and I do not wish at this stage to become immersed in statistical estimates There are, however, factors with which I have recently had much to do, notably in the deliberations of a Committee of the Institute of Physics and the Physical Society on some of the problems facing university physics departments in the current expansion programme This Committee has just reported[6], and among the many factors to which it directs attention there is one that I would single out as especially relevant to Playfair's views on science and the State

Universities and Research Establishments

I would first go back to Strange's 1868 paper to the Association In that paper[7], he had proposed "that there should be established a system of national institutions for the sole purpose of advancing science by practical research, *quite apart from teaching it*" Strange's proposal was afterwards supported by the Royal Commission on Scientific Instruction. and Playfair alluded to it in his address

"Such laboratories supported by public money, are as legitimate subjects for expenditure as galleries for pictures or sculpture, but I think that they would not be successful, and would injure science if they failed It would be safer in the meantime if the State assisted universities or well-established colleges to found laboratories of research under their own care Even such a proposal shocks our Chancellor of the Exchequer The widening of the bounds of knowledge, literary or scientific, is the crowning glory of university life Germany unites the functions of teaching and research in the universities, while France keeps them in separate institutions. The former system is best adapted to our habits, but its condition for success is that our science chairs should be greatly increased so that teachers should not be wholly absorbed in the duties of instruction "

Strange and Playfair thus held rather differing ideas on where research should be concentrated They were expressing, in fact, the two sides of an argument that lies at the base of one of our difficulties in physical science in modern Britain The difficulty has arisen because of an intense interaction between science and the State, brought about by the very dependence of our national survival on our technological achievement that Playfair and Strange had both foreseen

It may be vital for us to realize what the difficulty is—so let us look at what happened There were, of course, a few Government scientific institutions before the time of Playfair and Strange, the Royal Observatory at Greenwich being a distinguished example There were also, of course, universities; and despite a contrary argument by Cardinal Newman[8], Playfair held that most of the important British discoveries in science had been made by men who were also teachers As twin results of the efforts of the 1870's and 1880's, new universities were started, and new Government laboratories began to appear The most notable of the latter was the National Physical Laboratory, which opened in 1900 Once again, this was in part due to the belated following of a foreign example, this time that of Germany, where the Physikalisch-Technische Reichsanstalt had been founded in the 1880's, but much of the stimulus for the new laboratory came from the British Association, where Sir Oliver Lodge devoted his presidential address to Section A in 1891 to a strong plea for a "Physical Laboratory precisely comparable to Greenwich Observatory [where] accuracy should be the one great end"

Science and the First World War

The National Physical Laboratory succeeded, and various other Government laboratories were started, but the First World War revealed our national weaknesses resulting from an inadequate awareness of the value of natural science Disquiet among men of science had been simmering for many years After Playfair's death the struggle was carried on, notably under the leadership of Lockyer, whose presidential address to the British Association in 1903 was instrumental in getting the State grant to universities raised from £40,000 in 1902 to £115,000 in 1906 But these were small figures alongside the £500,000 or so provided by the German State for its universities[9] Indignation became so great that on May 3, 1916, a conference was held under the chairmanship of Lord Rayleigh in the rooms of the Linnean Society, following the publication of a memorandum in *The Times* of February 2, 1916, signed by 36 of our most distinguished scientists The meeting was attended by about one hundred and fifty supporters of the memorandum; and some further supporters were unable to pack into the hall They deplored the fact[10] that Britain had "suffered checks since the War began, due directly as well as indirectly to a lack of knowledge on the part of our legislators and administrative officials of what is called 'science' or 'physical science' ", and they pointed out that "In the whole history of British Governments there has only been one Cabinet Minister who was a trained professional man of science—the late Lord Playfair" There were not only men of science supporting the memorandum—to their shining credit, it is worth mentioning that among those who spoke in strong support were Dr Robert Bridges, the poet laureate, Dr Macan, Master of University College, Oxford, Dr Farnell, Rector of Exeter College, Dr Strong, Dean of Christ Church, Dr Shipley, Master of Christ's College, Cambridge, and Mr Nowell Smith, the headmaster of Sherborne School Dr Gow, the headmaster of Westminster School, was also to have spoken, but such was the crowd in the meeting that he was unable to get in These gentlemen were in the main classical scholars, and we should remember them with gratitude alongside Dr Jowett who, as Master of Balliol in an earlier day, had tried against the general run of his fellows to further the cause of science in Oxford

I am not sure of exactly what influence this conference had on Government policy A Committee of the Privy Council for Scientific and Industrial Research had been formed in July 1915 with an advisory council, but things were evidently not moving fast enough for two members of this council, Lord Rayleigh and Sir Charles Parsons, who were among the signatories of the 1916 protest Many influences, of course, were at work The extension of warfare to submarine and aerial operations demanded that scientists be enlisted in support, and new Government establishments began to grow rapidly The Royal Aircraft Establishment at Farnborough, for example, gathered an absolutely brilliant collection of men of science to work on problems of flight, and another distinguished group worked with the Royal Navy on the detection of submarines On the civil side, several research associations came into being

The Inter-War Period

The lessons of the First World War in science, as in international affairs, were only partly learnt Despite various official enquiries, and the formation of the University Grants Committee in 1919, the universities were inadequately supported in science, and the Government establishments became retarded through 'red tape' and parsimony On balance, if one wanted to do research, the universities even with their drawbacks were much more attractive than the establishments In general, the latter were allowed only to undertake research of a specifically applied nature, and the universities were almost the only places where pure research could be pursued A university man's research would inevitably be held up by lack of funds for apparatus and technical support, and by his teaching duties, but so was that of nearly everyone else working in the same field, because they too were working in universities with similar handicaps Thus, one's chance of discovering an interesting result was no less than anybody else's, and so there was little temptation for staff to leave the universities

Effect of the Second World War

The experience of the Second World War brought home to Britain the lessons so imperfectly learnt during 1914–18 Above everything else, the outcome of the Battle of Britain had depended jointly on the devotion of the Royal Air Force and on the imagination of a small band of scientists and engineers This was a lesson that no post-war Government could ignore, and there were plenty of other examples to re-inforce it by the time the War ended, culminating in the release of nuclear energy Now these results had largely been achieved by bringing in scientists from the universities, and seconding them to Government establishments When the War ended, some of these men, perhaps the majority, returned to the universities, but the Government learning from pre-war mistakes, now made its own establishments much more attractive Salaries were made higher than those in the universities, technical assistance was on a much more generous scale, as was also the provision of equipment Some of the differences were defensible matters of national defence could not afford to be handicapped in the way that university research was It should have been equally obvious that university research should be freed from the handicaps of the past But since the Government scientific organizations were administratively nearer to the seat of power, their claims were more clearly heard by the Treasury So the worker in a Government establishment was often able to devote more of his time to research than was his university counterpart and since it had been argued, very reasonably, that the pre-war establishments had been rather dull places because they had little contact with pure research, the better post-war establishments were encouraged to allow a proportion of their staff to spend much of their time on pure research Thus, in some instances, the Government establishments have been able to offer keen research workers better facilities, higher salaries, and a greater proportion of time for pure research than they would find in a university These observations apply particularly to physics, because this branch of science has been involved more than any other in matters of national defence in the past thirty years, but the same thing could happen to any branch of science which should begin to produce results recognized as essential to national survival

Now all this would not matter so much if we were producing enough able physicists (or mathematicians or engineers) to be able to man both the Government establishments and the universities When to these requirements we add those of industry and school-teaching, we find that there are not enough physicists to go round

Science and Industry

Playfair rated industry at its true value to Britain "It may be", he said, "that the engines of Watt and Stephenson will yield in their turn to more economical motors, still they have already expanded the wealth, resources, and even the territories of England, more than all the battles fought by her soldiers, or all the treaties negotiated by her diplomatists" He speculated on the time when coal might be exhausted in England "It may be that the supply of petroleum from America or the borders of the Caspian Sea, will be so easy and cheap that the increased technical education of our operatives may largely overbalance the disadvantages of increased cost in fuel" This was a courageous forecast at a time when oil engines were barely practicable, and when coal was the base for England's greatness

Playfair saw more clearly than most of his contemporaries that the British Empire might be a transient organization, and that the possession of colonies producing cheap supplies of raw materials and providing easy markets for our manufacturers might lull us into doing too little about technical education and industrial development We now all realize that he was right, and neither Government establishments nor universities can survive healthily, unless industry earns enough abroad to provide the necessary money It is therefore essential that industry should use science to the utmost, and that the universities and colleges should provide the scientists and technologists that industry needs Here, incidentally, it is interesting once again to see Playfair hitting the target "But while England has never lacked leaders in science, they have too few followers to risk a rapid march We might create an army to support our leaders in science, as Germany has done, and France is now doing, if education in this country would only mould itself to a scientific age" It has recently been encouraging to read the report of a Working Party of the Federation of British Industries[11], entitled *Civil Research Policy* and published in June, 1963, which shows that the Federation is now very much aware of the need for scientific support in the universities, the Government establishments, and the research associations It calls for proper co-ordination of the overall effort

Demands on Universities

A similar call has come from the Institute of Physics and the Physical Society, who deemed it important to assess the prospective demands on university physics departments in the expansion, in the light of adverse factors such as the drift of staff to Government establishments and to the United States, and to see whether the demands could be met

In the past twenty years, the position of university physics departments has been getting more difficult partly because the teaching load on members of staff has gone up, and partly because the attractions of non-university laboratories have increased As regards the teaching load, the staff/student ratio in science as a whole was rather better than 1/8 in 1939, and worsened to rather less than 1/10 in 1960, according to the University Grants Committee[12] In physics in 1960 the ratio was substantially worse than the average for science, being less than 1/14 While no one can dogmatize about the optimum value of the staff/student ratio, it is clear from the experience of our universities in the past ten years or so that the ratio has fallen too low, judged by the simple but vital criterion of whether the staff find conditions sufficiently attractive to remain in the universities These conditions could be even less attractive in the 'broiler universities' that could result from an uncontrolled attempt to produce university graduates in bulk each with as little flavour as the 'broiler chicken' and supermarket meat that now assault our palates It may be that such devices as teaching machines and other new methods of imparting knowledge may ultimately result in some economy in staff But this prospect, for the advanced subjects at least, is some way off, and even in the United States, which we might expect to be ahead of us in this respect, the staff/student ratio does not appear to be always more economical than our own, at Princeton, for example, the ratio is impressively better than the British average, being about 1 staff to 6 undergraduates

Taking all these factors and others into account, the Institute of Physics report shows that whereas the universities have annually recruited over the past few years a number of physicists equal to about 20 per cent of the national output of Ph D s in physics, they will need to recruit about 40 per cent of the annual output over the next few years, if they are to raise the staff/student ratio to the level where staff will stay in the universities and to meet the demands for physics teaching in the expansion of the universities planned by the University Grants Committee Even though this expansion, from 111,000 students in 1961 to 150,000 by 1967, may be thought by many to be too small, the opinion of a highly representative body of physicists is that it demands the absorption of nearly half of our Ph D output over the next few years The conclusion is important, since it results from what may be the only survey of the national situation in any of the key subjects, and it shows the large scale of effort required

Loss to the United States

The position would not be quite so difficult if Britain did not lose an appreciable fraction of her best science graduates to the United States Thirteen per cent of British Ph D s emigrate permanently, while a similar additional number go to the United States temporarily for post-doctoral work This year, as an external examiner at one of our universities, I set an essay question in the final honours examination, asking the students what they thought about this matter Their views are more important than those of us who are older, because it is primarily the loss of our young research workers that is most serious They did not put pay and standard of living as the paramount attraction It was, of course, important, but their main reasons were the greater liveliness in the United States and the greater public regard for scientists These are not new arguments but the spontaneity and vigour with which the students spoke might have convinced even the Minister for Science that emigration had nothing to do with faults in the American system of education

Teaching and Research

The staff of our expanded universities would not, we hope, be devoting their entire efforts to teaching Indeed, the underlying assumption is that they would have sufficient time to undertake enough research to ensure that their teaching was infused with the spirit of a living subject, and that sufficient research should be going on to make the universities attractive to research workers I just do not believe that teaching at the honours-level can survive without contact with research There are, indeed, some devoted teachers who succeed in inspiring their pupils without undertaking any research themselves, but the numbers of such teachers are in any event too small to undertake more than a fraction of the teaching Much of the teaching must therefore be done by men who want also to carry out research, and I believe it good that this should be so

The question then arises Since the expansion of university teaching must make such demands on the limited numbers of research-trained graduates, how can Britain most effectively use these limited numbers so that their full potential can be realized both in teaching and in research? Here we face a difficulty which by an accident of recent history is greater in Britain than in the United States Whereas the Government establishments have much increased in strength in Britain relative to the universities, the opposite has happened in America The accident was that Britain foresaw the Second World War just in time to enlist science in support of air defence and to recruit scientists to the Government establishments In the United States, far less preparation was undertaken, and the devices that Sir Henry Tizard's mission took to the United States in 1940 were such a surprise that the American authorities decided that the quickest way of exploiting them in the emergency was to set up teams in the universities rather than wait to build up Government establishments The Radiation Laboratory at the Massachusetts Institute of Technology was an outstanding example, its British forerunner, by contrast, was the Telecommunications Research Establishment, a Government establishment The legacy of this accident is that in the United States the universities are now strong, the Government establishments relatively weak In 1960 the position was surveyed by a Presidential committee[13] which thoroughly endorsed the practice of getting the universities to undertake a great proportion of the national research, even that of a defence nature, on the grounds that, as President Eisenhower himself said "The process of basic scientific research and the process of graduate education in universities must be viewed as an integrated task if the nation is to produce the research results and the new scientists that will maintain the leadership of American science"

In Britain, the Government establishments are separated from the universities, and such different conditions have been created in them that the interchange of staff is very restricted the establishments play very little part in teaching the new generation of students This is despite the fact that, owing to smaller numbers in Britain, it is necessary to be even more careful than are the Americans to husband British scientific potential, both for research and for teaching Britain just cannot afford to let this present dichotomy continue It is just as important to realize the teaching potential locked up in the Government establishments as it is to realize the research potential of the universities This is not a problem to be solved piecemeal It needs to be tackled on a national basis, and a better balance sought not only between universities and Government establishments, but also between these and the requirements of industry, the technical colleges and the schools This is one of the most important problems confronting both science and the State in modern Britain Unless it is solved, no substantial expansion can take place, paradoxically, in any subject where there is an acute national interest and since this now involves the very science subjects which are the educational bases of all the others, the whole of the proposed expansion in science is threatened

Science and Defence

The last part of Playfair's address dealt with the theme that the flourishing of abstract science is the condition for progress He had been discussing, he said, the relations of science to the public weal, adding "That is a very old subject to select for the year 1885" Yet we can to-day recognize that it is still worth looking at what he said, partly in admiration of his foresight and partly in regret that the lessons that he preached have still not been entirely learnt

If we look critically at his address, it was so comprehensive that we are challenged to ask the question 'What did he leave out?' For myself, there are two subjects on which I should like to have heard him speak One is that on which a former president of the British Association, Sir Henry Tizard, spoke at the Royal United Services Institution in 1946 This[14] was "Science and the Services" Playfair did not ignore this subject through any question of conscience In the Crimean War he had invented a cipher that bears his name, and he had submitted inventions for incendiary shells and poison-gas shells These were turned down, the latter being rejected on the grounds that they would not be a legitimate method of warfare "Why", he wrote[15] in his *Memoirs*, "a poisonous vapour which would kill men without suffering is to be considered illegitimate warfare is incomprehensible War is destruction, and the more destructive it can be made with the least suffering the sooner will be ended that barbarous method of protecting national rights "

This observation shows that Playfair would have been involved in matters of national defence, had they arisen in his time, and that the omission of them from his address was probably due to their comparative unimportance in the 1880's Yet, as has been seen, some of the drive for science came in fact from the military side As far back as the Renaissance, some of the interest in dynamics sprang from the new problems in ballistics arising from the invention of the cannon In the nineteenth century, Strange's efforts for science were as much devoted to the military applications as they were to the civil and, in the event, it was much more the demands of the two World Wars than anything else that brought about the advancement of science in the public eye This is to be regretted, since the greatness of science lies in its constructive achievements, both intellectual and material, and not in its destructive applications It is, therefore, an unhappy reflexion that it should have taken two vicious wars, rather than the skilled advocacy of men like Playfair, to bring home to mankind the power of science But, accepting the facts as they are, we must congratulate ourselves in Britain that the partnership between science and the Services has been such a cordial one in the past thirty years To quote[14] from Sir Henry Tizard

"The first time, I believe, that scientists were ever called in to study the needs of the Services, as distinct from their wants, was in 1935 and then only as a last resort, The Air Staff were convinced of the inadequacy of existing methods and equipment to defeat air attack on Great Britain, and a committee was established for the scientific survey of air defence I want to emphasize that this committee, although it consisted on paper only of scientists, was in fact from the first a committee of scientists and serving officers, working together

"When I went to Washington in 1940 I found that radar had been independently invented in America about the same time as it had been invented in England We were, however, a very long way ahead in its practical applications to war The reason for this was that scientists and serving officers had combined before the War to study its tactical uses This is the great lesson of the last war "

Sir Henry concluded "What is needed is a scientific staff attached to the central Chiefs of Staff organization that will have no executive duties, nor be overburdened by administration, but will devote its whole time to the study of the influence of advancing scientific knowledge on the problems of defence "

Such an organization as Sir Henry wanted to see is at last coming about The recent White Paper on *Central Organization for Defence* (Cmnd 2097 H M S O , July 1963) [see *Nature*, 199, 1021; 1963] much increases the strength of the Chief Scientific Adviser, and places him on the Defence Council alongside the Chiefs of Staff of the three Services By a wise arrangement the three Services will keep enough of their individualities and traditions to preserve the loyalties which we know from experience to have contributed so much in emergencies to their fighting spirit At the same time, the merging at the higher levels should make for more national and economic planning This had to come, as the Services became more interdependent, and jointly more dependent on the limited resources of science and technology It is thus the growth of science and technology that has been one of the main factors responsible for unifying British Defence Services, and lowering the barriers between them We hope that this same growth may ultimately lower the barriers between nations, so that international relations proceed on a basis of respect by each nation for the constructive achievements of the others

Science and Government

All is not yet well, even with the national organization of science in Britain Attempts are now being made to improve this organization, but they have to face difficulties that would not have existed had organization in Britain been planned at the time when Playfair and Strange were pressing it Instead of having a coherent organization, we have to assimilate various pieces of independent organization which have grown up, not altogether logically, through the absence of a basic plan If we take civil research in electronics, for example we find that responsibility is uneasily divided between the Department of Scientific and Industrial Research and the Ministry of Aviation, while in the important field of computers the Board of Trade has formal responsibility While the Admiralty has its own research establishments, such as the Admiralty Research Laboratory, the Royal Air Force has its research done for it by the Ministry of Aviation, which is also responsible for civil aviation These asymmetries and anomalies arise because individual Ministries were forced to start research projects for themselves because of the absence of a national plan I am certainly not recommending a single elephantine organization for research, since there are many dangers to such a course; but much rationalization of our present arrangements needs to be made, and the very strength of some of the individual growths may be resistant to change, however desirable such change may be It was for Britain, I think, a fortunate source of weakness to Germany in the Second World War that so many clandestine military research centres were set up before German rearmament could come out into the open By the time this occurred many of the individual centres were just too strong and independent to be amalgamated into a coherent organization, so that German research effort could never afterwards be economically directed We are in danger of repeating this error in Britain, for entirely different reasons, and it will take strong leadership from the Ministry for Science if we are to avoid it

Now all these considerations entail that good central planning and leadership are forthcoming, and that great responsibility rests on a relatively small number of men I do not see how to avoid this, but I am not unduly frightened by it Even in limited fields of research, such as elementary particle physics or space investigations, expenditure on a single project may be so great as to make appreciable demands on the national exchequer, and so some degree of central foresight and control is required It is on this general matter of wisely making and implementing scientific policy that I should most like to have heard Playfair speak

His experience of science and Government was quite unrivalled, in this century his mantle fell jointly on Sir Henry Tizard and Lord Cherwell Anyone who had the fortune to know them must be struck by the way that Playfair seems to have embodied the best points of them both In the past few years Sir Charles Snow has made the story of their differences an occasion for investigating the relations of science and Government Actually, their similarities were, from our point of view, much more significant than their differences, and, by the public service that they each unstintingly gave, they both stood for the better incorporation of science and Government, and for the greater recognition of science in national life They were thus both in the Playfair tradition I had the privilege of working with each of them, and from this experience, I venture to give my own thoughts on how decisions on scientific policy might best be taken

Many decisions are taken by open committees, and when time and security considerations allow, this is a reasonable method, even though it sometimes produces a result less sensible than would have been arrived at by any single member of the committee The railway gauge of Ireland, for example, seems to have been settled in this manner[16]—the average of all other national gauges was taken, and the result was 5 ft 3 in the Irish thus had a new gauge, and their rolling stock was not interchangeable with that of any other railway The common agreement necessary for members of a committee to come to a conclusion often does not go far enough, especially in those important decisions where an element of uncertainty and risk has to be accepted This is not always so, and we must remember with gratitude, for example, the courage shown by the Tizard Committee before the Second World War But I have seen many occasions when a committee has been wrong and an individual right, anyone who doubts this has only to read Chatham's last speech on the American War of Independence So while I would accept the committee approach on as public a basis as possible, whenever it is feasible, I am not certain that it is always the best approach

The Scientist in Government

These considerations are especially relevant to the process of incorporating scientific advice into national policy and executive decisions Sir Charles Snow[17], speaking of Lord Cherwell's work in the Second World War, says "We must never tolerate a scientific overlord again"; but Sir Charles proposes no alternative My own view is that, high above the details of controversy, we should be grateful to Sir Winston Churchill for being the first British Prime Minister (if we except Peel, who used Playfair's talent in investigating the Irish potato famine) to rate the contribution of science so highly that he insisted on having personal scientific advice at the highest level The fact is that, especially in matters of defence, executive decisions have often to be taken by individuals If science is not organized so that some of our best-suited men of science are in key administrative positions in Government, then those policy decisions affecting science will be taken by non-scientific men who will at best base their judgments on how they have been convinced by particular scientists as individuals This is certainly not better than having scientific opinion injected at the highest level by an able representative who is himself a man of science, and it could be a good deal worse It may be argued that this task would place great responsibility on the man of science who was selected and the field of science is certainly now so wide that no one man could cover it In his day, and indeed almost right up to the present day, I would have had implicit trust in Lyon Playfair single-handed in this task It would now require more than one man, but surely we are still great enough to produce men of the right calibre

Even so, I would agree with Sir Charles Snow that there is danger in having any man of science in a position of isolated power But the danger really lies much more in the isolation than in the power; and this observation is not peculiar to science—it is general for all aspects of Government How then can we ensure that the individual does not become isolated? For myself, I should try to do this by providing a small forum for each of the principal scientific advisers to the Government, the forum would consist in the main of other men of science as nearly his equals as possible, chosen so that the membership covered in knowledge and experience the full range of his activities The forum should include some relatively young men, older men may be wiser, but their wisdom may well not be enough In the problems that we now face, imagination, energy, and working contact with the most modern technology are essential, in these qualities youth has the advantage, and so the forum should be challenged and stimulated by the presence of some younger men in their thirties or even twenties If the adviser were as wise as he should be, he would consult the forum before taking any key decision, so that while the power to make the decision, or to frame the relevant advice to the Government, was his alone, he would take that decision only after having heard the relevant arguments and he would have to have enough confidence in his decision to be able to justify it in any subsequent face-to-face encounter with his forum. The justification would not be an inquisitorial matter, but would save the individual from the slow drift away from the primary standards of scientific truth and morality that too easily attends the acquisition of unchallenged power Any man brought up in scientific method can scarcely help recognizing scientific truth, whatever the status of man stating it A suitable forum may therefore be the only sanction needed to keep the individual from abusing his power, beyond the sanctions already set by our form of Parliamentary Government I am not claiming, of course, to lay down a rigid procedure for the injection of scientific advice into Government This is a relatively new problem, and none of us knows the full answer, if such an answer exists But I did myself use the procedure I advocate, in my own work in a limited field during the Second World War, and it saved me from error on several occasions, without restricting my speed of operation, I would always use it again

In this story of a few far-sighted men, the part of the British Association for the Advancement of Science has been an honourable one, indeed, as the Prince Consort pointed out, one of its declared aims was "to obtain a more general attention to the objects of Science, and a removal of any disadvantages of a public kind which impede its progress" This aim still exists, and I have therefore directed attention to some of the problems that remain These include the relationship between universities and Government establishments and, more generally, the rational distribution of scientific effort among the universities, establishments, colleges, schools and the vital fields of industry in which we have to earn our national living The need to expand education in science and to reduce the loss to the United States are other parts of the same body of problems Finally, the method of injecting scientific consideration into matters of State requires most careful thought But it is gratifying to find that there is now a general appreciation at least of the existence of these problems and that, on this account, we may be reaching "the end of the beginning". Although Britain has thrown away the lead that men like Bacon and Playfair tried so hard to give her, I believe that Britain still has the talent, the resilience, and the humanity to harmonize the relationship between science and the State This will be not only to their mutual benefit, but also to the good of men as individuals the world over.

[1] Lyon Playfair's presidential address can be found in the British Association Report for 1885, and in his *Subjects of Social Welfare* (Cassell and Co , London, 1889), see also *Nature*, 32, 438 (1885)

[2] Royal Commission on Scientific Instruction and the Advancement of Science, 3 vols (1872–5)

[3] Strange, A , *J Roy United Services Inst* , No 64, 15 May, 1870 *J Soc Arts*, July 7, 1871

[4] Strachey, Lytton, *Eminent Victorians*, 193 (Chatto and Windus, London, 1918)

[5] Playfair, Lyon, "Universities in their Relation to Professional Education" (1873) Reprinted in *Subjects of Social Welfare* (Cassell and Co , London, 1889)

[6] *Problems facing University Physics Departments* (Inst Phys and Phys Soc , Lond , August 1963)

[7] Strange, A , British Association Report for 1868 Mathematics and Physics Section, 6

[8] Newman, J H , *The Idea of a University*, Preface p xiii (Pickering, London, 1873)

[9] Cardwell, D S L , *The Organization of Science in England*, 155 (William Heinemann, Ltd , London, 1957) This book is a valuable survey and reference source

[10] *The Neglect of Science*, Proc Conf May 3, 1916, Lord Rayleigh presiding (Harrison and Sons, London, 1916)

[11] *Civil Research Policy* (Fed Brit Ind , London, June, 1963) See also *Nature*, 199, 725 (1963)

[12] Returns from Universities and University Colleges in receipt of Treasury Grants, Academic Year 1960–61 (Cmnd 1855) (H M S O , London, 1962) See also *Nature*, 197, 1 (1963)

[13] Scientific Progress, the Universities and the Federal Government (U S Gov Printing Office, Washington, 1960)

[14] Tizard, H T , *J Roy United Services Inst* , 91, 333 (1946)

[15] Reid, Wemyss, *Memoirs and Correspondence of Lyon Playfair*, 160 (Cassell and Co , Ltd , London, 1899)

[16] O'Dell, A C , *Railways and Geography*, 16 (Hutchinson and Co , Ltd , London, 1956)

[17] Snow, C P , *A Postscript to Science and Government*, 35 (Oxford University Press, London, 1962)

SOME ATTITUDES TO VEGETABLES, AND THEIR IMPLICATIONS

By Dr. R HARPER

Department of Psychology, University of Leeds

At a time when, for various reasons, so much emphasis is being placed on the importance of foods it is surprising that so little 'cross-cultural' information has been placed on record about attitudes to foods This article draws together available information from a number of different sources about attitudes to vegetables Vegetables are of special interest because of the wide divergence in attitudes which are encountered. Several different methods of collecting results have been used, including simply counting the number of persons who dislike a particular item or express lack of familiarity with it, or the analysis of views expressed by children about vegetables in the course of a school essay, or—more recently—the use of a simple Hedonic Rating Scale, which gives some indication of the extent to which a particular item is liked or disliked The Hedonic Scale[1], which has a long history behind it, is simply a form of rating scale with the addition of a category of response 'never tried' to allow for this contingency The results of a number of different studies are compared, the potentialities of the Hedonic Scale as a device for routine surveys are considered along with the general implications of the data available from different sources

Two Early Studies

Hall and Hall[2] examined the attitudes of 700 American students in three different universities to some 150 food items Responses were restricted primarily to whether a particular item was disliked or unfamiliar The following information refers to a selection of vegetables from the whole group of foods

Percentage disliked		Percentage unfamiliar	
Parsnips	24	Leeks	65
Egg-plant	22	Okra	51
Hominy	20	Endive	43
Turnips	19	Chard	35
Rutabagas	18	Lentils	31
		Rutabagas	29

For comparison with later studies information about a particular group of five vegetables is also as follows

Percentage disliked		Percentage unfamiliar
Potatoes	1 4	*
Cabbage	5 9	*
Leeks	5 6	64 8 'never tried'
Spinach	8 2	*
Asparagus	3 5	*

(* denotes figures not available)

Vegetables were somewhat arbitrarily divided into strong-juiced versus sweet-juiced, but these two groups overlapped considerably in the extent to which they were disliked

Some regional differences were noted, but similarities between different groups were more noticeable than were differences 50 per cent of the respondents referred to taste as the reason for not liking a particular food, and naturally some of the reasons given were highly personal Women were familiar with more foods than men and had more aversions

Le Gros Clark[3] examined *The School Child's Taste for Vegetables* in war-time England by means of school essays on "The Vegetables we Eat", written by some 432 children The teachers limited their influence to writing a short list of vegetables on the board, including cabbage, sprouts, cauliflower, lettuce, turnips and carrots Table 1 gives a selection of the available data including both the number mentioning and the number disliking a particular vegetable

Many of the children were indifferent to how potatoes were served As a rule, attitudes tended to be rather clearly defined (for or against a particular item) rather than neutral Attitudes to green vegetables (cabbage, sprouts and cauliflower) were fairly uniform, but there were some inconsistencies in the attitudes of particular individuals to the separate items in this group Over the limited age-range considered there were few trends with age The only marked exception again concerned green vegetables in so far as there was a progressive reduction in the percentage disliking them of some 25 per cent when the 10–11-year-olds were compared with the 8–9-year-olds and a further 50 per cent reduction among the small number of 12–13-year-olds who took part in the study No single sensory feature is uniquely associated with

Table 1 ANALYSIS OF DATA FROM SCHOOL ESSAYS ON THE VEGETABLES WE EAT (after ref 3)

	(*N*) No mentioning (out of 432)	Percentage actually disliking	Comments
Potatoes	388	Under 1	
Carrots	379	8	40 liked them raw
Cabbage	329	25	Many individual inconsistencies
Sprouts	305	31	
Cauliflower	302	24	
Onions	262	21	
Leeks	86	51	
Marrow	236	61	
Turnips	270	60	
Parsnips	242	62	(six persons expressed abhorrence—parsnips disliked with monotonous regularity) *
Swedes	220	63	
Broad Beans	142	34	
Haricot Beans	116	20	
Runner Beans	Generally favoured, with exceptions		
Salad vegetables	Generally favoured by about one-third of the children		
Spinach	Referred to occasionally, but many describe it as being strong and bitter		

Note that in all instances where a percentage disliking is quoted, this is calculated in terms of the children who actually mentioned a particular item An unspecifiable adjustment would have to be made to get a better idea of the percentage of the total group who disliked a particular item

* A personal observation about parsnips is relevant here As a child, parsnips stand out in my memory as having an unpleasant, bitter taste Nowadays, they taste rather sweet to me Whether I have changed, or whether the parsnips have changed cannot be resolved Of course if parsnips are stored too long starch turns to sugar anyway

what is liked or disliked but there was a strong tendency for the children to dislike "slimy, mushy, stringy or slippery things". Some bland tastes, especially when combined with these rather distinctive and unpopular textures—as in the case of marrow—also seemed to cause difficulties. Many strong tastes also caused difficulties for some children, and idiosyncrasies were evident in so far as a few children even developed a disliking for peas, and many children found lettuce bitter

Use of the Hedonic Scale

The two investigations outlined here did not involve a systematic attempt to determine the extent to which the various food items were liked or disliked, although some qualitative information naturally emerged from descriptions and comments Dove[4] was probably the first to introduce this more systematic approach and included a number of vegetables in his investigation He was concerned with "Appetite levels of food consumption a technique for measuring foods in terms of their psychological and nutritional values combined" This title directs attention to one of the implications of this whole series of studies in so far as vegetables (and other foods) which may be low in nutrients may be important sources of those nutrients if the foods concerned have a high appeal and are available and assimilated in appreciable quantities

(This observation may prove to be totally irrelevant in the context of an excess of attractive though undesirable foods in conditions of affluence, but such an observation would be scarcely likely to apply to vegetables) In any event, some old people in particular have been known to get their total vitamin C requirement from eating comparatively large amounts of potatoes—about 250 g per day In Dove's pioneer survey 129 individuals expressed their views about 228 food items including 63 vegetables, using the following seven response categories never tried the food—allergic to the food—dislike the food—tolerate only when hungry—desired the food moderately well—considered the food good—considered the food very good Table 2 gives the frequency of responses to seven selected vegetables Results from three separate groups of respondents *A*, *B* and *C* are included Dove has stressed the importance of the number of different ways of preparing various food items For example, he[5] refers to 99 ways of preparing potatoes Even this familiar vegetable may be obtained in a number of different varieties each with their distinctive features and special appeal Different varieties may also serve different purposes For example, in the United Kingdom it has been said that the Majestic variety is particularly suitable for making chips, whereas for other purposes a floury potato is much preferred here, in contrast to a waxy type on the Continent of Europe (Strictly speaking, any statement of this type should be given a date to allow for changes in food habits) The floury Idaho potato is also very popular in restaurants in widely separated parts of the United States, but the fact remains that for the American Serviceman French fried potatoes ('chips' in Britain) are one of the most often and most strongly liked of all vegetables[6] Further attention will be directed later to the precise effects of different methods of preparing the same vegetable or food The fact that the British and the Americans refer to different modes of preparation by the same word 'chips' directs attention to the uncertainties of simply presenting a list of names of food items This difficulty might well be overcome by the more time-consuming procedure of actual taste-testing but it would no longer be possible to evaluate 50–60 food items in a single session

Table 2 DATA RELATING TO SELECTED VEGETABLES (after ref 4)

Vegetable	Group	G or VG	Tolerate	Dislike	Never tried
White potatoes	*A*	84	3	0	0
	B	82	2	4	0
	C	95	0	0	0
Tomatoes	*A*	95	0	0	0
	B	92	4	0	0
	C	88	2	0	0
Cabbage	*A*	92	3	0	0
	B	74	2	4	0
	C	88	2	0	0
Broccoli	*A*	81	0	3	0
	B	42	14	12	16
	C	60	3	8	8
Turnips	*A*	68	13	3	0
	B	60	6	8	4
	C	54	17	0	0
Kohlrabi	*A*	22	14	5	51
	B	8	8	4	72
	C	15	13	5	45
Collards	*A*	0	0	0	95
	B	0	4	0	90
	C	10	2	5	75

Note Percentages quoted do not add up to 100 Nos /Sex *A* 38F, *B* 50F *C* 41M

Table 3 ORDER OF VITAMIN C CONCENTRATION IN A SELECTION OF VEGETABLES AS COMPARED WITH ACTUAL CONTRIBUTION TO DIET, BASED ON AMOUNTS EATEN (after ref 4)

	Percentage contribution from 100 g	Rank	Actual percentage contribution from named source	Rank
Cabbage	143	1	21	3
Collards	100	2	4	7
Broccoli	93	3	18	4
Kohlrabi	71	4	5	6
Turnip	43	5	8	5
Potato	40	6	37	1
Tomato	39	7	23	2

Note in particular that although potatoes and tomatoes are at the bottom of the list in terms of actual vitamin C content, as sources of vitamin C they are at the top of this list

An Exploratory Investigation (United Kingdom, 1956)

IN 1956 a small pilot investigation was undertaken by a group of undergraduates to explore the potentialities of the Hedonic Scale and to provide some provisional information about attitudes to vegetables in the United Kingdom The scale used consisted of only five categories in addition to that of 'never tried' Fifty people of different ages, selected according to availability, gave their views on about 23 vegetables and a selection of the data is given in Table 4

Although the number of respondents is too small to warrant the presentation of a detailed analysis some interesting conclusions emerged Without considering their statistical significance the following points may be noted

(1) Many vegetables, including peas, lettuce, brussels sprouts, carrots, potatoes and cabbage, gave no indication at all of changes with age

(2) Spinach also showed little change with age, but in contrast to the more recent market research survey (see following) opinions about spinach were about equally divided and formed a bimodal distribution

(3) With increasing age, respondents' attitudes became more favourable to parsnips, broad beans and kale, and in the case of kale increasing age brought noticeably increasing familiarity.

(4) Asparagus and artichokes show a similar increase in familiarity with increasing age, but in the case of artichokes, attitudes continued to be fairly well divided, whereas among the over-sixties group no one said they disliked asparagus

Table 4 SELECTED DATA FROM SMALL-SCALE PILOT STUDY
(U K , 1956—50 respondents)

	Like		Neutral	Dislike		Never tried
	very	moderate		moderate	very	
Tomatoes (raw)	62	26	2	4	6	0
Potatoes	40	38	20	2	0	0
Cabbage	36	32	14	14	4	0
Brussels sprouts	50	28	4	10	4	4
Parsnips	22	20	10	12	28	8
Broccoli	14	20	4	6	8	24
Kale	10	14	10	2	6	58
Onions (boiled)	44	14	14	8	12	8
Leeks	24	24	10	8	16	18
Spinach	10	22	2	12	28	26
Artichokes	4	8	4	2	14	68
Asparagus	16	18	4	4	10	44
Corn on the cob (included for comparison)	6	4	8	6	4	72

Quartermaster Food and Container Institute

(1) *Preliminary report (1953)* The Hedonic Scale has now been in regular use by the Food Acceptance Branch of the (U S) Quartermaster Food and Container Institute for the Armed Forces for at least ten years Some eight separate surveys have been carried out with samples of between 3,000 and 6,000 in each investigation Methodological investigations have been included, and these have dealt with such questions as the largest number of items which can reasonably be included on a single occasion, how many categories of response should be permitted, what are the most appropriate verbal formulations of these categories A preliminary report of the first few surveys was presented by Wood and Peryam[7] The number of food items on the forms was limited to 60 or less and a nine-point scale covered the 'Hedonic range' from 'like extremely' to 'dislike extremely', with the additional category 'never tried' The main conclusions include the following

(*a*) There was a marked degree of consistency between the different surveys For example, the 'percentage disliked' ranged from 2 8 to 4 4 for fresh sliced tomatoes, whereas buttered turnips ranged from 40 2 to 51 4, these closely grouped figures for each distinct item point to the stability of the technique

(*b*) A distinction was made between items with 'good acceptance characteristics' and those with 'poor acceptance characteristics' The 'good' group included foods which are disliked by less than 10 per cent of the respondents, the 'poor' group are disliked by 40 per cent or more

(*c*) Vegetables as a class were at the bottom of the list in order of acceptability with only 15 per cent 'good' items and as many as 26 per cent 'poor' items

(*d*) There were 12 vegetables in the 'good' groups and these consisted of only five different items prepared in different ways, that is tomatoes, potatoes, green peas, corn on the cob, green beans

(*e*) Similarly, the 20 vegetables in the 'poor' or 'problem group' consisted of 11 different items prepared in different ways These included squash, parsnips, brussels sprouts, egg-plant, broccoli, spinach, turnips, rutabagas, cauliflower

(*f*) As a general rule, there was no uniformity of opinion about what is disliked, only about what is liked

(2) *Main report (1960)* The most extensive report on attitudes to foods is contained in the 160-page document prepared by Peryam *et al*[6] for the Quartermaster Food and Container Institute for the Armed Forces in Chicago This report combined the results of eight different surveys carried out between 1950 and 1954 The number of food items in a single survey ranged mostly between 45 and 55 In addition to the response category of 'never tried' the following categories were used like extremely—like very much—like moderately—neither like nor dislike—dislike slightly—dislike moderately—dislike very much—dislike extremely These categories were assigned numbers and simply averaged Various possible forms of numerical transformation were considered but these were rejected either on theoretical or on practical grounds Detailed results about individual items are not given in this report so that comparisons with other studies are to that extent restricted The most satisfactory way of presenting the data is to report the 'mean preference values', the 'centile rank', and the 'percentage not tried', for a selection of vegetables (Table 5)

Table 5 ATTITUDES TO SELECTED VEGETABLES
(In rough hierarchical order U S data from ref 8)

	Mean preference	Centile rank	Percentage not tried	
Fresh buttered corn on the cob	8 0	96	1	
White potatoes				
Baked	6 7	55	1	White potatoes appear in 16 modes of preparation ranging from 'mean preference values' of 5 8 (boiled in their skin) to 8 2 (french fried potatoes, i e , chips)
Creamed	6 9	63	2	
French fried (chips)	8 2	97	1	
Mashed	7 6	86	1	
Fresh sliced tomatoes	7 9	94	1	
Fresh grilled tomatoes	6 4	40	12	
Brussels sprouts	4 8	6	10	
Cabbage (boiled)	5 5	16	4	
Cabbage (baked with cheese)	4 3	2	30	
Carrots	5 3 to 5 9	13 to 32	4 to 11	According to mode of preparation
Broccoli	4 6 to 5 6	4 to 18	16 to 38	According to mode of preparation
Turnips	4 6 to 5 0	4 to 8	8 to 11	According to mode of preparation
Rutabagas	4 7	5	39	
Spinach	4 7 to 4 8	5 to 6	5 to 37	According to mode of preparation
Asparagus	4 2 to 4 9	2 to 6	4 to 17	According to mode of preparation
Cauliflower with cheese sauce	4 1	1	12	

Strictly speaking, the mean preference values are the average ratings on the like – dislike scale These ranged from 8 2 for the most popular item to 4 1 for the least Somewhat surprisingly (to me) one of the least popular items was cauliflower with cheese sauce, but perhaps this particular dish is one which is difficult to prepare properly for mass, as opposed to individual, serving

Cauliflower with cheese sauce was at the bottom of the hierarchy with a centile rank 1 The meaning of centile ranks is self-evident, but for clarity a centile rank of 96 would simply mean that only 4 per cent of all items classified would be liked more (on the average) than this one With the exception of broccoli, cabbage baked with cheese and spinach, most of the items included in Table 5 show surprisingly high acceptance characteristics Examples in which information is quoted about more than one mode of preparation indicate just how important this can be Thus, for example, baked white potato (presumably this refers to the Idaho potato) shows a mean rating of 6 7 and a centile rank of 55, whereas French fried potatoes (chips) were extremely popular with an average rating of 8 2 and a centile rank of 97 Note also the very considerable shift when the position of fresh sliced tomatoes is compared with that of fresh grilled tomatoes

Table 6 MASS OBSERVATION (PILOT SURVEY, SPRING 1962)

	Potatoes	Cabbage	Leeks	Spinach	Asparagus
	Like / Dislike	Like / Dislike	Like / Dislike	Like / Dislike	Like / Dislike
A Percentage responses	(i) 84 (ii) 8 (iii) 4 (iv) 1 (v) 1	(i) 66 (ii) 16 (iii) 8 (iv) 3 (v) 1	(i) 34 (ii) 7 (iii) 4 (iv) 1 (v) 1	(i) 13 (ii) 4 (iii) 2 (iv) 1 (v) 1	(i) 9 (ii) 2 (iii) 1 (iv) * (v) *
B Percentage of people who had not eaten a particular vegetable last year	2	6	53 Marked regional variation	79	88
C Percentage 'like very much' under various circumstances					
Age groups (*a*) 18–24 (*b*) 25–44 (*c*) 45–65	*a* 80 *b* 79 *c* 77 No appreciable change	*a* 58 *b* 65 *c* 69	*a* 24 *b* 32 *c* 39	*a* 6 *b* 12 *c* 17	*a* 6 *b* 8 *c* 12
Sex	M (81) F (76)	M (62) F (69)	M (33) F (35)	M (12) F (14)	M (10) F (9)
Marital status	S (75) M (79) WD (72)	S (57) M (67) WD (71)	S (26) M (34) WD (42)	S (7) M (13) WD (25)	S (9) M (9) WD (16)
Socio-economic status	*AB* 71 *C1* 79 *C2* 81 *DE* 82	*AB* 61 *C1* 62 *C2* 64 *DE* 73	*AB* 39 *C1* 35 *C2* 32 *DE* 33	*AB* 29 *C1* 16 *C2* 8 *DE* 11	*AB* 34 *C1* 12 *C2* 6 *DE* 3 (Effect more marked in higher age group)
Variation with frequency of eating out Less than or equal to 5 times a month *v* – greater than 5	79/78	67/63	33/35	11/18	7/17
Town size	1 76 2 73 3 81 4 85	1 65 2 61 3 67 4 70	1 28 2 24 3 42 4 43	1 14 2 9 3 13 4 14	1 9 2 10 3 9 4 11
Regional variations					
London and South-East	77	65	27	23	10
South and East	76	59	39	18	14
South-West and Wales	80	72	36	9	10
Midlands	77	66	33	13	9
North and North-East	79	67	18	6	6
North-West	80	63	32	6	8
Scotland	84	72	65	6	10

Key

Size of towns, etc

1 More than 250,000
2 More than 50,000
3 Less than 50,000
4 Rural districts

Category of response

(i) Like very much
(ii) Not very much
(iii) Mixed feelings
(iv) Dislike a little
(v) Dislike a lot

Socio-economic group

A, *B*, *C1*, *C2*, *D*, *E* From Registrar-General's classification

Marital status

S = Single
M = Married
WD = Widowed or divorced

* Denotes less than ½ per cent

Mass Observation Study, 1962

The final study noted in this series is in some respects still a pilot investigation in so far as only five vegetables are included The data were collected by Mass Observation, Ltd (London), through the co-operation of Mr L R England In the course of a routine enquiry in the spring of 1962 questions about attitudes to these five vegetables were answered by some 2,000 respondents selected according to usual practices taking account of age, sex, marital status, and socio-economic status and other basic conditions A summary of the main conclusions is given in Table 6

Again, only the more striking observations are emphasized The investigation was restricted to questions about potatoes, cabbage, leeks, spinach and asparagus These were selected in the light of previous experience so as to include the widest possible range of variation in types and patterns of responses, with a view to relating these to the distinctive sub-classes in the sample In this case as few as 1 per cent (or less) of all respondents strongly disliked any of these vegetables This is in marked contrast with the high frequency with which spinach was disliked (28 per cent) in the previous British sample, with its bimodal characteristics Restricting attention here to the 'like very much' category of response the following points stand out

(1) Potatoes show no change at all with age

(2) Whereas the 'like very much' response shows a slight and gradual increase with age of the respondent groups in the remaining instances (that is, other than potatoes) in no case is this trend marked

(3) Sex differences are trivial

(4) Changes with marital status mostly follow the sequence, single, married, widowed and divorced, in increasing order of 'like very much' response Since these trends are very much the same as those with age, perhaps there is some confusion (confounding) of the effects of age and marital status

(5) Socio-economic status only slightly affects attitudes to cabbage and potatoes As might be expected, this variable shows greatest effect on the 'like very much' response for asparagus, varying from 34 per cent for class *AB* to a mere 3 per cent for class *DE* Similar though less marked effects are to be seen with spinach

(6) With the possible exception of its effect on leeks, town size has only a trivial effect on the 'like very much' response

(7) There is no regional variation in 'like very much' response for potatoes

(8) The most marked regional variation is to be found with leeks in the very high frequency of 'like very much' response in Scotland (65 per cent) as opposed to the low value of 18 per cent in the north and east

(9) London and the south-east show the greatest frequency of 'like very much' response for spinach (18–23 per cent) as compared with the north, the north-west and Scotland all with a frequency of 6 per cent

(10) Only in the case of the more unusual vegetables in the group (spinach and asparagus) is there any suggested association to link frequency of 'eating out' in a positive manner with the frequency with which a particular item is liked

(11) Finally, there is in general an inverse relationship between the frequency with which a particular item is liked and its unfamiliarity

The available results have been presented in sufficient detail to make it unnecessary to recapitulate points of detail What, then, are the main conclusions to be drawn from the collation of the information from these very different sources ? Points of special interest include the following

(a) In practically all these investigations potatoes are widely familiar, usually liked and only rarely disliked

(b) Although exact figures for particular vegetables may vary considerably from one investigation to another, with some exceptions relative positions show a considerable measure of concurrence

(c) Comparisons are not simple to make, in view of the fact that no single index provides full and adequate information about a particular vegetable In practice, other variables not discussed here have to be considered in attempts to predict patterns of acceptance

(d) In this context note may be taken of the report by Pilgrim[8] or Pilgrim and Kamen[9] that about 75 per cent of the variability in acceptance of a wide range of foods in the Army situation can be predicted by three variables These were the average Hedonic rating, the satiety value or 'fillingness' of a particular food, and its basic nutrients (protein and fat) This particular observation does not refer exclusively to vegetables but to the complete range of items in the American Army surveys

(e) In particular, this work stresses the potential value of the Hedonic Scale and its use as a method of distinguishing between different foods and vegetables They point to the need for more extensive surveys in the United Kingdom, and to the possibility of making regular comparisons to trace out changes with time in patterns of likes and dislikes

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FREQUENCIES FOR RADIO ASTRONOMY AND SPACE SCIENCE

By Dr R L SMITH-ROSE, C B.E.

President, International Scientific Radio Union

FOR several centuries astronomers have been exploring the heavens with the aid of optical telescopes which have been steadily developed to give greater resolving power and precision of observation In more recent times, the astronomer has increased the facilities available to him by the aid of photographical and other recording techniques, thereby extending the range of wave-lengths under observation to include the infra-red and ultra-violet portions of the spectrum Even so, the basic range of wave-lengths available for use by the optical astronomer is limited to approximately the band 1,000–30,000 Å, corresponding to wave-lengths from 10^{-7} to 3×10^{-6} m (or frequencies from 3×10^{15} to 10^{14} c/s) The development of radio astronomy has more than trebled the extent of the electromagnetic wave spectrum now available for scientific research, the frequencies in present-day use ranging from 2 5 to more than 30,000 Mc/s (wave-lengths 120 m to less than 1 cm)

Radio Frequency Allocation

In the past decade or so, the use of radio waves for all purposes such as communication, navigation, broadcasting and television has expanded on an enormous scale, with a consequent increasing demand for allocations of various portions of the radio-frequency spectrum It has therefore become essential for scientists engaged in radio astronomy or space science to establish their claim to the protection of certain bands of frequencies for their use in experimental research

For this purpose, an Inter-Union Committee on Frequency Allocations for Radio Astronomy and Space Science (IUCAF) was formed in 1960 under the auspices of the International Council of Scientific Unions, to examine in detail the number and width of the various frequency channels in which protection from interference is desired, and to report its conclusions to the International Telecommunication Union and its International Frequency Registration Board The Inter-Union Committee consists of representatives of the three international scientific bodies concerned (namely, the International Scientific Radio Union, the International Astronomical Union and the International Committee on Space Research), together with members of the International Council of Scientific Unions, the International Telecommunications Union and the International Frequency Registration Board, and with one or more independent consultants, who are specially experienced in the international radio field Dr J F Denisse of the Observatoire de Paris is the chairman, and Dr R L Smith-Rose is the Secretary-General

The Committee on Frequency Allocations for Radio Astronomy has given serious study to the problem of frequency protection for radio astronomy and space science, and its representatives have participated in both study group meetings and the recent (1963) Plenary Assembly of the Comité Consultatif International des Radio-communications, at which the technical issues involved have been discussed on an international scale Both radio astronomy and space research are recognized services within the sphere of the International Telecommunication Union, and some progress was made at Geneva in 1959 towards ensuring the protection of frequencies required for such services

At the forthcoming Extraordinary Administrative Radio Conference of the International Telecommunications Union, which opens at Geneva on October 7, the rapid progress made in recent years in both radio astronomy and space science and communications will be reviewed in detail, in order that the allocation of specific bands of frequencies may be made more definite

Radio Astronomy

The International Telecommunication Union in its Geneva, 1959, Radio Regulations recognizes the Radio Astronomy Service in the following two definitions

"No 74 *Radio Astronomy* Astronomy based on the reception of waves of cosmic origin",

"No 75 *Radio Astronomy Service* A service involving the use of radio astronomy"

It should be noted that the Radio Astronomy Service differs from all other radio services in two important respects (a) it does not itself originate any radio waves and therefore causes no interference to any other service, (b) a large proportion of its operation is conducted by the use of reception techniques, which are several orders of magnitude more sensitive than those used in other radio services

The radio astronomer continues to improve his reception techniques, aerial systems and receiving apparatus, in order to obtain the utmost sensitivity for recording the very weak signals forming the various radiations he is studying The conduct of this work involves him in the development and construction of very expensive equipment, and it is important to ensure that the use of this apparatus should not be unduly impaired by interference from other radio services

In order to pursue his scientific research successfully, the radio astronomer seeks protection from interference, first, in a number of bands of frequencies distributed at approximately harmonic intervals throughout the spectrum, and secondly, more complete and specific protection for the exact frequency bands in which natural radiation from, or absorption in, cosmic gases is known or expected to occur An example of the latter is the emission line of hydrogen at 1,400–1,427 Mc/s, which is already given an exclusive allocation in the existing Radio Regulations (1959) In all other cases in these Regulations, reference to frequencies for radio astronomy is made on a basis of sharing with other radio services What is now sought is an improved measure of protection for a number of these frequencies distributed throughout the spectrum, from the guard bands associated with the standard frequencies of 2 5, 5, 10, 15, 20, and 25 Mc/s, to a number of other frequencies at or near the values of 40, 80, 150, 408, 610, 2,700 and 5,000 Mc/s The widths of the bands in which, so far as practicable, an exclusive allocation is desired at these frequencies, range from 0 5 Mc/s at the lower end to 10 Mc/s at the upper end of the spectrum In addition, and to provide for future developments in research, it is requested that radio astronomy should be regarded as a primary service in four higher regions of the spectrum at 10 6, 15 4, 19 4 and 31 5 Gc/s, where bandwidths ranging from 0 1 to 0 3 Gc/s are suggested as being necessary for the efficient pursuit of research with the most advanced type of apparatus at present available

Space Research

The need of the space research scientist for protection of radio frequencies is somewhat different from that of the radio astronomer Some satellites are already in use for direct application to the extension of world-wide communications Many other satellites are, however, being placed in orbit around the Earth with the view of extending our knowledge of the upper atmosphere at increasingly great heights, and to measure various physical quantities in outer space, such as solar radiation, cosmic rays, and the density and orbits of electrons trapped in belts determined by the Earth's magnetic field In some cases the frequencies required here are for control of instruments in the satellite or for locating it during its orbit, and, in such instances, the frequencies could be conveniently shared with the corresponding requirements of, say, communications satellites But, bearing in mind the inaccessibility of the scientific equipment carried in the space vehicle and the need to use the measuring equipment for the maximum proportion of the available time, it is important that the results of the scientific measurements can be communicated to the ground controlling station or laboratory with the minimum of delay or interference It is thus desirable that a number of bands of frequencies in appropriate parts of the spectrum should be allocated, without danger of interference, to the needs of scientific research being conducted with the aid of satellites in orbit

At the recent meeting of the International Committee on Space Research (COSPAR) in Warsaw, the following general resolution was adopted "Considering that the transmission of data from space vehicles to the ground is essential to the use of such vehicles in space research, COSPAR recommends that through IUCAF every effort should be made to guarantee the continued allocation of suitable frequency bands for this purpose "

While in some cases frequencies required by those concerned with communications by means of satellites can be shared with the scientist interested solely in scientific measurements, in many instances it is desirable to have separate allocations, so far as is practicable, in order that the elaborate and expensive equipment used in space research can be used to the utmost extent It is thus desired that bands at approximately the following points in the spectrum should continue to be available for the purposes of space research on at least a primary, if not on an exclusive, basis 136, 400, 1,427, 1,700 and 2,300 Mc/s, and 5 2, 8 5, 15 2 and 31 5 Gc/s A number of other frequencies are also required for tracking and controlling satellites and for communication and telemetry services, but in some or most of these cases the actual frequency bands may be allocated on a shared basis among the various users

Conclusion

It will be seen from the foregoing brief survey that the rapidity with which advances in both radio astronomy and space science are taking place at the present time renders it most important that careful consideration should be given to the allocation, on either an exclusive or on a primary basis, of various bands of frequencies within the radio spectrum The forthcoming Conference at Geneva will have the most difficult task of debating the claims of the various users, anxious to advance radio astronomy and space science as well as the practical operation of world-wide communications by means of Earth satellites But it is the decisions and recommendations of this Conference that will determine the ease with which scientists will be able to pursue their work with the maximum degree of protection from interference from other radio services

OBITUARIES

Prof. E D Hughes, F.R S.

THE untimely death in London on June 30, after a short illness, of Prof E D Hughes, head of the William Ramsay and Ralph Forster Laboratories, and professor of chemistry in University College, London, deprives British science of a man of wisdom and authority at the height of his powers

Edward David Hughes was born on June 18, 1906, of a farming family in Criccieth He studied chemistry at the University College of North Wales, at Bangor, under Prof K J P Orton, whose pioneering work in mechanistic organic chemistry inspired his early enthusiasm for this field His first scientific papers, on kinetic aspects of keto-enol tautomerism, were published with Orton and with H B Watson He took a Ph D degree of the University of Wales in 1930, and in that year he joined Prof C K Ingold in the Chemistry Department of University College, London, as a post-doctoral Fellow In 1936 he became Ramsay Memorial Fellow, and then successively assistant lecturer and lecturer on the staff of that Department This period marked the beginning of the long scientific collaboration which, continuing as it has until his death, is almost without parallel in the history of English science, and inextricably links the names of Hughes and Ingold as the foremost contributors to a change in approach to organic chemistry, which now is manifest both in the terminology and teaching of the subject, and at the forefront of progress in the field

His first investigations at University College, London, were in the field of nucleophilic displacement and elimination reactions The simple concept that several limiting mechanisms for such reactions could be identified and characterized kinetically led quickly to the recognition of fundamental patterns of behaviour which until then had been obscured by apparently conflicting observations Perhaps the major triumph of this period was the elucidation of the main factors determining whether racemization, retention or inversion accompanies nucleophilic displacement at a saturated carbon atom—the problem of the 'Walden inversion' His brilliant reviews in the *Discussions of the Faraday Society* in 1937 and 1941 still provide the fundamental basis of any mechanistic account of these reactions They anticipate also many of the later developments in this field, stereochemical, structural and environmental factors, determining the course of these reactions and the rearrangements which can accompany them, are all discussed in these early summaries, though the elaboration of details required many years of later investigation, to which he and his associates no less than other workers in the field have since contributed

From 1939 he was one of a very small staff maintaining the Department of Chemistry of University College, London, through its evacuation to Aberystwyth and Bangor In 1943 he was appointed to the chair of chemistry in the University College of North Wales at Bangor where he had first studied There he spent five years, and was responsible for the rapid post-war rebuilding and expansion of his Department At the same time he gathered around him a group of enthusiasts in the application of isotopes to the study of reaction mechanisms Their pioneering work on the large-scale separation of the heavy isotope of oxygen now has fruitful offshoots in several departments and countries

Through this period, Hughes maintained his interest and participation in the work in progress in the Chemistry Department of University College, London His direct and critical approach led during these years towards simplification of the interpretation of the kinetic forms observed for aromatic substitution, and to the general acceptance of the view that the nitronium ion is the most important entity involved in aromatic nitration

In 1948 he was appointed professor of chemistry at University College, London Here he remained until his death His scientific distinction was marked in 1949 by his election to fellowship of the Royal Society In 1961 he became head of the Department of Chemistry at University College, London His contributions to the elucidation of reaction mechanism ranged in these years over a wide field He initiated important and imaginative studies of structural and stereochemical effects in nucleophilic and aliphatic replacement reactions, of Wagner-Meerwein and related rearrangements, of anionotropic rearrangements, of electrophilic aromatic and aliphatic substitution, of aromatic rearrangements, and of prototropic shifts Alongside these developments he maintained a policy of encouraging and fostering independence in his younger colleagues, who owe a great debt to his generosity and to his faith in their ability and promise

In 1961 he undertook editorship of a series of monographs to be published by Elsevier on special topics in physical organic chemistry, the first of these was at the proof stage at the time of his death

His services to academic life, which were marked by his election to fellowship of the University of Wales and of University College, London, were by no means solely in the field of research A valued teacher and colleague both at Bangor and at University College, London, where he was dean of the Faculty of Science from 1958 until 1961, he contributed also in a most valuable and important way as an appointed teacher of the University of London, particularly through his chairmanship (1955–60) of the University Board of Studies in Chemistry Here his wise and efficient guidance gained him many friends, and he will be much missed from the academic councils of the University during the times of change ahead He was greatly in demand as external examiner for first degrees and for higher degrees of universities throughout Britain He performed an extremely important task as honorary secretary (1949–61) and then as chairman of the Advisory Council of the Ramsay Memorial Fellowship Trust, and on committees of selection for Imperial Chemical Industries, Ltd, and other senior research fellowships, where his judgment, common-sense and powers of assessment were regarded highly.

Outside the University he played many important parts He was a governor of the Northern Polytechnic (1950–60) He was a Fellow (1938) and member of the Council (1961–63) of the Royal Institute of Chemistry, and acted for the Institute as assessor in organic chemistry for Higher National Certificates and Diplomas, and as a special examiner, he was honoured by the Society as a Meldola Medallist in 1936 Perhaps his greatest contribution, however, was to the Chemical Society, to the publications of which he personally contributed so much He served on almost every sub-committee of this great Society, being chairman of the Library Committee from 1959 onwards, an honorary secretary from 1950 until 1956, and a vice-president (1956–59) He was honorary secretary of the Chemical Council (1953–55)

Hughes's scientific lifetime has seen an enormous change in organic chemistry All its many aspects have been affected by the realization that the complicated patterns of yield and reactivity can be interpreted and already in part predicted in terms of the accessibility of the various transition states leading from starting-materials to products Few have contributed to the acceptance of this approach, bitterly contested as it has been, more than Hughes His work has always been characterized by steadfast belief in the importance of mechanistic investigations, an insistence on experimental verification of each point as it has come under scrutiny, and an intuitive recognition of the quality of crucial experimental observations

He was a man of outstanding humanity His actions and his advice led directly to the heart of any problem through clear recognition of general principles His friends will remember him for his deep sense of loyalty, for his unshirking sense of responsibility, and for his devotion to the Department where so much of his work was done

In 1934 he married Ray Fortune Christine, daughter of the Rev Ll Davies, of Brecon She survives him with their daughter P B D DE LA MARE

Dr. Annie Porter

PARASITOLOGISTS the world over will mourn the loss of Annie Porter, who died in London at the advanced age of eighty-three on May 9 after a protracted and debilitating illness

Born at Shoreham-on-Sea, Sussex, on February 20, 1880, Annie was the elder daughter of Samuel Porter The family early moved to Brighton where she received her school education, whence she proceeded to University College, London There her first love was mycology—a subject in which she retained an interest throughout her life—although animal parasitology, and especially protozoology, demanded most of her professional time in later years

From University College she proceeded in 1914 for a time to the Quick Laboratory in Cambridge, where she met and worked with H B Fantham (and whom she married in 1915) At Cambridge Dr Porter held a Beit Memorial research fellowship for two years and taught as an assistant helminthologist

In 1917 she accompanied her husband to Johannesburg and became head of the department of parasitology in the South African Institute for Medical Research, serving also as senior lecturer in parasitology in the University of the Witwatersrand and as examiner in zoology and animal parasitology at both institutions She remained in South Africa until 1933 when she accompanied her husband to Canada, where she remained until 1938 as research associate in zoology at McGill University, Montreal On the death of her husband (during October 1937) she was invited by the secretary of the Zoological Society of London (Dr (now Sir) Julian Huxley) to become honorary parasitologist at Regent's Park, a post she filled with great success until a few months prior to her death

The furtherance of zoology and especially animal parasitology was almost the sole interest in Annie Porter's long life Always ready to help anyone interested in animals, she will long be remembered by her many pupils and friends who are now widely scattered over the globe Her dedication to zoological science transcended all other interests and amounted to a passion when stimulating her co-workers in the several team projects with which she was associated

Her early interest in mycology found expression during the lean periods of the Second World War when she combed the areas around her home for edible fungi, samples of which she generously bestowed on her friends She spent long hours propagating the edible species by spreading their spores in suitable localities in Regent's Park

Apart from her scientific work Dr Porter devoted her time to social welfare and particularly to the education of spastic children, for whom once a year she borrowed live animals from the Zoo for demonstration

Out of her large estate in Great Britain, Dr Porter willed £10,000 to Christ's College, Cambridge, to endow a research scholarship in animal parasitology and protozoology, £5,000 each to University College and to the Zoological Society of London for similar research scholarships, besides £5,000 to the University of the Witwatersrand for a research scholarship "to be held in any University in the British Empire" and £1,000 to McGill University for a research scholarship W C OSMAN HILL

NEWS and VIEWS

The U.S. National Science Foundation: Dr Alan T. Waterman

ON the retirement of Dr Alan T Waterman as first director of the National Science Foundation, the three Presidents of the United States under whom he had served paid tribute to his skilled leadership in establishing the Foundation and guiding it through the crucial years of its existence as a Federal agency in support of basic research and education in the sciences The statements of the three Presidents, as well as tributes from the scientific community, were read at a dinner given for Dr and Mrs Waterman by the National Science Board on June 21, 1963 President Kennedy observed "Through the work of the Foundation in sponsoring basic research, the Nation has embarked on exciting and critical adventures in science that will contribute importantly to human progress The NSF has helped extend our horizons to the innermost workings of man and his society and the outermost reaches of our planet and the universe" Dr Waterman, who continues as a consultant to the Foundation, is also president of the American Association for the Advancement of Science, and next year will become chairman of the Board of Directors Dr Waterman has deferred for the time being further plans for the future until he avails himself of the holiday which he consistently neglected in order to devote himself to the work of the National Science Foundation

Dr. Leland J. Haworth

DR LELAND J HAWORTH succeeded Dr Alan T Waterman as director of the National Science Foundation on July 1 He has been one of the five commissioners of the U S Atomic Energy Commission since 1961 Prior to his appointment as commissioner, Dr Haworth was associated with the Brookhaven National Laboratory, one of the United States' major facilities devoted to high-energy physics He became director of the Laboratory in 1948, and in 1960 he was, in addition, made president of Associated Universities, Inc, a non-profit corporation of nine eastern universities which operates the Laboratory for the U S Atomic Energy Commission and the National Radio Astronomy Observatory for the National Science Foundation A native of the Middle West, Dr Haworth was educated at the University of Indiana, where he received his A M degree, and at the University of Wisconsin, where he was awarded his doctorate in physics in 1931 During 1938–47 he was professor of physics in the University of Illinois During the Second World War he was on leave of absence from Illinois to work on radar development at the Radiation Laboratory of the Massachusetts Institute of Technology Dr Haworth's intimate knowledge of Government research and development problems has been further enhanced by membership of a number of advisory committees and panels His most recent personal research interests include high-energy physics, and high-energy accelerator design In earlier years he worked on secondary emission of electrons, Joule–Thomson effects at very low temperatures, nuclear physics, and electronics

Mathematical Physics at Birmingham: Prof T H. R Skyrme

MR T H R SKYRME has been appointed to the chair of mathematical physics at the University of Birmingham as from October 1, 1964 Mr Skyrme succeeds, as professor and head of the Department of Mathematical Physics, Prof R E Peierls, who has been appointed Wykeham professor of physics in the University of Oxford (see *Nature*, 196, 1038, 1962) Mr Skyrme's academic career started with scholarships at Eton and at Trinity College, Cambridge, where after taking a double first in mathematics he became a Fellow of his College During the Second World War he was with Prof Peierls for two years as a member of a British mission sent to work with American scientists in New York and later in New Mexico, he returned to the United Kingdom with Prof Peierls as a Research Fellow in the Department of which he is now to be the head Here he met and married Dr Dorothy Millest, at that time lecturer in the Department of Physics Since leaving Birmingham in 1948, Mr Skyrme and his wife have been on the staff of the Atomic Energy Establishment, Harwell, except for two years when he held fellowships at the Massachusetts Institute of Technology and Princeton, a period when he was a visiting professor at the University of Pennsylvania, and the time since 1962 when they have been teaching at the University of Malaya Thus Mr Skyrme, who is aged forty, goes to Birmingham in 1964 with experience of eight universities and research institutes in three continents He has carried out research in the theory of atomic nuclei and of sub-nuclear particles, especially in field theory

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Memorial to the Late Dr. A. T. R. Mattick, C.B.E.

COLLEAGUES and friends of the late Dr. A. T. R. Mattick, for many years head of the Bacteriology Department and deputy director of the National Institute for Research in Dairying, University of Reading, wish that some form of memorial be established to commemorate his work and perpetuate his name. Everyone who knew Dr. Mattick will be aware that he set himself high standards in his scientific work and in its presentation. They will also remember that he always had in the forefront of his mind the benefits to the Institute's research programme which came from discussion with scientists in other research centres both at home and abroad. It is proposed, therefore, to establish a memorial fund, the income from which, in the absence of financial support from other sources, would be used to enable members of the research staff of the Institute to visit research centres either in the United Kingdom or abroad or to attend specialist conferences. It might also be appropriate on occasion to invite a distinguished scientist to give a memorial lecture on a topic which would be of interest to people connected with dairying. Individuals and organizations within the dairy industry who have reason to be grateful to Dr. Mattick for his help and guidance would no doubt wish to be associated with this proposal and to subscribe towards a fund to commemorate the life work of a man who is spoken of as "the father of dairy bacteriology". Contributions towards the Memorial Fund should be sent to the Director, National Institute for Research in Dairying, Shinfield, Reading.

Bicentenary of the Birth of John Dalton

THE bicentenary of the birth of John Dalton falls in September 1966, and to mark the occasion a programme of events will be held in Manchester, where Dalton lived and worked for most of his life. The celebrations are being sponsored by the Royal Society, the Manchester Literary and Philosophical Society, the Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry, with the collaboration of the British Society for the History of Science and the Director of the Science Museum. The detailed arrangements are being made by the Manchester Literary and Philosophical Society. Although the details are not yet fixed, the celebrations will probably be held during the third week in September 1966, and are likely to consist of a specialist conference for the historians of science, followed by a series of meetings of more general interest. The programme will include an exhibition and a number of social events.

Science and the Policies of Governments

Science and the Policies of Governments presents the report of the *ad hoc* Advisory Group on Science Policy of the Organization for Economic Co-operation and Development (Pp. 55. Paris: Organization for Economic Co-operation and Development, 1963). The Group was appointed in 1961 by the Secretary-General to advise him on policy issues of science and technology that are increasingly demanding attention from Governments and the scientific community, as well as on the general orientation and long-term objectives of the Organization in this field. The Advisory Group, of which M. Pierre Piganiol was chairman and Sir Willis Jackson a member, after reviewing generally the new dimensions of science and technology, the public stake in science and the relation of science to economic growth and objectives, points out that science policy is not an abstract concept but of the same nature as economic policy or foreign policy. There has developed on Governments a major and continuing responsibility to make choices about the issues that involve science. The second part of the report discusses science and national policy, including its bearing on scientific policy and on other factors that enter into the effective management and pursuit of scientific work and scientific communication, as well as some of the implications of science in making economic or foreign policy or its bearing on manpower and educational policy. This section of the report insists on the need to formulate a national policy for science, to co-ordinate the various scientific activities of a country, and to integrate science with general policy, and advocates the constitution for these purposes of a Science and Policy Office, the specific functions of which are detailed. From this, in the third part of the report, the Group proceeds to a discussion of international affairs, emphasizing first the need for policy and recommending specifically for the Organization for Economic Co-operation and Development a meeting of Ministers for Science. The agenda visualized would be concerned with national science policy, the concept of which should be clarified, with international scientific co-operation, and with science, economic growth and the role of the Organization for Economic Co-operation and Development. [Such a meeting was, in fact, held in Paris during October 3–4, when the report was considered.]

London and Home Counties Regional Advisory Council for Technological Education

THE fourteenth annual report of the London and Home Counties Regional Advisory Council for Technological Education, covering the period from September 1, 1961, to December 31, 1962, claims that the Council is a vital link in the chain of communication and partnership between education and industry (Pp. 39. London: London and Home Counties Regional Advisory Council for Technological Education, 1963). The Council has been intimately connected with many developing features of technical and technological education, and a working party, sponsored by the Council and the Institute of Education, University of London, has just completed a report on educational research into technical education. Concern is expressed as to the effect on the constitution and functions of the Council of the Government proposals for the reorganization of local government in Greater London. Some 289 courses were supported during the year, 51 being in applied science and mathematics, 98 in engineering and 140 in other subjects. Twelve colleges in the region have been recommended for designation as regional colleges, with a reserve list of three others for future designation. Reports from the Regional Academic Board, the Distribution of Courses Committee and the fifteen Regional Advisory Committees are included.

Centralization and Documentation

A FINAL report to the National Science Foundation made by Arthur D. Little, Inc., on *Centralization and Documentation*, dated July 1963, does not support the large-scale centralization of facilities for storing and retrieval of scientific documents at the present time (Pp. iv+70. Cambridge, Mass.: Arthur D. Little, Inc., 1963). It is considered that a large centralized facility drawing on present-day techniques for retrieving documents is unlikely to achieve the main objective of providing an effective exhaustive capacity for searching the literature to supplement efforts to prevent duplication of research or development. The report recommends, however, a comprehensive programme to indicate the real needs of scientists and engineers for information. Such a survey, conducted with imagination and insight, is an essential pre-requisite to the support of any centralized searching facilities in future, if such facilities are to serve useful functions. Before extensive efforts are made to develop such aids as elaborate word thesauri for existing retrieval systems, the report recommends also the further investigation of the use of statistical techniques for the automatic generation of thesaurus lists and for the automation of some functions at present performed by human intermediaries. To this end, the report suggests that one of the existing medium-sized co-ordinate retrieval systems should be operated experimentally on a statistical association basis Although a body of theory for the design of such systems has been developed and tested successfully on a small scale, there are many practical technical problems to be solved before a useful system incorporating these concepts can be developed Existing co-ordinate searching systems do not possess three necessary characteristics a capacity for generalizing a user's request to make it more compatible with the vocabulary of the retrieval system, a capacity to match automatically the user's depth of search requirement to system parameters, by ranking the documents presented to users in decreasing order of probable relevance, a potential capacity of almost instantaneous reaction between user and searching machine, without need for a human intermediary

The Eastern European Academies of Sciences

PUBLICATION 1090 of the National Academy of Sciences–National Research Council, *The Eastern European Academies of Sciences a Directory*, represents a first attempt by the Office of the Foreign Secretary of the Academy to compile information on academies and research councils throughout the world (Pp v+148 Washington, D C National Academy of Sciences–National Research Council, 1963 2 dollars) The academies of sciences described are those of Bulgaria, Czechoslovakia, Hungary, Poland, Rumania and Yugoslavia The academies of sciences of the U S S R are not included since they have been the subject of other studies, the results of which are already available in the United States The *Directory* presents a brief history of each academy, its present organization, responsibilities, and the titles of its main periodicals Rosters of leading academy officials and of affiliated institutes and their directors are also included, along with biographical sketches of prominent academicians in each country It is claimed that the survey reflects, as accurately as possible, the status of the academies and their principal personnel and programmes in December 1962 As a 'directory' the information supplied is not extensive, and does not supplant that available from, for example, *The World of Learning*, especially as the latter gives full lists of academicians and far more titles of periodicals Thus the use of the present publication will be as a reference guide to the policies and personalities of academies in eastern Europe

Perkin Centenary Awards

APPLICATIONS for the awards of the Perkin Centenary scholarships are invited by the Trustees Each award, tenable for one or two years and renewable for a further year at the discretion of the Trustees, is offered to candidates employed in an industrial firm or other institution concerned with the manufacture or the application of colouring matters to study at a university or technical college Two types of award are available, some to the value of £100, to be used in conjunction with a local education authority grant, and others to the value of £350, which may be increased to £400 for a student living away from home Perkin Travel Grants are also available However, the Perkin Centenary Fellowship will not be awarded in the year 1964–65 Further information can be obtained from the Secretary, Perkin Centenary Trust, c/o the Chemical Society, Burlington House, London, W 1

Does Instrumentation Pay?

FOLLOWING the success of its symposium last year, when more than 100 applicants had to be turned down because of lack of space, the South Yorkshire section of the Society of Instrument Technology is organizing another one-day event in Sheffield on October 24 The theme of this year's symposium is "Instrumentation and Control—Does it Pay?" Intended as the South Yorkshire section's contribution to National Productivity Year, the Memorial Hall of Sheffield City Hall has been booked in anticipation of a large attendance Nine speakers will talk on various aspects of instrumentation and control, drawing extensively from their own practical experience Special emphasis will be directed towards the needs of the smaller firm, where lack of knowledge tends to restrict the opportunities for the application of relatively inexpensive control systems Speakers will include R Barber (Land Pyrometers Ltd, and chairman of the South Yorkshire Section of the Society of Instrument Technology), M A Thomas (Steel, Peech and Tozer), B O Smith (Honeywell Controls Ltd), Dr P Bacon (Guest Keen Iron and Steel Co Ltd), H B Western (National Industrial Fuel Efficiency Service), J Hancock (Unbrako Steel Co Ltd) A Barker and R F Wright (Thos Firth and John Brown Ltd), I C Ross (Steel, Peech and Tozer) The symposium will conclude with an open forum under the chairmanship of Mr Barber Further information can be obtained from C G Hague, A C E-Hartons Ltd, 109, Rockingham Lane, Sheffield, 1 An attendance fee of one guinea is being charged for the meeting

Announcements

THE twenty-first annual Pittsburgh Diffraction Conference will be held at the Mellon Institute, Pittsburgh Pennsylvania, during November 6–8 Further information can be obtained from W M Biagas, Crucible Steel Company, P O Box No 7257, Pittsburgh 13, Pennsylvania

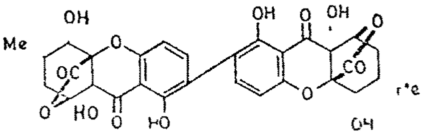
A SYMPOSIUM on "The Toxicology of Cosmetic Materials" will be held at the Town Hall, Leamington Spa, during November 19–20, by the Society of Cosmetic Chemists of Great Britain Registration forms together with full details can be obtained from the general secretary Mrs E Millman, 2 Lovers Walk, London, N 3

THREE short courses are being organized by Bradford Institute of Technology during November on "Developments in Polymerization" (November 8–9), "Recent Industrial Applications of X-ray and Electron Diffraction" (November 14–15), and "New Techniques in the Study of Materials" (November 22–23) Further information and application forms can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7

A SERIES of courses of special lectures in higher technology will be held at Woolwich Polytechnic during the academic year 1963–64 These will include courses in mathematics, physics and telecommunications, chemistry, biology, civil, mechanical and production engineering, electrical engineering, economics and management Further information can be obtained from the Secretary, Woolwich Polytechnic, Woolwich, London, S E 18

THE ninth annual conference on "Magnetism and Magnetic Materials", sponsored by the American Institute of Physics and the Institute of Electrical and Electronics Engineers, will be held in Atlantic City during November 12–15 In addition to the usual topics the Committee is encouraging papers relating to superconductivity and magnetism Further information can be obtained from Mr William D Doyle, Franklin Institute Laboratories, Philadelphia 3, Pennsylvania

ERRATUM There is an omission of two OH groups from the formula of ergoflavin given in the summary of Prof J Monteath Robertson's presidential address to Section B (Chemistry) at the Aberdeen meeting of the British Association for the Advancement of Science, which appears on p 883 of the August 31 issue of *Nature* The formula should be



INDUSTRIAL AND AGRICULTURAL RESEARCH IN BELGIUM

THE annual report of the Belgian Institute for the Encouragement of Scientific Research in Industry and Agriculture for 1962* records the award during the year of 62 subsidies totalling 303 million francs, on which 319 research workers and 930 technicians were engaged Of this total, 65 million francs went to the National Centre for Metallurgical Research for work in physical and chemical metallurgy, on constructional steel and special steels and the metallurgy of iron, etc Eight grants, totalling some 20 4 million francs, also went to the Centre for Scientific and Technical Research on the manufacture of metals The Committee for Mapping the Soil and Vegetation of Belgium received a subsidy of 23 5 million francs, and the Committee for the Study of the Diseases and Nourishment of Cattle 16 7 million francs, while another 16 million francs went to the Centre for Applied Electronics for investigations on impulsion circuits, industrial control systems and of amplifiers, modulators and oscillators for transistors The Road Research Centre received a subsidy of 14 75 million francs, while the Scientific and Technical Centre of the Belgian Textile Industry was granted 11 5 million francs for research covering cotton, wool jute, linen, rayon and acrylic fibres Apart from a subsidy of 11 35 million francs to the Centre for Scientific and Technical Research of the Metal Manufacturing Industry included among the eight already noted, and one of 10 75 million francs to the Belgian Society for Nitrogen and Chemical Products of Marly, only the subsidies of 9 9 million francs to the National Committee for the Study of Fruit Cultivation and of 9 5 million francs to the Committee for the Study of the Solid State are worthy of special mention The Committee for the Scientific and Technical Study of Milk received 5 8 million francs, the Committee for the Study of High Polymers 5 6 million francs for research on the behaviour of high polymers to irradiation on the degradation of high polymers used as insulators, and on photopolymerization, the Research Centre for Animal Breeding and Fodder in High Belgium 5 27 million francs, and the Scientific and Technical Research Centre for the Explosives Industry 5 25 million francs

The only other grant of 5 million francs or more was to the National Centre for Herbage and Fodder Research but 4 7 million francs went to the Belgian Institute for Improvement of Beetroot, 4 3 million francs to the Belgian Centre for the Study and Documentation of Water, for research including, besides investigations on potable and industrial waters, on corrosion and effluents, some work on atmospheric pollution, and 4 million francs to the Committee for the Study of the Structure of the Soil Among other large subsidies may be mentioned 3 8 million francs to the Technical and Scientific Centre for the Brewing, Malt and Related Industries, 3 4 million francs for tobacco research, 3 4 million francs for investigations of vegetable reproduction, 3 1 million francs for work on the conservation of fruit and vegetables, and 3 1 million francs for research on vegetable parasitology, 2 2 million francs went to the Belgian Wood Centre and almost 2 million francs each to the Belgian Centre for the Study of Medicinal Plants, the Research Centre of the Belgian Ceramic Industry and the Committee for the Study of Agriculture Grants totalling almost 3 million francs went to the National Centre for Scientific and Technical Research for the Cement Industry, of which some 1 3 million francs were jointly for the Scientific and Technical Centre for Construction for an investigation on the measurement of the resistance of bitumen to compression The report indicates briefly the extent of the investigations for which the subsidies are given and includes a list of publications during the year dealing with the subsidized investigations

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* Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Bruxelles Rapport Annuel, Exercice 1962 Pp 277 (Bruxelles Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1963)

CIVIL RESEARCH POLICY

THE report of the F B I (Federation of British Industries) Working Party on Civil Research Policy* is the outcome of a remit from the Federation to consider recommendations as to how the Government should support civil scientific research and development in industry, bearing in mind the possible lessening of such support for defence research and development, which has had valuable civil application Part of the memorandum is addressed to industry and part to the Government

The prime concept of the report is the presentation of a case intended to find ways and means of increasing very substantially the financial resources to be put into civil research and development The magnitude of the target is a sum of £100 million per annum A considerable proportion of this, it is advised, should be spent as direct support of research and development in industry The responsibility for determining the allocation of such funds to the many claimants is recommended to be assigned to the Minister for Science, advised by a Council having presumably wide representation

The following comments on the report in question are based on a long experience in three distinct fields of scientific research in industry with a company noted for its technological development; in the university, in charge of a technological department; and in research associations, covering no less than seven in various capacities from member of research committees to Department of Scientific and Industrial Research visitor

A very important statement of the Working Party amounting almost to a reservation, is its expression of concern as to whether its ideas would be practicable in terms of man-power This question, apart from the problem of finding the money, becomes the crucial one of the whole matter Mere multiplication of numbers will not give a corresponding increase of achievement in respect of fruitful research though it may give acceleration of the rate of progress at possibly a relatively increased price Rather should the means be sought of strengthening existing organizations This is in some measure advocated in the report, but it is not suggested to apply in any marked degree to the industrial Research Associations Indeed as a broad generalization the Working Party says "We are bound to confess that the opinion in industry about Research Associations is not universally favourable" But how could one in all seriousness expect a unanimous opinion from industry on so wide a matter? I suggest that on balance industry is favourably impressed by the system of Government-aided research associations and by their achievements although it no doubt feels, as the report suggests, that methods of financing may need recasting and particularly that the measure of Government financial support is inadequate It is true that in the past there has been a serious lack of means in Britain to put to their fullest use the results of research For their part, the research associations have not had in general the means to pursue active development in this respect This function has been regarded as the responsibility of industry, but it becomes increasingly clear that the large-scale development of important research results must in the future become the responsibility of the scientist, the technologist, industry and Government, each playing an appropriate part

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* *Civil Research Policy a Report of an F B I Working Party* Pp iii+11 (London Federation of British Industries, 1963) 2*s* 6*d*

The research associations have in this connexion an important potentiality which a number of them have developed very successfuly, namely, the practicability of close collaboration between the technical elements of industry and the scientific staffs of the associations The technical staffs of industry become closely associated with the progress of the work of projects in which they are interested There can be developed active collaboration by many avenues; co-operation of member firms in joint researches, conferences, joint committees and frequent contacts between the technical staff of the works and the research station The intelligence service of the research association alone is a powerful instrument in developing the growth of that research-mindedness which, as the report says, "cannot be achieved until all heads of companies become fully research minded nor until adequate status is accorded to those responsible for research and development in the business" This is done successfully in some industries in Britain and more often in some countries abroad, notably France On the other hand, the scientist should not expect the advocacy of his case to be undertaken by his lay brethren The attitude to be adopted should be that a worthy cause deserves support

Noteworthy is the point in this respect that the many achievements of the research associations have been made with only a small fraction of the public support that the Working Party now suggests as being necessary

In the scheme advocated there could be new problems arising from lack of co-ordination, particularly in the privately inspired research and development contract, while at the same time there could arise a danger of over-rationalization by centralization of civil work at fewer centres The secret of successful research I believe to be freedom from external interference of the skilled team, for team it must be to-day in much applied research The largest organizations inevitably become unwieldy in administration To be able to remain successful they have to resort ultimately to decentralization There is a point of balance between the extremes of insufficient and over-control, which may become a critical issue

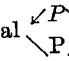
It should of course be realized that the elements of progress are at work all the time Often the most important are those about which there is least publicity It is to be hoped that those who are called on to operate the 'priming-pump' may have the wisdom to guide the stream rightly

R J SARJANT

THE MELLON INSTITUTE, PITTSBURGH

THE fiftieth annual report of the Mellon Institute*, covering the year ended February 28, 1963, includes a historical sketch of the development of the Institute written by Dr E R Weidlein

Dr Weidlein traces the growth of the Institute and of the industrial fellowship established by Dr R K Duncan at the University of Kansas in 1907, and the Department of Industrial Research he established at the University of Pittsburgh in 1911 with the support of A W and R B Mellon The Mellon Institute of Industrial Research and School of Special Industries was first established in 1913 as a part of the University of Pittsburgh, becoming independent as the Mellon Institute of Industrial Research (shortened to Mellon Institute in 1962) in 1927, although it still maintains close co-operation with the University Functionally a centre for pure and applied research, it is also a clearing house for advanced scientists and a clearing house on specific scientific information At the end of the year, out of a total personnel of 573, 118 held Ph D s or other doctorates, 33 were M Sc s and 303 were employed in scientific activities, while in addition 49 specialized technicians and craftsmen were mainly concerned with servicing research projects Operating expenditure on independent and sponsored research amounted to 6 2 million dollars, and 600,000 dollars were also spent on improvement of existing or purchase of new equipment

In biochemistry, investigations of acid-fast bacteria established that *p*-aminobenzyl alcohol is hydroxylated to *p*-aminophenol, and a new pathway for the metabolism of *p*-aminobenzoic acid has been established chemically by the use of radioactive isotopes The carboxypeptidase *N* of human plasma fraction IV–I that activates the hypotensive peptides, bradykinin and kallidin, have been further investigated, and a mica replica technique used to examine macromolecules and various polymers of myosin in the electron microscope In organic chemistry the rotational isomerism of some mono- and di-substituted propenes has been investigated by nuclear magnetic resonance spectroscopy, *cis, cis, cis*-1 4 1-*cyclo*nonatriene synthesized from indane in seven steps and also the pseudo-aromatic hydrocarbon, hexaphenylpentalene, and dioxocyanogen, the first difunctional nitriloxide A multi-state mechanism has been proposed for homogeneous ionic polymerization, studies of the statistical thermodynamics of dilute polymer solutions continued in an examination of the equation of state for solutions of branched polymers, and the light-scattering behaviour of dilute solutions of macromolecules was studied as a function of temperature with a precision photometer having close temperature control, high angular resolution and signal stability In geochemistry, investigation of the origin of stony meteorites was a major project, and in inorganic chemistry the study of the geometric isomerism of the diphenylalkylphosphine complexes of nickel halides continued, new routes to compounds containing four-membered heterocyclic rings of the type metal  metal were discovered for nickel, cobalt, iron, manganese and molybdenum, and a unique chemical system capable of carrying molecular oxygen or hydrogen

In physical chemistry, investigations of the scattering of electrons by molecular gases were initiated and experiments on the vibrational spectra of various compounds completed, while the structures of tetraphenylsilicon and tetraphenyltin were refined by full-matrix 'least-squares' technique using anisotropic temperature parameters for all atoms In radiation chemistry, investigations of the reaction kinetics of halogenated radicals have been extended to the examination of the relative rates of combination and disproportionation of trichloromethyl radicals with various other radicals In continued investigations of the normal stress effect, the concentration-dependence of solutions of polyisobutylene in cetane has been studied in steady-study torsional shearing between two parallel circular disks and between a cone and a disk Work continued on the development of new methods for obtaining accurate solutions of the molecular Schrodinger equation, and in metal physics tensile deformation investigations were carried out of the close-packed hexagonal copper-germanium phase, while the deformation stacking fault probability was measured in dilute intranoble metal alloys containing up to 5 atomic per cent solute A list of publications is included

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* Fiftieth Annual Report of the Mellon Institute for the fiscal year ended February 28, 1963 Pp 48 (Pittsburgh Mellon Institute, 1963)

WHEAT GENETICS

THE second International Wheat Genetics Symposium was held during August 19–24 at the University of Lund, Sweden The meetings produced a broad survey of many recent advances in knowledge of the genetical architecture of the hexaploid wheat of commerce, *Triticum aestivum*, and of some of its relatives in the genera *Triticum*, *Aegilops*, *Agropyron* and *Secale* Naturally, considerable attention was directed to ways in which the new information could be exploited in the improvement of the crop, but since wheat offers many unique opportunities for genetical work there was no restriction to topics of immediate practical importance

For example, R C F. Macer (Great Britain), R G Anderson (Canada), D R Knott (Canada) and A T. Pugsley (Australia) considered the genetic control of resistance to stripe rust, leaf rust, stem rust and powdery mildew respectively In the main, similar patterns of major gene effects are responsible for differences in resistance to all four fungal pathogens, and, as was pointed out by W Q. Loegering (United States), alleles for resistance or susceptibility in the host could be matched by alleles for avirulence or virulence in the fungus, provided the latter had a sexual stage Basic information of this type has a direct practical value since the recognition of the mode of inheritance greatly facilitates the use of genetically determined resistances in breeding programmes Moreover, in all these instances the genes for resistance are being isolated, in otherwise equivalent genetic backgrounds giving full susceptibility, so that differential hosts will be available that will permit accurate assessments of the virulence genotypes of pathogens isolated in the field

The practical importance of incorporating, in wheat, the genetically determined disease resistance of other species was widely accepted during the Symposium The isolation against the wheat background was reported of single chromosomes from *Agropyron* giving resistance to stripe, stem and leaf rust by A Wienhues (Germany), Y Cauderon (France) and D. R Knott (Canada), and of one giving resistance to the wheat streak mosaic virus by E E Sebesta (United States) R C F Macer (Great Britain) and C J Driscoll (Australia) reported that single chromosomes of *Secale*, isolated against a wheat background, would give resistance to stripe rust and leaf rust In several instances, use was being made of translocations induced by irradiation to transfer to a wheat chromosome the critical segment of the alien chromosome giving disease resistance

A remarkable piece of engineering of this type was described by D R Knott (Canada) It involved the transfer of *Sr*6, a gene giving stem rust resistance carried on chromosome 2*D* of the *D* genome of *T aestivum*, to *T durum*, a tetraploid in which chromosome 2*D* has no homologue Chromosome 2*D* was isolated, by backcrossing, against the *T durum* background and a satisfactory translocation of *Sr*6 to a chromosome of *T durum* was induced and made homozygous Not only is a new form of resistance now available at the tetraploid level, but also the *Sr*6-carrying tetraploid can be crossed to *T aestivum* and the gene, in its new position, back-crossed into the hexaploid, and so obtained in four doses Whether duplication of the gene will show a dose effect that improves disease resistance remains to be seen A possible alternative to the introduction of alien variation by translocation might be to substitute a pair of alien chromosomes for a pair of wheat chromosomes This can be achieved by sequences of hybridizations between various aneuploid lines A Wienhues (Germany) described the substitution of *Agropyron* pairs for wheat pairs, and B C Jenkins (Canada) the substitution of *Secale* pairs for wheat pairs There was some discussion as to whether an alien pair could only be expected to replace a homoeologous wheat pair with which it had a common evolutionary origin, but insufficient evidence is as yet available to clarify the issue

H Kihara (Japan) described the production of male sterile forms of wheat by the substitution, by backcrossing, of the wheat nucleus into the cytoplasm of various other species, while J. W. Schmidt and V A Johnson (United States) outlined the discovery and use of genes that restore male fertility This work clearly indicates that the commercial exploitation of hybrid wheat may soon be possible It is not yet known whether there will be sufficient hybrid vigour to produce an increase in yield adequate to cover the increased cost of seed production—an increase in yield of about 20 per cent is estimated to be necessary Moreover, many hold that genotypes giving the greater yields should be fixable by selection in the derivatives of hybrids showing heterosis, although this ignores the probable difficulties of breaking some repulsion linkages in a few generations of recombination

The accumulation of conventional linkage data has been very slow in *T aestivum*, but E R Sears (United States) demonstrated how this could be remedied by using telocentric chromosomes to estimate linkage distances between single major genes and the centromere In this way some linkage information has already been obtained about chromosomes 3*B* and 6*B* Although aneuploidy has been of the utmost value in the genetic analysis of *T aestivum*, as was demonstrated by R I Larson (Canada) and J Kuspira (Canada), it has been less useful with the tetraploid wheats—presumably because they have fewer duplicate loci and are consequently intolerant of aneuploidy However, M Noronha-Wagner and T Mello-Sampayo (Portugal), working with *T durum* (a tetraploid), have been able to hold the nullisomic or monosomic condition for some chromosomes provided that there was compensation for the deficiency by extra dosage of the homoeologue in the other genome Of interest was the fact that there is some tendency for homoeologous chromosomes to pair at meiosis when in the monosomic–trisomic condition, despite the normally diploid-like meiotic behaviour of the tetraploid wheats

R Riley (Great Britain) provided confirmation of the hypothesis that homoeologous meiotic pairing and recombination occur when the restricted pairing specificity, imposed by the activity of chromosome 5*B*, is relaxed Moreover, the relaxation of specificity permitted the incorporation in wheat chromosomes, by recombination, of segments from the chromosomes of other species in a manner that was otherwise impossible In a parallel investigation of the same system, M Okamoto (Japan) had selected induced mutants that altered the activity of chromosome 5*B* in the regulation of meiosis He indicated the likelihood that such mutants could be stabilized in stocks that would have considerable potential value in practical breeding work

C F Konzak (United States) and R E Scossiroli (Italy) considered the value of induced mutations in wheat breeding and both emphasized the particular value of changes that affect quantitative characters. The genetic analysis of the protein quality of the grain was discussed in relation both to the physical causes of the different properties of flours and of their assessment in small-scale tests Finally, no ultimate verdict was possible by J Mac Key (Sweden) or M. S. Swaminatham (India) on the true nature of the genes on the pronounced effects of which are erected many taxonomic categories in *Triticum.*

Wheat is, with rice, the principal food crop of the world and to a considerable degree its present pre-eminence is owed to its extreme genetic versatility. The second International Wheat Genetics Symposium marked a further advance in our comprehension of this versatility and confirmed that the cytogenetic structure of *T. aestivum* continues to be the subject of intensive investigation in many parts of the world. Indeed there were several workers from the U S S R. present, all of whom treated the genetics of wheat from the Lysenkoist point of view. That some of the fundamental cytogenetic investigations of the past have already been turned to practical account is gratifying, and promises well for the future of the more thorough understanding that we are now attaining.

RALPH RILEY

TEACHING OF BIOLOGY

By DR GARTH UNDERWOOD
University of the West Indies, Trinidad

THERE has been considerable recent discussion of the effect on the teaching of biology of the growing field in which the traditional distinction between botany and zoology is disappearing Catcheside[1] and Darlington[2] put forward proposals for teaching the biological sciences which bring together microbes, plants, animals and man Their proposals, however, still retain the divisions of biology into such aspects or "disciplines" as genetics, ecology, taxonomy and physiology

These several biological disciplines grew up separately at a time when there was no general body of biological theory to relate them to one another They started as inductive sciences Investigators at this stage were trying to ask the right questions and to devise techniques to obtain the answers Discussion of techniques figured largely in the discussion of the results and theories were fragmentary and controversial The attention of each investigator was understandably turned in on his own field, at this stage other fields had little to offer him. This was the period during which there developed a deep gulf between field and laboratory This period also produced some biologists who now look rather strange the biochemist to whom a rat was the animal reared in the room at the end of the corridor, the taxonomer in a dusty museum whose study was, as Philip Gosse pointed out a hundred years ago, not so much biology as necrology, the geneticist who had no idea where to start looking for a *Drosophila* in the field, the physiologist who did not know the systematic relationships of (or sometimes even how to identify) the organisms which he studied Parenthetically it may be remarked that this is perhaps why the origin of new adaptive features remains an obscure problem[3] An account of the origin of a new feature would require a knowledge of its structure and function, of its genetical and developmental determination and of the habits and ecology of the organism; in other words, a synthesis of the disciplines which have usually been studied separately.

I would like to offer some suggestions aimed at achieving a greater merging of these several aspects of biology as well as a merging of plant and animal studies We are still far from being able to proceed in a series of deductive steps from simple molecules to twentieth-century human society, however, we can now suggest the lines along which large areas of biology may be linked together Organisms have been most studied as individuals Bacteria, protists and higher plants and animals represent three grades of individual organization Genetics has an important contribution to make to the discussion of cellular structure and function and, in the case of the higher organisms, to the organization of the development of the individual Consideration of the higher organisms leads to the structural and physiological differentiation of tissues and organs Some topics such as animal behaviour and locomotion remain frankly zoological Such relationships as that between insect and flower, host and parasite, mimic and model or between the bees in a hive can be looked on as special cases of relationship between individuals

Studied as individuals organisms can, however, be understood only in part This leads us to the study of populations A consideration of populations involves such topics as population dynamics, territory, living space, overt and concealed polymorphism, natural selection, geographic variation, reproductive isolation and the nature of species The rules for dealing with lower taxonomic categories follow quite naturally on discussion of species A number of special relationships already examined at the individual level may now be studied at the population level, insect and flower, host and parasite, mimic and model are viewed from a different angle

From the relatively simple relationships between populations just mentioned we can proceed logically to the complexity of major communities Here a number of ecological ideas such as niche, trophic-level and pyramid of numbers can be introduced The grades of individual organization and the metabolism of plants and animals, already examined, now take on a new significance in the new context From the examination of community structure we may proceed to a review of the adaptive diversity of plants and animals, a review of the niches which are actually filled

Living organisms will now have been investigated as individuals, in populations and as members of communities It remains for the student to recognize that recent living organisms have a long evolutionary history behind them that the present cannot be fully understood without reference to the past This leads us to consider temporal changes in the physical environment, the fossil record and the evidence on which phylogenetic schemes are constructed, here morphology has a major place and biogeography and the comparative approach to physiology, biochemistry and cytology can be considered To present the findings of phylogenetic studies, rules for the treatment of higher taxa are necessary. The actual results of phylogenetic studies can now be reviewed in outline in the form of a historical survey of plants and animals and a classification of recent organisms

Man will have furnished good illustrative material for some of the earlier parts of the course such as the nature of gene action, natural selection and population dynamics An important part of the general educational value of a biology course, however, lies in the light which it throws on man himself Such a course could well conclude by taking a look at man in the light of biological knowledge the origin of man, genetics and physical anthropology, the domestication of plants and animals, population studies, food supplies, disease, radiation hazards and, finally, man in space

The conventional 'packaging' of biological teaching made it easier for the student to leave out important aspects genetics because he had trouble with ratios, physiology because his experiments never seemed to work, ecology because the lectures of Dr *X* were dull A greater merging of these branches of biology will help to create an atmosphere in which ecology or genetics could no more be left out than the cell theory or water and carbon dioxide The greater maturity of the branches of biology means that the presentation of conclusions of general significance is less dependent on an explanation of techniques, there is therefore less chance that a student will fail to appreciate important conclusions because of his difficulties with the way in which the conclusions were reached Biology can thus be presented as a subject which has matured to the point that every branch has some bearing on every other branch, the student can be brought up to feel that the physiologist who knows nothing of genetics, the taxonomer who is unacquainted with the science of ecology and the biochemist who is ignorant of evolutionary theory are biologically uneducated

[1] Catcheside, D G , *Nature*, **197**, 427 (1963).

[2] Darlington, C D , *Nature*, **199**, 117 (1963)

[3] Berry, R J , and Searle, A G , *Proc Zool Soc London*, **140**, 577

3 : 5-DIHALOGENO-4-HYDROXYBENZONITRILES AS HERBICIDES

3 : 5-Dihalogeno-4-hydroxybenzonitriles : New Herbicides with Molluscicidal Activity

2 4-DINITRO-6-METHYLPHENOL (DNC) is a herbicide with high contact phytotoxicity which has been used in agriculture for many years, especially for the destruction of broad-leaved weeds in cereal crops The activity of this molecule is specifically related to the presence of $-NO_2$ groups in the 2- and 4-positions to the –OH group though the 6-methyl grouping can be replaced by hydrogen or certain other alkyl groups without appreciable loss of phytotoxicity

I have investigated the capacity of DNC and other aromatic nitro compounds to form intermolecular complexes with amines and hydrocarbons[1,2] In the first of these investigations[1] it was established that the $-NO_2$ groups in 1 3 5-trinitrobenzene can be replaced by –CN without destroying the capacity to form molecular compounds, though the effect of the $-NO_2$ group in promoting compound formation was found to be greater than that of –CN It was concluded from this work that the inductive effects exerted by these groupings in the benzene ring are similar in magnitude

Such considerations led me to make a systematic investigation of the effects on herbicidal action of replacing the $-NO_2$ groups in DNC by –CN A number of derivatives of 2- and 4-hydroxybenzonitrile were therefore synthesized and their activity as weed-killers was assessed by standard methods In general, poor performance only was shown by derivatives of salicylnitrile, but a number of compounds derived from 4-hydroxybenzonitrile were shown to possess outstanding herbicidal activity The most promising of these, first tested in June 1959, was 3 5-diiodo-4-hydroxybenzonitrile (later referred to as ioxynil)

The 3 5-dibromo analogue (broxynil) also showed high activity but the corresponding dichloro-derivative was much less active The water-soluble sodium, potassium and ammonium salts of ioxynil and broxynil and certain of their esters were prepared during 1959–60 and tested against a range of crop and weed species It was soon established that they are capable of destroying the tissues of many plants when applied as a spray at concentrations as low as 250 p p m The effect is entirely that of a contact herbicide and no systemic activity has been observed The compounds become inactivated by contact with soil Against most plant species the phytotoxicities of ioxynil and broxynil are considerably greater than that of DNC, but, as with this compound, the differential wetting concept enables them both to be successfully used for destroying broad-leaved weeds in certain monocotyledonous crops Ioxynil was tested for me by P H Rosher in Trinidad against a range of tropical crop and weed plants in December 1959 Most species were seriously affected or killed by a spray containing 1,000 p p m , grasses, millet and maize showing the least damage Since that time a number of trials have been carried out by P Cozens, Miss L MacDonald and D H Bartlett at this College both with ioxynil and broxynil The former has always proved to be the more effective compound In two trials designed to examine their capacity for the destruction of potato haulm, both substances gave excellent results

Investigations of the mode of action of these compounds are being carried out by my colleagues and me and it has now been established by M Kerr that the substances are strongly active in uncoupling oxidative phosphorylation within plant tissues Since this property is shown also by DNC and by pentachlorophenol, both of which are good molluscicides, steps were taken to examine ioxynil and broxynil for their capacity to kill snails This work was carried out for me by J Duncan, of the Tropical Products Research Unit, Department of Technical Co-operation, who has consistently shown ioxynil to have an LC_{50} of about 0 5 p p m and broxynil about 2 p p m when tested as their sodium salts against *Australorbis glabratus*, a snail host of *Schistosoma mansoni*, the causative agent of intestinal bilharzia Against another species, *Biomphalaria sudanica*, also a snail host of *S mansoni*, the LC_{50} was $<$ 1 0 p p m for ioxynil and $<$ 3 0 p p m for broxynil Ioxynil has also been shown in immersion tests to be some ten times more effective than metaldehyde against several mollusc species by I F Henderson and P F Newell at Rothamsted Experimental Station The compound, however, has no attractant properties A small-scale test of ioxynil for controlling water hyacinth (*Eichhornia crassipes*) has been carried out in Egypt with promising results The destruction of this weed, which impedes water movement in the irrigation canals, is an urgent problem, and supplies of the compound have been sent to Egypt for further trials to be undertaken The possibility of destroying water hyacinth and controlling the vector of bilharzia simultaneously with ioxynil would appear to warrant investigation

R L WAIN

Wye College
(University of London),
Near Ashford, Kent

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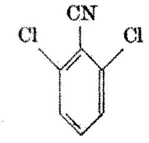
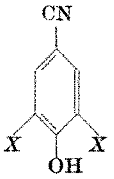
Herbicidal Action of 3 : 5-Dihalogeno-4-hydroxybenzonitriles

THE isolation of the plant growth regulator indolyl-3-acetonitrile from cabbages by Henbest *et al* [1] in 1952 directed some attention on the role of the nitrile group, more particularly when it was later shown by a number of workers that this molecule was active *per se* and that activity was not solely dependent on the conversion of the nitrile into the carboxylic acid Since then several herbicidally active nitriles have been described, for example, diphenylacetonitrile[2], triazine nitriles[3], and more particularly 2 6-dichlorbenzonitrile[4] (I) It seems clear that there is no rational or predictable relationship between the spectrum of herbicidal activity (if any) of the aromatic nitriles and the corresponding carboxylic acids. Nor indeed has any clear pattern of structure-activity relationship yet emerged in this class. This topic has recently been reviewed by Barnsley and Yates[5].

A considerable number of nitriles was accessible in our laboratories as a result of earlier chemotherapeutic work on amidines, and a search for useful herbicidal activity among these was, therefore, attractive experimentally, albeit empirically. One outcome of this work has been to uncover a high level of activity in 3 : 5-dihalogeno-4-hydroxybenzonitriles (II).



(I) dichlorbenil



(II)

X = I M and B 8,873
X = Br M and B 10,064
X = Cl M and B 9,895

Although some formal chemical resemblance exists between I and II in that each contains a benzene ring, an aromatic nitrile and two halogen atoms, it seems that the type of herbicidal activity is so different as to imply fundamentally different modes of action. Thus I is active as a pre-emergent herbicide whereas the 4-hydroxy benzonitriles so far examined possess much greater activity when applied as a foliar spray. Moreover, the position of the halogen atoms in relation to the nitrile group which is required for maximum activity is quite different. Thus, if the halogen atoms in I are in the 3 : 5 positions or in II are in the 2 : 6 positions the nitriles are very much less active. That the nitrile group is an important part of the molecule is shown by the twenty-fold diminution of activity of the corresponding 4-hydroxy 3 : 5-dihalogeno benzoic acids.

Field trials of M and B 8,873 and M and B 10,064 as a foliage spray are in progress on cereals and some leguminous crops. The results so far available confirm the earlier work in the glass-house that most annual weeds, including the 2,4-D-resistant weeds, are controlled at low dose rates.

The details of recommendations for commercial use must await the outcome of the further investigations, now in progress, into practical applications of these compounds in a variety of crop-weed situations. Residue examinations, with the aid of radioactively labelled material, are in hand and the results so far indicate that there is a fairly rapid disappearance of both compounds from treated wheat, oats and barley.

K. CARPENTER
B. J. HEYWOOD

May and Baker, Ltd.,
Research Laboratories,
Dagenham, Essex, and
Agricultural Research Station,
Ongar, Essex.

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SOIL COMPACTION AND MANGANESE DEFICIENCY

By DR. J. B. PASSIOURA and PROF. G. W. LEEPER
Department of Agricultural Chemistry, University of Melbourne

MANGANESE deficiency disease, best known as the characteristic 'grey-speck' of oats, has long been a scientific puzzle. It occurs characteristically on soils in the *p*H range of 6·5–8·0 (sometimes outside), and characteristically (though not exclusively) on sandy and on peaty soils. Two reasons have been given for the unavailability of manganese to oats and other sensitive plants growing on these soils. Firstly, that practically all the manganese has been biologically oxidized to the higher insoluble oxides (collectively denoted here as MnO_2). Secondly, that the manganese retains its valence of two but is held in such a strong covalent link by organic colloids that the root cannot obtain its necessary supply of an ounce per acre. This second reason does not concern us here, and we have produced much theoretical and experimental evidence against it elsewhere[1].

The first explanation, depending on the insolubility of MnO_2, will not do either. Against it we have the many successes reported in curing 'grey-speck' with finely divided insoluble oxides[2] including the product of microbial oxidation[3]. Sensitive plants growing on neutral and alkaline soils may be healthy at very low contents of exchangeable manganese so long as there is ample MnO_2 extractable with the gentle reducing agent quinol[4]. However, there are still many exceptions to this last relation. Some neutral soils are healthy in spite of a low content of quinol-soluble manganese, and some, especially organic soils, are deficient in spite of a high content. Now organic soils are typically loose and open, and their exceptional behaviour suggests that the availability of manganese may be related to the degree of compaction.

We have compacted two soils on which 'Algerian' oats (a local variety) showed severe 'grey-speck', and have found, both in pots in the greenhouse and in plots in the open, that the disease was mostly cured, with up to a 50-fold increase in grain production. The two soils were: (*a*) the fine sandy loam from Mount Gambier, South Australia, a young soil[5] formed on calcareous basaltic tuff, *p*H 7·4; its bulk density was 0·85 uncompacted, 1·0 compacted: (*b*) the sandy loam of the grounds of the University of Melbourne, an imported soil[6] which has been *in situ* for more than fifty years, *p*H 7·4; bulk density 1·2 uncompacted, 1·5 compacted. Even in its compact state this soil was permeable, and in the plot test on the morning after a rain of 4 cm there was no water lying on its surface.

The pot tests were done either in an unheated glasshouse or under a glass canopy in a bird-proof cage, using 15 cm × 20 cm glazed ceramic pots in duplicate. For compacting, successive lots of 700 g moist soil were added to the pot and given a set number of 2 kg × 10 cm blows with a Proctor hammer, namely 10 for the University soil, and one lot of 15 and one of 25 for the Mount Gambier soil. Water was added to keep the pots to constant weight, using a figure found to equal the percentage of water at *p*F 2·5.

With the University soil in pots, the grain yield of Algerian oats (a sensitive variety) was 0·04 g on loose and 2·3 g on compacted soil; that of Palestine oats (a tolerant variety) was 1·3 g on loose and 9·1 g on compacted soil. On the plots in the open, the general effect of compaction was healthier growth and three weeks advance in heading. Quantitative results are not quoted since germination was damaged by compacting on one of the six square-yard plots.

Two soils from Mount Gambier were used, *C*, with no manganese added at any time, and *M*, coming from the same source, but having been mixed 27 years previously with a synthetic reactive MnO_2 at the rate of 80 p.p.m. of manganese. This residual manganese was almost useless on the loose soil (its initial good effect having disappeared after about ten years), but it was valuable on the

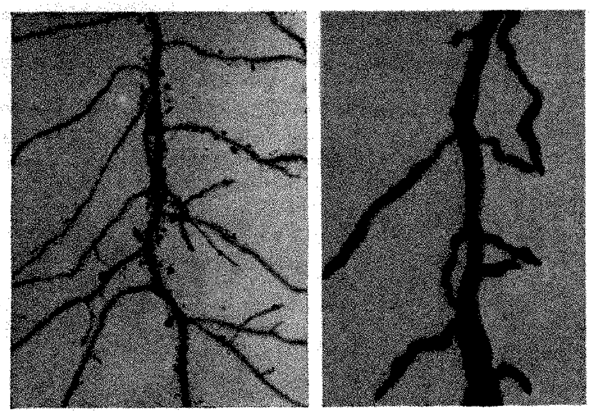

Fig. 1. Roots of Algerian oats growing on University soil. Left, loose soil; right, compact soil (both *c.* × 2)

compact soils. Two degrees of compaction were used, as here. With the greater degree of compaction (*B*) there was much more growth than with the lesser (*A*), both of Algerian on soil *M* and of Palestine on the control soil (Table 1). While the bulk density of *B* was only 4 per cent greater than that of *A*, the resistance to a penetrometer needle was almost twice as great.

Table 1. YIELD OF DRY MATTER (g) OF OATS GROWN ON MOUNT GAMBIER SOIL, WITH AND WITHOUT COMPACTION

Soil	Oat variety	Treatment of soil Loose	Compact *A*	Compact *B*
C	Algerian	0·55	1·05	0·92
C	Palestine	1·87	2·13	4·16
M	Algerian	0·91	2·66	3·57
Bulk density		0·85	0·99	1·03

The distribution of pores in the potted soils of Mount Gambier was measured by pressure-plate (Table 2). The loose and compact soils have much in common, the main difference being in the largest pores of all (drained at 10 cm tension, equivalent to 300μ diameter). The two degrees of compaction give almost identical curves; the difference between these two soils is in their shear strength, not in their aeration. The aeration of both appears from Table 2 to be adequate.

The conventional explanation for thus overcoming the deficiency would be as follows. The more compact soil is supposed to be less freely aerated, so that more carbon dioxide accumulates, so lowering the *p*H of the soil solution; and less oxygen is available for the normal microbial oxidation which converts the mobile manganous ion into MnO_2. This explanation is mistaken. First, the manganous ion extractable with HNaCaEDTA at *p*H 8 after Beckwith[7] was 0·3 p.p.m. on loose and compact alike. Secondly, the *p*H of the soil solution is not lowered sufficiently to account for the effect; when carbon dioxide is circulated through the Mount Gambier soil in amounts such as to reach twice the concentration (0·5 per cent) found in the corresponding compact soil, the plants show no improvement. Thirdly, the diffusion of oxygen in the compact University soil has been measured using a platinum electrode after Lemon and Erickson[8] and is no less than in the loose soil. Fourthly, the bulk density of the Mount Gambier soil after compaction was only 1·0. Further, all such explanations ignore the fact that manganese deficiency is typically a disease of the wetter and cooler climates of the world, where aeration might generally be expected to be less than in the drier and warmer climates.

Table 2. AMOUNTS OF WATER HELD AT VARIOUS TENSIONS BY MOUNT GAMBIER SOIL, LOOSE AND TREATED TO LOWER DEGREE (*A*) AND HIGHER DEGREE (*B*) OF COMPACTION. g WATER PER 100 g DRY SOIL

Tension cm water	Loose	Compacted *A*	*B*
0	70	63	64
10	60·6	57·0	57·4
40	59·4	57·2	57·6
100	41·9	41·4	42·5
340	32·9	31·5	31·7
1,000	29·4	26·8	26·6

A more satisfactory explanation follows from the theory that the reserve of available manganese in a neutral soil consists of reactive MnO_2, which is insoluble in water but capable of rapid dissolution by mild reducing agents such as neutral quinol[4]. We suggest that the root itself acts by a 'contact reduction', analogous to the 'contact exchange' proposed for cations in general by Jenny *et al.*[9], and that compaction works by increasing the area of contact. As is shown in Fig. 1, the roots growing in the compact soils were short and stubby as compared with those in the loose soils. Their irregular outlines indicate moulding by the tight-fitting soil. They also had far fewer hairs; this observation is at first surprising, but what seems to matter is the total area of contact rather than the existence of numerous points of contact. The total dry weight of root was always greatly superior in the compacted soils, being proportional to the above-ground weight.

Occasional observations have previously been made of better growth after rolling[10,11], but have been explained in the conventional way which we think is mistaken. Grey-speck of oats has always been known to be a patchy disease. This can be easily understood if it depends mainly on the compaction of the soil.

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ISOLATION OF MALTOTRIOSE AND MALTOTETRAOSE FROM STARCH HYDROLYSATES

By A. G. CLARK

Beecham Food and Drink Division, Ltd., Beecham House, Great West Road, Brentford, Middlesex

AND

K. R. L. MANSFORD

Beecham Research Laboratories, Ltd., Brockham Park, Betchworth, Surrey

IN order to carry out gut perfusion investigations on the components of liquid glucose it was necessary to isolate dextrose oligosaccharides in 50-g quantities. The charcoal-'Celite' column chromatographic technique of Whistler and Durso[1] was applied to selected starch hydrolysates.

Analysis of commercial- and laboratory-made starch hydrolysates by the technique of Patterson and Buchan[2] revealed that the richest source of maltotriose was a laboratory-made starch hydrolysate using salivary amylase and maize starch. The richest source of maltotetraose was obtained by fermenting liquid glucose (high dextrose equivalent) with *Saccharomyces cerevisiae*. By these means the starting materials were prepared so as to contain about 30 per cent w/w of each oligosaccharide respectively.

The maltotriose-rich material was prepared as follows. Human saliva was collected, during stimulation from chewing paraffin wax, and centrifuged to remove the mucin. The saliva was stored at 0° C under toluene and used without further purification. About 15 ml. saliva was allowed to act on 200-g maize starch for 24 h at 30° C and *p*H 4·8. The reaction was stopped by boiling, followed by centrifugation to remove insoluble dextrins. The cyclone concentrated hydrolysate was stored at 0° C until required for application to the charcoal column. To prepare the maltotetraose rich material a seed culture of *Saccharomyces cerevisiae* (N.C.Y.C. 240) was sub-cultured in corn (maize) steep liquor (20 ml.), dextrose (50 g), and water (to 1 l.). The sub-culture was incubated with 100-ml. portions of high dextrose equivalent liquid glucose (special enzyme syrup prepared by Manbré and Garton, Ltd.) (200 g) containing urea (1·1 g), dipotassium hydrogen phosphate (1·1 g), potassium dihydrogen phosphate (0·7 g), magnesium sulphate (0·7 g), calcium chloride (0·2 g), and water (to 2 l.), at 20° C and *p*H 6·9. After 150 h the fermentation liquors were filtered to remove the yeast cells and cyclone concentrated prior to storage at 0° C.

The 36-in. column used for the separation work was composed of 460 g of charcoal (supplied by Sutcliffe, Speakman, Ltd., Leigh, Lancs., quality 130 in air-tight canisters) and 230 g of 'Celite', Johns-Manville grade 545, supported in a glass pipe of 2-in. diameter, and resting on a grade 2 porosity sintered glass disk. (The column is fabricated from standard Q.V.F. parts.) The whole column was surrounded by a water jacket, the connectors of which were coupled to the external circulator pump of a temperature-controlled bath, the temperature of which could be varied between 20 and 60° C. The top end of the column was closed by a reducing piece, held in place by standard flanges, and topped by a glass tube entering through a *B*24 standard joint secured by springs. The glass tube reached near to the surface of the carbon column. This tube was connected by a Y-piece to the lower liquid connexions of the two solvent reservoirs, containing the eluant, which were immersed in the water of the temperature-controlled bath. The gas connexions to these reservoirs were connected to a compressed air supply at 5 lb./in.^{2} and to a manometer and bubbler. The manometer reading served to confirm the gauge reading and the bubbler gave visual confirmation of a continuing gas supply. The lower end of the column was closed by a *B*50 standard joint fitted with a sintered glass disk which supported the column. This was in turn supported by a slotted panel cushioned with rubber. Below this section was a *B*19 standard joint supported by springs and fitted with a glass tap. The outlet of this tap led to a chain of fraction cutting T-pieces which operated with hydrostatically controlled non-return valves. These were fitted to litre collecting flasks, and the last flask was open-ended.

The column with the bottom parts well greased and securely connected was prepared as follows: the charcoal and 'Celite' were intimately mixed to a homogeneous powder, 2 l. of boiling distilled water were added with stirring, and the whole was thoroughly mixed to a homogeneous slurry. This was poured into the column and any entrained air was removed by agitating with a glass drumstick-shaped plunger, which was worked vertically to give a smoothly mixed slurry in the column. As more slurry was added, the plunger was removed and finally the last few portions of slurry were added as the column settled. When the column was full, the top fittings were well greased and securely connected and cold boiled distilled water passed through until at least 5 l. were collected. The top surface of the column should be just below the end of the main glass pipe. This surface was covered with a circular wire gauze disk supporting a filter paper disk.

The column was loaded by allowing the water-level to fall until it was immediately above the filter paper disk. The syrup or sugar solution containing about 25 g carbohydrate was added, by way of the opened standard joint, and allowed to drain into the column. As the level of the surface layer of syrup approached the column surface it was washed in with successive small amounts of boiled distilled water. The standard joint was then replaced securely and the eluant passed through the column at 5 lb./in.^{2} above atmospheric pressure. The eluant composition and the temperature were varied in accordance with requirements. The litre fractions were successively analysed by the orcinol method[3] for the presence of carbohydrate.

In order to elute maltotriose from the maltotriose-rich starting material, dextrose and maltose and any other mono- and di-saccharides were eluted simultaneously with 5 per cent aqueous ethanol at 40° C. When there was no further trace of carbohydrate in the eluate, maltotriose was eluted with 8 per cent aqueous ethanol at 40° C. Under these conditions the flow rate was about 450 ml./h and the maltotriose was collected in about 12 l. of eluate.

To obtain maltotetraose from the maltotetraose-rich material the lower oligosaccharides were eluted with 10 per cent aqueous ethanol at 40° C. Maltotetraose was then eluted with 12 per cent aqueous ethanol at 40° C. The elution of maltotetraose under these conditions was slow, and in order to complete the separation and preparation of dry material in a weekly cycle an attempt was made to accelerate the procedure by raising the temperature of operation to 50° C. This increased the flow rate to 600 ml./h, but the separation was incomplete and the early and late fractions had to be rejected because of traces of lower and higher oligosaccharides. The loss of maltotetraose involved was about 10 per cent. It was necessary to keep strict paper chromatographic control of the fractions and this was achieved with an overnight run on Whatman No. 1 paper in *n*-propanol, ethyl acetate, water (7 : 1 : 2) or more rapidly (3–4 h) on Whatman No. 1 paper in *n*-butanol, ethanol, water (2 : 1 : 1) at 60° C. The solution of isolated oligosaccharide was concentrated to about 200 ml. in a cyclone evaporator boiling at 20°–24° C under reduced pressure obtained by a water pump. The rate of evaporation was about 1 l./h and the concentrated eluate was freeze-dried.

Up to 30 per cent of the carbohydrate applied to the charcoal-'Celite' column was lost, apparently by irreversible adsorption. Even after desorption with hot 95 per cent ethanol complete recovery was not achieved. For this reason, and because the flow rate gradually decreases, the adsorbent was discarded after each run and the column reformed from fresh charcoal and 'Celite'.

Although the maltotriose and maltotetraose obtained as described were sufficiently pure for the purpose required there were isomeric oligosaccharides present in trace amounts, which were only detected by paper chromatography using Whatman 3*MM* paper and applying a 10 mg load. These substances appeared during the enzymatic treatment of the starch hydrolysate, whether by amylolysis or yeast fermentation. Maltotriose and maltotetraose can be obtained pure from commercial liquid glucose, but the yields will be very much smaller. It is possible to separate the maltotriose and maltotetraose from the contaminating oligosaccharides by the method of Barker *et al.*[4] using a charcoal column in presence of borate, but this technique has not been applied on a large scale. Another possible source of maltotriose is from the action of *Aureobasidium pullulans* on the polysaccharide pullulan[5], prepared from the organism *Pullularia pullulans*[6], which under suitable conditions hydrolyses this material specifically to maltotriose only

We thank Mr R K Opie and Mr A B Leah for assistance and Dr M Richards of the Microbiology Department at Brockham Park, who carried out the fermentation work We also thank Dr G Harris of the Brewing Industry Research Foundation for advice on the enzymatic and fermentation procedures and for arranging the supply of yeast from the N C Y C

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ENZYMATIC ACTIVITY OF MOUSE HISTOCOMPATIBILITY ANTIGEN PREPARATIONS

By Dr A R. SANDERSON and Dr. D. A. L. DAVIES

Microbiological Research Establishment, Experimental Station, Porton, Nr Salisbury, Wilts

IT would be of interest to know what function transplantation antigens may serve in the cell and helpful for further purification and interpretation of the mechanism of graft rejection It is generally assumed that the same substance is being detected or measured by different test systems, for example, hastening skin-graft rejection, inducing hæmagglutinins or cytotoxins, inhibiting hæmagglutination or cytotoxic antibody and enhancing tumour growth in allogeneic mouse strains It appears that a more complete or undegraded form of material is required to affect skin grafts but the specificity can be followed further by serological methods, which have revealed that the substances are lipoproteins associated with the insoluble fraction of cells[1-3]

Fully active preparations have yet to be produced in a demonstrably homogeneous form; histocompatibility antigens can be prepared free from normal mouse serum components and cell-bound species-specific antigens[4], but evidence for physical homogeneity is not convincing It is not difficult to obtain a single schlieren band in the ultracentrifuge at alkaline pH or in the presence of some dispersing agent, but similar patterns can be obtained with preparations known from other evidence to be relatively crude So far preparations are probably mixtures of lipoproteins from various sub-cellular particles and membranes, some of which are likely to be inactive Such mixtures would be difficult to resolve on account of their essentially insoluble form, this suggests that the appropriate sub-cellular particle or fraction would make a more suitable starting material for purification The appropriate fraction is elusive, however, activity was largely in the nuclear fraction but also spread over mitochondrial, 'fluffy' and microsomal fractions when tested by tumour enhancement[5] By inhibition of cytotoxins, activity followed lysosomes, as indicated by acid-phosphatase activity[6], by skin-graft rejection, the microsome fraction was active[7], but in this case other sub-cellular fractions were not tested By hæmagglutinin induction microsomes and 'fluffy' layer are most active, but some activity is associated with nuclei and mitochondria though not with the soluble fraction[8] The suggestion[2] that activity is associated with external cell membranes the fragments of which may be distributed over a range of g time standard sub-cellular fractions has to be seriously considered

The preparation of high H-2 histocompatibility specific activity used here was prepared from ascitic fluid[1] and has these properties hastens skin-graft rejection[8], induces hæmagglutinins and cytotoxins[4,8,9] inhibits H-2 hæmagglutination and cytotoxic antibody[9,10] and carries non-H-2 specificity[11] Similar preparations from mouse leukæmic cells carry 'X' tumour specific antigenicity[12]

The lipoprotein nature of the material and its association with insoluble cell fractions suggested an examination for enzymes which are frequently associated with membranes Electron micrographs (× 62,000) have shown precise localization of nucleoside phosphatase in membranes[13], and the presence of 'ATPase' activity in our histocompatibility antigens was therefore not unexpected. In Table 1 are shown the activities which have been determined, it will be seen that nucleoside diphosphatase and triphosphatase levels are high compared with the other activities tested There is no consistent preference for any one of the four common ribonucleotide bases when used as substrate at the tri-, di-, or mono-nucleotide-level The triphosphatase activity figures are inaccurate to the extent that inorganic phosphate release was actually measured and the other product of hydrolysis, the nucleoside diphosphate, is then broken down (at a slower rate) with further inorganic phosphate release This error is minimized under the conditions used, where only a few per cent of the substrate was hydrolysed in order to avoid product inhibition, inaccuracy would only be significant with dissimilar K_m values

Table 1 PHOSPHATASE ACTIVITIES OF HISTOCOMPATIBILITY ANTIGEN PREPARATIONS

Substrate	pH	Activity*		
Adenosine 5′-phosphate	8 4	0 5		
Cytidine 5′-phosphate	8 4	0 8		
Guanosine 5′-phosphate	8 4	1 0		
Uridine 5′-phosphate	8 4	0 5		
Adenosine 3′-phosphate	8 4	0 6		
Adenosine 2′-phosphate	8 4	1 3		
Nicotinamide adenine dinucleotide	8 4	0 9		
Adenosine 5′-pyrophosphate	8 4		14 2	
Cytidine 5′-pyrophosphate	8 4		7 0	
Guanosine 5′-pyrophosphate	8 4		5 2	
Uridine 5′-pyrophosphate	8 4		7 0	
Adenosine 5′-triphosphate	8 4			20 0
Cytidine 5′-triphosphate	8 4			19 0
Guanosine 5′-triphosphate	8 4			13 5
Uridine 5′-triphosphate	8 4			15 3
Glucose 6-phosphate	6 2	0 4		
Glycerol 2-phosphate†	8 4	1 4		
Glycerol 2-phosphate‡	5 0	0 1		

Hexokinase was similarly shown to be absent (pH 8 4)

* All activities are expressed in mμmoles H_3PO_4 liberated per min/mg dry wt of enzyme preparation and are averages of several determinations done under optimal conditions of pH and metal ion requirements for the enzymatic activity being followed

† Conditions for alkaline phosphatase

‡ Conditions for acid phosphatase

Using ATP as substrate, the way in which nucleotide triphosphate activity followed histocompatibility activity on purification is shown in Table 2 A proper comparison of the two activities is difficult because one is using a highly accurate enzyme assay and a relatively inaccurate histocompatibility assay, hæmagglutination inhibition, where doubling dilutions give 50 per cent activity increments between adjacent tubes However, it can be seen that in each purification step the fraction having highest histocompatibility activity also has highest enzymatic activity The relatively poor histocompatibility activity of '*TM*' compared with '*SP*' fractions, which have more

Table 2. CORRESPONDENCE OF HISTOCOMPATIBILITY ACTIVITY AND TRIPHOSPHATASE ACTIVITY DURING ANTIGEN PURIFICATION (*C3H/BP8* PRODUCT)

Fraction[1]	Histocompatibility activity*	Triphosphatase activity†
AF	1	1
DP	100	8
DS	0	0
SP	500	33
SS	0	0
*TM*1	200	28
*TM*2	200	31
TT	0	/
TB	0	10

* Determined by *H*-2 hæmagglutination inhibition.

† Determined at optimal *p*H (8·4) and optimal metal ion requirement.

impurity, is probably due to their different states of dispersion[1].

Further properties of the triphosphatase are shown in Table 3. These indicate that the activity resembles that of a mitochondrial[14] and also of a seminal enzyme[15]. Differentiation from the activity described for another mitochondrial enzyme preparation[16] is afforded by the lack of dinitrophenol stimulation and sucrose inhibition, while the insignificance of the sodium and potassium enhancement of hydrolysis distinguishes this triphosphatase from others[17,18]. Finally, neither progesterone nor hydrocortisone[19] exerts any effect on hydrolysis.

Table 3. PROPERTIES OF THE ADENOSINE TRIPHOSPHATASE ACTIVITY

*p*H optimum (*tris*-buffer)		8·4	
Activated by	Mg^{++}	3·4 fold	over metal-free conditions
	Mn^{++}	2·3 fold	
	Ca^{++}	3·1 fold	
	Co^{++}	1·9 fold	
	Fe^{++}	1·8 fold	
Km (ATP)	$4\cdot0\times10^{-4}$M		

Activity unaffected by 2: 4 dinitrophenol, progesterone, cortisol, sucrose, sodium ions, potassium ions.

The *p*H stability, heat denaturation, periodate sensitivity, formaldehyde sensitivity and resistance to trypsin are similar for histocompatibility activity and enzymatic activity although it must be remembered that measurement of the former is subject to the limitations of a test using two-fold dilutions.

The very low acid phosphatase activity (Table 1) does not support the suggestion of an association of histocompatibility activity with lysosomes[6]; on the other hand, our antigen, being obtained from ascitic fluid, cannot correspond to any cell fraction prepared by normal cell disruption procedures in sucrose. Many different nucleotidases have been found in sub-cellular particles and membranes, distinguishable by various criteria. The characteristics may differ from one cell type to another (isozymes) so that our information does not yet indicate a cellular location. Nucleoside triphosphatase, as tested *in vitro*, is a purely destructive activity and presumably reflects a cellular process where an appropriate receptor is available, for example, transferase or permease. The impingement of an immune environment on such vital cell processes in grafted tisue might explain the dramatic nature of histocompatibility responses.

We thank Miss P. D. Marshall for excellent technical assistance.

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HYPERPLASIA OF THE ISLETS OF LANGERHANS IN BREEDER RATS WITH ARTERIOSCLEROSIS

By PROF. BERNARD C. WEXLER and DR. CHARLES T. FISCHER

May Institute for Medical Research, Jewish Hospital of Cincinnati, and Department of Pathology, University of Cincinnati College of Medicine, Ohio

IT has been demonstrated that the glucocorticoids of the adrenal cortex exert a very significant effect on carbohydrate metabolism, that is, increased gluconeogenesis[1–3]. Chronic hyperadrenocorticism will produce diabetes, that is, steroid diabetes[3–4]. The diabetes seen in Cushing's syndrome[5], during pregnancy[6], in cases of excessive steroid treatment[7], or in experimentally induced steroid diabetes[8], does not respond readily to insulin. Further, steroid diabetes is different from pancreatic diabetes in that it is seldom associated with severe hyperglycæmia or ketosis, and the diabetes is often transient, disappearing promptly when the patient is relieved of steroid excess[9]. Despite these specific metabolic differences between steroid diabetes and diabetes mellitus, pronounced islet hyperplasia is found in both conditions[2,9].

The islets of Langerhans of the pancreas become hypertrophied and hyperplastic in diabetes[2], during pregnancy[6] in offspring of diabetic mothers[6], in patients with Cushing's syndrome[5], and in man and animals treated with ACTH or adrenal steroids[3,4,10]. This increase in islet size and number has been associated with increased demand and secretion of insulin. Chronic demand for insulin results in islet hypertrophy, progressive degranulation of the insulin-secreting beta cells, eventual hydropic degeneration and culminates with complete islet cell exhaustion[12,13].

We have been investigating experimental arteriosclerosis, which develops spontaneously in male and female breeder rats after repeated breeding[14–17]. The arteriosclerotic breeder rats also show low glucose tolerance when challenged with a glucose load[18]. These animals also showed evidence of hyperadrenocorticism and fatty infiltration of the liver, which became progressively more pronounced with successive breedings. It was of interest to learn if there were any alterations in the morphology of the islets of Langerhans which could be correlated with the number of breedings and the other pathological changes. Therefore, in the experiment reported here a number of animals were mated and afterwards killed so that a serial investigation could be made of the insular changes occurring in the pancreas of rats after 1–5 breedings. During the course of this experiment, it was found that these animals showed definite islet changes, arteriosclerotic lesions of the pancreatic vessels and pancreatitis.

Young adult males and female Sprague–Dawley rats were actively bred in our laboratory under controlled conditions. The time period for mating, gestation, nursing, and the few days of rest after weaning was kept constant throughout the breeding period. The mating partners were kept together throughout the experiment and were allowed to breed *ad libitum*. Of the initial 200 mating pairs there were 100 pairs which had had a similar breeding history, that is, the day of conception, delivery and size of litter being relatively constant. Most of the male breeders were so debilitated by the time they had sired four litters that it became necessary to kill them before they became further deteriorated. (Deterioration was evidenced by inactivity, loss of appetite, yellowing and loss of fur and the assumption of a humped position.) Male and female breeders were killed when the young were removed at weaning time (21 days after birth). Control animals consisted of virgin males (12 rats) and virgin females (12 rats) killed at the outset of the breeding experiment. Virgin rats of an age comparable to breeders do not develop arteriosclerosis. Further, to control the age factor, a similar group of twelve male and twelve female virgin rats was held, two rats of the same sex to a cage, and then killed as controls with the five-time breeder rats at the end of the experiment. All animals were housed in air-conditioned quarters. They were fed 'Rockland Rat Chow' *ad libitum*. No salt or fat was added to the diet.

Pancreatic tissue was removed and fixed either in 10 per cent buffered neutral formalin (Lillie) or in Zenker's fixative. Paraffin sections were cut at 3μ and stained with hæmatoxylin and eosin and with an aldehyde fuchsin trichrome stain to demonstrate beta cell granulation. Alcian blue, periodic acid Schiff stain, toluidine blue and Hale's stain were used to demonstrate metachromasia and mucopolysaccharides. Von Kossa's stain was used to demonstrate calcium.

To measure the increase in islet size and number with each breeding, sections were made of the pancreas of male and female rats in each breeding period, that is, 1–5 breedings. The number of sections examined microscopically in each case is given in Tables 1 and 2. The total number of islets larger than 25μ in any one diameter, in each section was counted. The long diameters of each of the first eight islets encountered in any given section were also measured and averaged. Finally, the total number of islets which exceeded 250μ in any given section was counted.

Table 1. EFFECT OF REPEATED BREEDING ON THE SIZE OF THE ISLETS OF LANGERHANS IN THE SPRAGUE–DAWLEY FEMALE RAT

	No. of sections*	Average No. of islets per section greater than 25μ	Size of islets in μ (mean ± *S.D.*)	Islets greater than 250μ (per cent)
Virgin controls (90 days old)	5	23·4	139 ± 29	7·7
First breeding	19	26·7	138 ± 34	7·5
Second breeding	14	24·9	136 ± 36	7·2
Third breeding	9	24·1	135 ± 27	7·8
Fourth breeding	15	26·4	156 ± 28	9·3
Fifth breeding	7	29·7	181 ± 36	18·3
Virgin controls (340 days old)	21	23·3	140 ± 28	7·3

* Each section of pancreas taken from a separate animal.

Table 2. EFFECT OF REPEATED BREEDING ON THE SIZE OF THE ISLETS OF LANGERHANS IN THE SPRAGUE–DAWLEY MALE RAT

	No. of sections*	Average No. of islets per section greater than 25μ	Size of islets in μ (mean ± *S.D.*)	Islets greater than 250μ (per cent)
Virgin controls (90 days old)	5	28·2	129 ± 26	8·5
First breeding	22	27·1	151 ± 36	10·6
Second breeding	10	21·9	155 ± 35	11·1
Third breeding	16	25·6	150 ± 35	12·2
Fourth breeding	13	28·1	171 ± 27	11·6
Fifth breeding	—†	—	—	—
Virgin controls (290 days old)	20	27·6	131 ± 25	8·2

* Each section of pancreas taken from a separate animal.
† Insufficient number of surviving animals for adequate sampling.

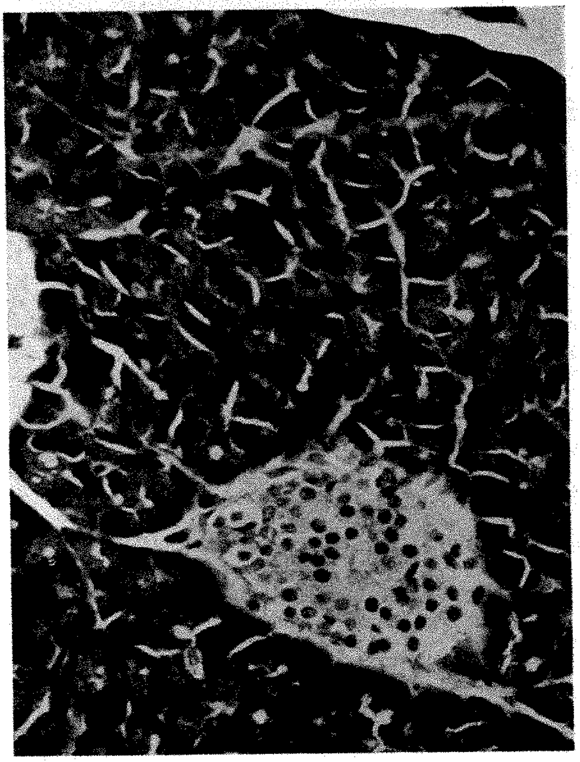

Fig. 1. Normal-sized islet in a virgin female Sprague–Dawley rat at 90 days of age. (Hæmatoxylin and eosin, × 200)

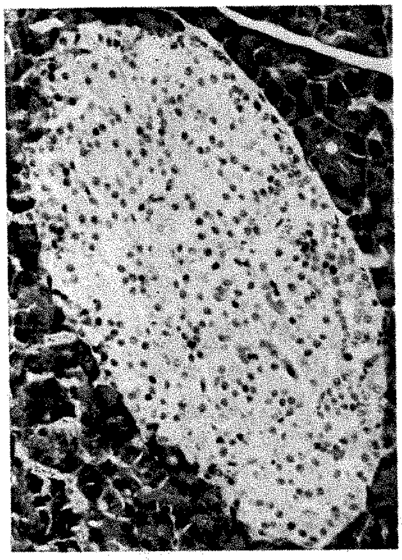

Fig. 2. Giant-sized islet in a female breeder rat (5 litters). (Hæmatoxylin and eosin, × c. 100)

Islet size. There was a definite increase in size and number of the islets of Langerhans in breeder rats compared with virgin controls (Tables 1 and 2). In the female breeders there was little change in average islet size after their first, second and third pregnancies. However, after their fourth and fifth pregnancies there was a definite increase in the number of islets larger than 25μ in each section, in the average size of islets in general, and in the percentage of giant islets, that is, greater than 250μ (Figs. 1–3). Male breeders, by contrast, had sustained (or developed) a marked increase in islet size and in the number of giant islets soon after their first litter. This situation continued throughout the course of the experiment, with an additional increase in the average size of islets after the fourth breeding. The increase in the average size of the islets in male breeders is even more meaningful if one considers the fact that male virgin control rats have smaller islets to begin with than female virgin rats (cf. Tables 1 and 2). That is, breeding effects a proportionately much larger increase in the size of islets in male rats, compared with non-bred males (virgins), than it does when non-bred females are compared with breeders.

Islet cytology. The alpha cells in the islets of the rat have a peripheral location. The more centrally located beta cells are much more numerous than the alpha cells. With continued breeding the alpha cells maintained their peripheral orientation and showed an increased definition of cell outline (Fig. 3). At the same time, the beta cells increased in number and had progressively increased in granulation. During this period of increased granulation, there was evidence of active ductular proliferation associated with generation of new islet tissue (Fig. 4). After the fourth to fifth breeding there was marked degranulation, hydropic degeneration and hyalinization in both male and female breeders. The hydropic beta cells were shown to contain glycogen, using Best's carmine stain.

Pancreatic arteries. Arterial lesions in the pancreatic arteries appear to be an extension of a more fulminating process which is initiated in the abdominal aorta[14]. In both the male and female breeders microscopic evidence of arterial lesions of the abdominal aorta and its branches appeared after the first breeding. Although the lesions in the main abdominal aorta showed involvement of both the intimal and medial layers, becoming more complicated with increased number of breedings, the pancreatic arteries showed only intimal changes (Fig. 5). These intimal changes in the pancreatic arteries consisted of either: (1) discrete endothelial cushions or endothelial proliferation involving a large portion of the intima accompanied by stretching of the internal elastic membrane; or (2) intimal basophilia without endothelial proliferation but accompanied by minor changes in the internal elastica (Fig. 5). Special stains of the basophilic material contained within the delicate intimal layer of the pancreatic arteries revealed that this basophilic material was intensely metachromatic and that it was an admixture of calcium and mucopolysaccharides.

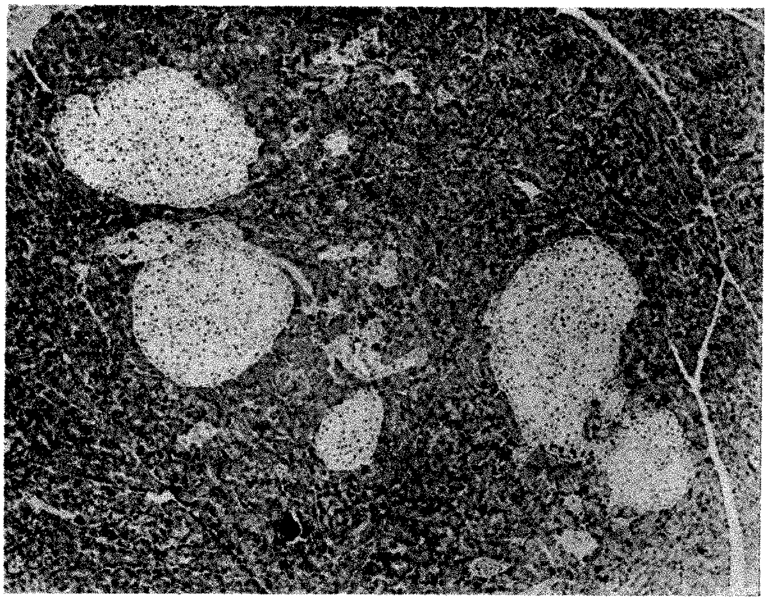

Fig. 3. Islet hyperplasia in a female breeder, bred five times. The peripheral alpha cells in the three giant-sized islets have prominent cell membranes. The beta cells are degranulated. (Hæmatoxylin and eosin, × *c*. 50)

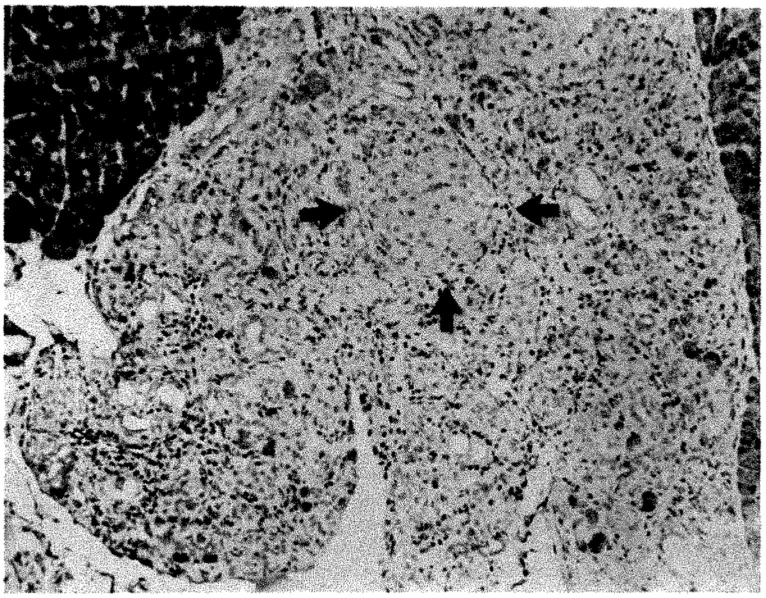

Fig. 4. Ductular proliferation in a male breeder rat showing newly formed islet tissue in the centre of the photo (see arrows). (Hæmatoxylin and eosin, × *c*. 100)

Pancreatitis and mixed islet and ductular proliferation. About the time of the third to fifth breeding, when islet hyperplasia and beta cell degranulation were becoming most pronounced, in many instances active ductular proliferation intermixed with hyperplasia of islet tissue occurred. In all instances, the ductular hyperplasia occurred in association with hyperplastic islet tissue (Fig. 4). The usual single intralobular ducts seen in the pancreas of the rat showed multiple branching. The hyperplastic islet tissue was often found entwined about these newly formed ductules. Islets of all sizes from small to giant size were found within these areas of ductular multiplication. The ratio of alpha to beta cells appeared to be normal in these newly formed islets.

After the fourth breeding, when definitive beta cell degeneration became evident, there was a concomitant reduction of the ductular proliferation which had become progressively more frequent during the second to fourth breeding. After the fourth breeding ductular proliferation began to wane until practically no cases of duct proliferation were encountered in animals bred five times.

Evidence of pancreatitis was found in 40 per cent of the male breeders and 25 per cent of the female breeders. The pancreatitis was characterized microscopically by œdema, necrosis, by marked infiltration of polymorphonuclear cells, some fatty infiltration, and, in more advanced cases, by calcification particularly about the periphery of the pancreas (Fig. 6).

The increase in number and size of islets with breeding shown here is particularly meaningful in that other experiments have shown that breeder rats have low glucose tolerance[16]. That is, the increases in islet size and number are either a response to the stimulus of the abnormal carbohydrate metabolism in these breeder rats, or the islet changes *per se* are the cause of the spontaneous development of diabetes. It is more likely that the islet

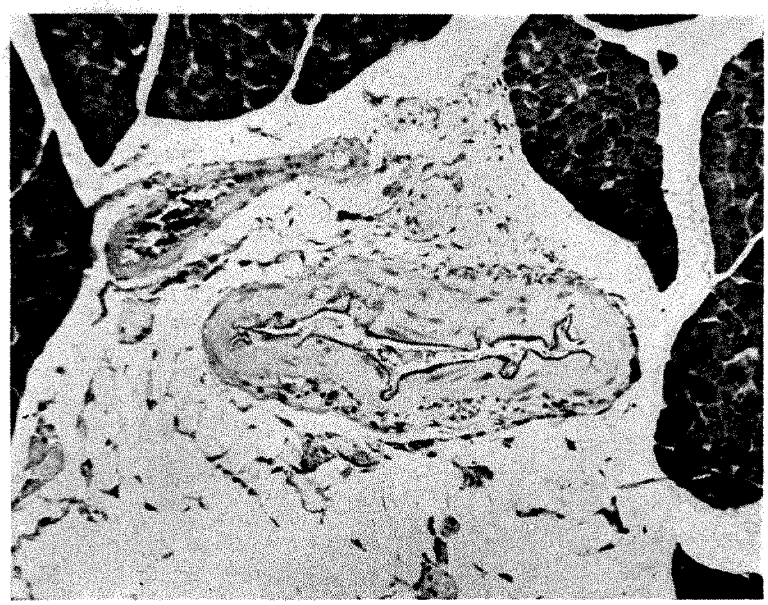

Fig. 5. Pancreatic artery showing heavy concentration of basophilic material in the intima. This material is intensely metachromatic, intensely positive with the Hale and Von Kossa stains and is believed to be an admixture of mucopolysaccharides and calcium. (Hæmatoxylin and eosin, × c. 100)

hyperplasia is an attempt by the pancreas to compensate for the increased adrenocorticoid activity which accompanies the progressive development of diabetes, hypertension and arteriosclerosis in repeatedly bred rats. In experiments to be published soon the adrenal cortices of these breeder rats have been found to show progressive hypertrophy and lipid depletion with successive breedings and concomitant with the development of arteriosclerosis. Also, the thymi in breeder rats become severely involuted during progressive breeding. Further, *in vitro* investigations of the alpha-ketolic steroid production of adrenal glands from breeder rats show definite derangement of adrenal steroid production. The changes in the adrenal glands, both biochemically and histologically, coupled with the severe thymic involution demonstrate to us that the adrenal glands of breeder rats are actually producing steroids in abnormal amounts.

Many investigators believe that the islets of Langerhans have a capacity for compensatory increased production of insulin and are able to meet unusual demands for insulin so long as this resiliency on the part of the islets is maintained[11]. However, under unusual conditions, for example, excess glucose mobilization due to overabundant secretion of glucocorticoids, this insulin reserve may break down. Or, if the islets are capable of hyperplasia and produced increased amounts of insulin, the hyperglycæmia may remain undetected. In this same connexion, ancillary studies show that male breeder rats probably have a more severe defect in their carbohydrate metabolism than female breeders because their glucose tolerance curve shows a much more severe hyperglycæmia 1 h after glucose loading than female breeders[18]. It is suggested that the islets of the female breeder probably have a greater functional reserve capacity than the male breeders. It is interesting that islet hyperplasia occurs soon after the first breeding in the male; but the stimulus for increased islet hyperplasia in the female does not become evident until well after the fourth or fifth breeding. Increase in islet size has been demonstrated by Hellman to occur with age[19]. However, the size of the islets in virgin rats (controls) of the same age as the breeders was only slightly larger than the younger virgin control rats killed at the outset of the experiment.

The specific cytological changes in the islets permit an even closer correlation with the physiological events taking place than the changes in islet size. Up to the fourth breeding the beta cells showed progressively increased granulation, in both sexes. This could be construed as being indicative of a compensatory response on the part of these cells to produce increased amounts of insulin. One could extrapolate, however, that this response was not adequate because: (1) these animals become hyperglycæmic[18]; (2) ductular elements showed proliferation to produce more islet tissue; (3) existing islets underwent progressive hyperplasia in an attempt to produce more beta cells. Further, it is felt that the demands of continued breeding finally caused exhaustion of these beta cells as demonstrated by their rapid degranulation after the fourth breeding.

Glucose tolerance tests conducted on breeder rats have revealed that these animals are not severely hyperglycæmic[18]. Rather, it appears that they are more likely in a mild diabetes-like state which would best fit the condition of hyperadrenocorticism found in these breeders. Although these animals do not have any marked elevation of serum cholesterol until arteriosclerosis is well advanced, they do have a progressive fatty infiltration of the liver at the same time that they show abnormal tolerance to glucose and begin to show evidence of arteriosclerosis[14]. One could perhaps best rationalize these many metabolic disturbances as being part of a syndrome which is much like that seen in Cushing's disease or in human pregnancy[5,6], that is, islet hyperplasia, fatty liver, diabetes and arteriosclerosis are all concomitants of Cushing's syndrome[5].

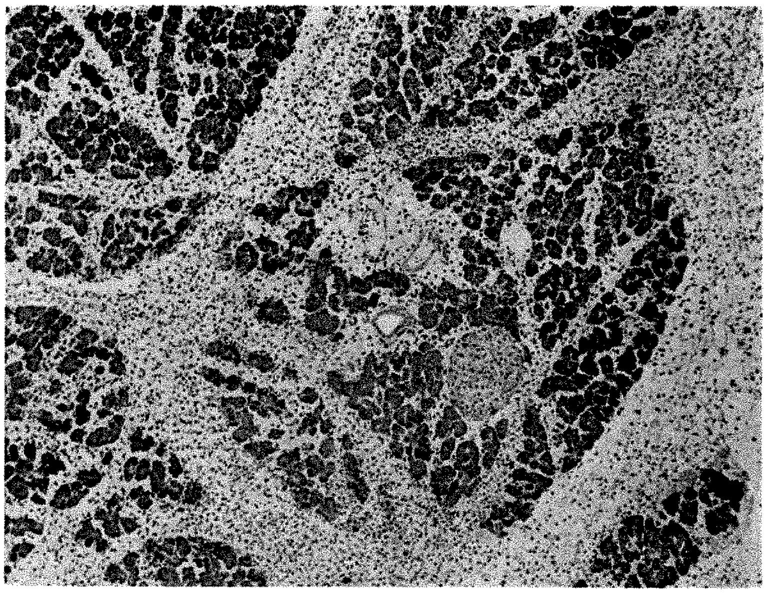

Fig. 6. Severe pancreatitis in a repeatedly bred male rat (sired four litters). Note the round cell infiltration and the marked œdema which separates individual acini. Fatty necrosis and calcification (not shown here) were found in peripheral portions of this organ. (Hæmatoxylin and eosin, × c. 50)

It is particularly interesting that the morphology of the arterial lesions in the larger branches of the abdominal aorta was distinctly different and much more severe than those in the parenchyma of the pancreas itself The intimal accumulation of mucopolysaccharide–calcium complex in these pancreatic arteries is typical of the arterial lesions in arteries of similar calibre in organs served by the intestinal arteries

Ductular proliferation and budding-off of islet tissue have been demonstrated to occur during fœtal life and in the post-natal period Several authors have cited evidence to indicate that this ductular response to form new islet tissue may be under hormonal control For example, Ogilvie and Maclean have shown striking increases in pancreatic duct growth and generation of islet tissue in rabbits in association with marked changes in the adrenal cortices of these same animals[20] The hormones of the anterior pituitary gland, for example, growth hormone and adrenocorticotropin, have also been demonstrated to elicit these changes[21,22] The connexion between the anterior pituitary gland and its hormones has been cited for two reasons First, the ductular proliferation becomes progressively more intense with each breeding, which would implicate pituitary gland participation, and also the ductular proliferation suddenly becomes quiescent at the time when these particular animals become incapable of producing more litters Secondly, it is well known that pancreatitis appears after periods of stress, or can be induced by ACTH excess[23], and in advanced cases of Cushing's disease purulent pancreatitis, fatty necrosis and infarction are evident in the pancreas[24,25] It is possible that the disappearance of ductular proliferation and appearance of many cases of pancreatitis in these breeders is connected with some marked change in the function of the pituitary gland The higher incidence of pancreatitis in the male breeders (40 per cent versus 25 per cent in females) may also explain the earlier demise of the male breeders after the fourth breeding

In conclusion, it is felt that repeated breeding in rats leads to metabolic alterations which resemble conditions seen in Cushing's syndrome and in human pregnancy The hyperglycæmia[18], the islet hyperplasia, the beta cell granulation and eventual exhaustion are all indicative of a persistent demand, most likely due to adrenal steroid excess; for increased secretion of insulin culminating in eventual exhaustion of the islet cells Further investigation will be required to correlate the possible relationship between the simultaneous development of hyperglycæmia and arteriosclerosis in these breeder rats The tentative explanation for this phenomenon will remain implicit within the broad concept that this arteriosclerosis and abnormal carbohydrate metabolism are part of a Cushingoid condition existing in these animals

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VISUAL PIGMENTS IN HUMAN AND MONKEY RETINAS

By PAUL K. BROWN and Prof GEORGE WALD

Biological Laboratories of Harvard University, Cambridge, Mass.

ONE of the major problems in the field of vision is to define the photosensitive pigments of the cones which serve for colour vision in man and other primates, and to establish the associated spectral sensitivity curves of the colour vision receptors One cannot derive this information from traditional types of sensory measurement, for an almost infinite variety of trios of theoretical sensation curves can be devised which satisfy virtually all the sensory data[1] The problem, therefore, is not to invent further models that might account for colour vision, but to find the actual mechanisms that serve this function

Stiles seems to have come closest to solving this problem with specially designed sensory measurements (his "two-colour threshold technique") Some years ago, using this method on his own eye, he derived three sensitivity curves maximal at about 440, 540, and 590 mμ (ref 2) Most measurements since have tended to conform with these values

So, for example, Auerbach and Wald[3], following adaptation of the eye to intense orange light, measured the spectral sensitivity of a 'violet receptor', maximal at about 440 mμ The spectral sensitivity of the human fovea, particularly when dark adapted or in dim light, is reported to display inflections at about 430–450, 540–560 and 590–620 mμ, suggesting the presence of sensitivity maxima within these wave-length ranges[4]

Rushton[5] made a notable advance in this field by measuring directly the difference spectra of human colour vision pigments with light reflected from the fundus in living human eyes He has described a green-sensitive pigment ('chlorolabe') maximal at about 540 mμ, and a red-sensitive pigment ('erythrolabe') at about 590 mμ Weale[6], using similar methods, reports pigments maximal at about 540 and 600 mμ

This article describes measurements of difference spectra of the retinal photopigments of man and the monkey (rhesus, *Macaca mulatta*), performed with the

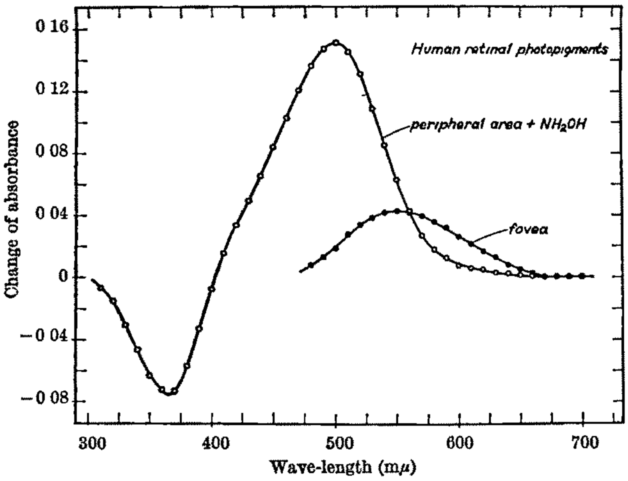

Fig 1 Difference spectra of photosensitive pigments in the human retina ●, total pigments of the rod-free area of the fovea The difference spectrum has λ_{max} about 550 mμ and E_{max} 0 042, ○, total bleaching of a rod-rich peripheral area of the retina, measured in the presence of 0 1 M hydroxylamine The difference spectrum is that of rhodopsin (λ_{max} 500 mμ), the bleaching of which yields retinal as product This combines immediately with hydroxylamine to yield retinaldehyde oxime, which accounts for the minimum at about 367 mμ

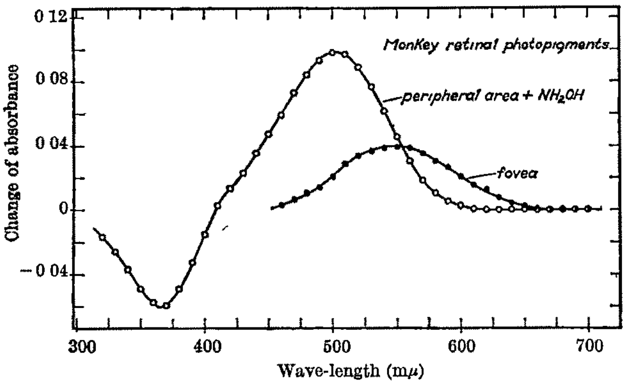

Fig 2 Difference spectra of photosensitive pigments in the retina of the rhesus monkey ●, the total bleaching of the central fovea (λ_{max} about 550 mμ) This fovea yielded also the data of Figs 3 and 5, ○, bleaching of a rod-rich peripheral area in the presence of 0 1 M hydroxylamine The difference spectrum is that of rhodopsin, with λ_{max} about 502 mμ The retinal liberated as a product of bleaching combines at once with hydroxylamine to yield retinaldehyde oxime, accounting for the minimum at about 367 mμ

recording microspectrophotometer already described[7] Circular areas of the retina were measured, 0 2 mm in diameter Such measurements made in a rod-rich area outside the fovea yielded the familiar difference spectrum of rhodopsin (Figs 1 and 2), earlier shown to match closely in the visible region the scotopic sensitivity of human vision, measured at the retinal surface[8] When the field of measurement lay inside the rod-free area of the fovea, which is about 0 5 mm in diameter in man and 0 2 mm in the monkey[9], a total difference spectrum was obtained, maximal at about 550 mμ (Figs 1 and 2) This matches closely the photopic sensitivity curve of human vision, corrected for absorption of light by the ocular tissues and the yellow macular pigmentation (Fig 8) On differential bleaching of the central fovea with lights ranging between deep red and orange, the total difference spectrum can be shown to include contributions from a red-sensitive pigment with λ_{max} about 565 mμ, and a green-sensitive pigment with λ_{max} about 535 mμ (man) or about 527 mμ (monkey) Some indication was obtained also of a small amount of a third pigment, bleaching maximally at about 440 mμ, though other evidence indicates that the fovea contains such a pigment, our measurements do not identify it reliably

Human eyes, obtained from an eye bank, were from persons 40–70 years of age All of them had been light-adapted to some degree at the time of removal Ordinarily they were placed in light-tight containers shortly after enucleation, and kept cold until called for, usually 4–5 h and never more than 12 h later We found that in fresh eyes the retina tends to cling to the vitreous, so the eyes were always kept cold for a total of about 12 h, after which the retina separates readily

Monkeys were blindfolded about 0 5 h before being killed, and the eyes were removed in deep red light, so that these retinas were genuinely dark adapted Measurements with one retina were begun within 2–3 h The other eye in a light-tight container was placed in a deep-freeze, and kept frozen until used, sometimes months later When such an eye is thawed quickly by immersion in warm water, the retina can be dissected out whole, and yields measurements not very different from those obtained with fresh retinas Fig 3 shows measurements made with such a previously frozen retina

All the operations to be described were performed in deep red light The eye, held by the stump of the optic nerve, is cut around the bulbus well behind the equator. The rear portion of the eye is immersed in normal saline solution in a flat glass dish, and the retina released by cutting through the point of attachment to the optic nerve The retina is spread out at the bottom of the dish, under saline, with the receptor cells pointing upward On viewing it over a very dim blue light, the macular region can be seen as a dark patch with a lighter spot near its centre that marks the fovea A more-or-less circular patch of retina about 3–4 mm in diameter containing the macular region is cut out with scissors and forceps and transferred to the microcell previously described (a quartz cover slip holding a plastic ring about 0 4 mm high)[7] One drop of 55 per cent glycerol in normal saline solution fills this cell, which is then closed with a second quartz cover slip The cell is placed on the stage of a low magnification microscope ('macroscope', see ref 7), and is aligned under deep red light (675 mμ) so that the central fovea lies just over a circular diaphragm, 0 08 mm in diameter, which delimits an area 0 2 mm in diameter at the level of the receptors After this preliminary alignment, a compound microscope of magnification 100 times is brought over the microcell, and the alignment further adjusted if necessary All these manipulations are carried

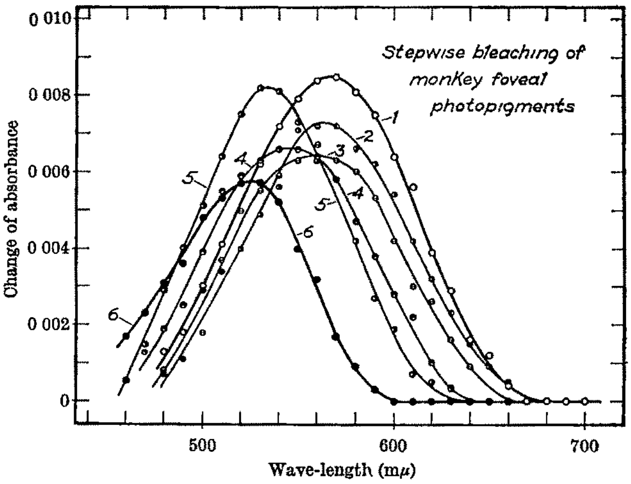

Fig 3 Difference spectra from a monkey fovea, associated with the bleaching of photosensitive pigments by short flashes of light transmitted through a progressive series of red cut-off filters The flashes had a half-duration of 120 msec The filters used in the successive bleaches were selected from the combinations listed in Table 1, and transmitted all wave-lengths longer than the following cut-off values (1) Combination 2 675 mμ, (2) Comb 3 665 mμ, (3) Comb 4 655 mμ, (4) Comb 5 645 mμ, (5) Comb 6 615 mμ, (6) Comb 7 595 mμ In constructing Fig 5, curve (1) has been taken as the spectrum of the red-sensitive pigment, and curve (6) as that of the green-sensitive pigment, while the sum of all the curves yields the total difference spectrum

out as rapidly and with as little exposure to red light as possible

Spectra are recorded in the microspectrophotometer as described previously[7]. It usually takes 0 5–1 h after the retina has been immersed in glycerol-saline to achieve a stable baseline The tissue is kept completely dark except during a recording, and the recording itself does not involve any measurable bleaching of the tissue The spectrum is recorded first using the ordinary 0–1 density range of the instrument in order to observe its general characteristics There is usually a large baseline absorption, mainly owing to the yellow pigmentation of the macula, shown some years ago to be due to the carotenoid xanthophyll[10] This is approximately balanced out in the reference beam by a solution of crystalline leaf xanthophyll in a mixture of carbon disulphide and chloroform (20 80), in a cell of adjustable depth[11] The xanthophyll had been heat-isomerized previously by melting the crystals, so as to produce a mixture of *cis* and *trans* isomers, the absorption spectrum of which in this solvent resembles closely that of the human macular pigmentation (cf Fig 8) After a retina had been balanced against the xanthophyll solution, the spectrophotometer was shifted to a 0–0 1 slide wire, so magnifying its absorbance scale ten times

The spectrum of the retina is recorded, first in the dark, and then after exposure to various lights By subtracting spectra of the bleached or partly-bleached tissue from spectra recorded earlier, the difference spectra shown in this paper are obtained

Bleaching was accomplished in one of three ways· (1) With 6 6 mμ wave-bands from a Bausch and Lomb monochromator, using a 100-W zirconium arc as source (2) With short flashes from standard *M*5 clear photographic flash lamps, transmitted through various colour filters Oscilloscope observations showed the output of these lamps to be remarkably constant, the half-duration of each flash being about 120 msec (3) With the focused light of a tungsten filament lamp in a Bausch and Lomb Nicholas illuminator, passing through colour filters.

The colour filters were all of the cut-off type, transmitting all radiation longer than a specified wave-length Each of them was tested carefully to be sure that it had no radiation leaks below the cut-off wave-length These filters were used always in combination with a Jena *KG* 1 heat filter Their characteristics are listed in Table 1

Table 1 THE CUT-OFF WAVE-LENGTHS ARE THOSE AT WHICH THE FILTERS TRANSMIT 1 PER CENT OF INCIDENT RADIATION J, JENA (SCHOTT), W, WRATTEN (EASTMAN KODAK), C, CORNING THESE FILTERS WERE USED ALWAYS IN COMBINATION WITH A JENA *KG* 1 HEAT FILTER

Filter	Cut-off wave-length (mμ)	Filter	Cut-off wave-length (mμ)
(1) J *RG* 10	690	(7) J RG 1	595
(2) J *RG* 8	675	(8) J OG 3	570
(3) W 36+C 3480	665	(9) J OG 2	550
(4) W 35+C 3480	655	(10) J OG 5	535
(5) J *RG* 5	645	(11) C 3384	490
(6) J *RG* 2	615	(12) C 3385	465
		(13) C 3387	442

All these measurements were carried out at room temperature, usually about 25° C No special attempt was made to supply the tissue with oxygen Though isolated retinas rapidly turn cloudy in saline or Ringer solution, in saline-glycerol mixtures they remain reasonably clear The base line was usually found to remain fairly stable for periods of about half an hour, sometimes longer

Peripheral retina rhodopsin Several measurements were made in areas of retina well outside the fovea In such cases hydroxylamine was added to the glycerol-saline in a final concentration of 0 1 M After recording the spectrum in darkness, the tissue was bleached with orange light, and the spectrum again recorded

Difference spectra so obtained from human and monkey retinas are shown in Figs 1 and 2 In both cases the familiar difference spectrum of rhodopsin was obtained The observation that human rhodopsin in the retina has λ_{max} 500 mμ agrees with measurements previously made on suspensions of human rod outer segments[8] The λ_{max} in the monkey came out slightly higher, at about 503 mμ The bleaching of rhodopsin yields a mixture of opsin and retinal (formerly retinene) as products, the latter immediately combining with hydroxylamine to yield retinaldehyde oxime (λ_{max} 367 mμ) The curves in Figs 1 and 2 display minima at about 367 mμ, owing to the formation of this product

The maximum extinction (E_{max}) of human rhodopsin varied considerably from retina to retina Our highest extinction, shown in Fig 1, was 0 15, equivalent to about 30 per cent absorption This agrees almost exactly with Rushton's estimate[12] of the extinction of rhodopsin in human rods

The highest extinctions of rhodopsin measured in monkey retinas were about 0 10, corresponding to about 20 per cent absorption It will be recalled that the monkey retinas were dark adapted, whereas we had no such assurance in the human tissues

When rhodopsin is bleached in solution in the presence of hydroxylamine, the rhodopsin maximum at about 500 mμ comes out almost exactly equal in height to the retinene oxime minimum at 367 mμ Recent spectrophotometric measurements on frog and cattle retinas yielded a quite different result—the retinene oxime minimum was considerably smaller than the rhodopsin maximum The reason is that in the rods the rhodopsin molecules are oriented so that their chromophores lie perpendicular to the long axis of the outer segment, and so absorb more strongly than in solution, whereas the retinene oxime formed on bleaching not only loses this orientation, but orients partly in the axial direction, so absorbing less strongly than in solution[13] The difference spectra of rhodopsin in human and monkey retinas display much the same type of relationships as observed in frog and cattle retinas, implying somewhat the same plan of molecular orientations.

Fovea: total bleaching. The difference spectra obtained by completely bleaching the photosensitive pigments of human and monkey foveas are shown in Figs 1 and 2 In both cases the difference spectra are maximal at about 550 mμ No hydroxylamine was used in these instances, so that the product of bleaching is retinaldehyde rather than retinaldehyde oxime Since, however, retinaldehyde does not absorb appreciably at wave-lengths longer than about 510 mμ, above this wave-length these difference spectra have the force of genuine absorption spectra

In both man and monkey the maximum density of the total foveal pigment that we observed was about 0 04, corresponding to about 9 per cent absorption This is about half the density reported for the total cone pigments by Rushton[5] One might ascribe this discrepancy to the fact that Rushton's measurements were made on wholly dark-adapted subjects, whereas our human retinas may have been partly bleached On the other hand, our rhodopsin measurements in human retinas agree exactly with Rushton's; and our monkey retinas, which were wholly dark adapted, yielded the same result

Fovea colour vision pigments We have attempted to fractionate the foveal photopigments into their components by differential bleaching with coloured lights This was done in two ways, illustrated in Figs 3 and 4

In the first procedure the retinas were exposed to the light from flash bulbs, as described in bleaching method (2) aforementioned, coming through a progressive series of red cut-off filters (cf Table 1)

Fig 3 shows such an experiment on a monkey fovea The first flash contained only wave-lengths longer than about 675 mμ, and caused a bleaching represented by difference spectrum (1), with λ_{max} about 568 mμ The second flash contained wave-lengths above about 665 mμ, and yielded difference spectrum (2), with λ_{max} about 564 mμ The third flash, cutting off at about 655 mμ, yielded difference spectrum (3), with λ_{max} about 560 mμ It should be noted that these first three bleaches with

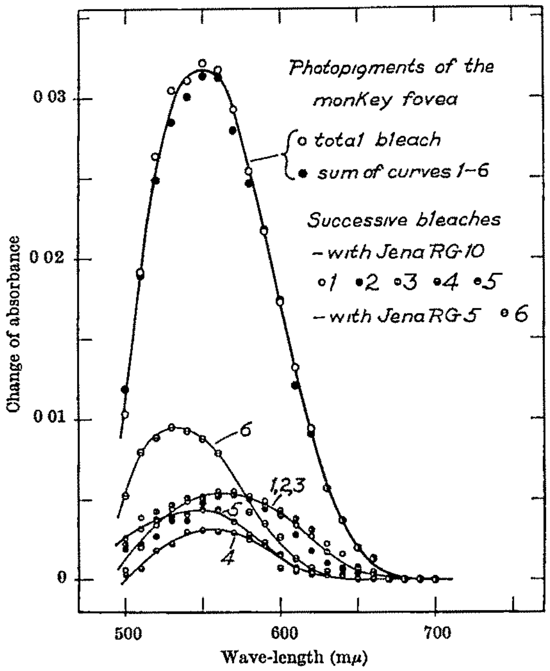

Fig 4 Difference spectra associated with the bleaching of photopigments of the monkey fovea in continuous red light. Curves 1–3, obtained by bleaching through a filter combination that transmits only wave-lengths longer than 690 mμ, involve primarily the bleaching of the red-sensitive pigment, with λ_{max} about 565 mμ Further exposure to the same deep red radiation bleaches in addition larger and larger proportions of the green-sensitive pigment (curves 4 and 5) The final bleaching with a filter combination transmitting above about 645 mμ involves virtually the green-sensitive pigment alone (λ_{max} about 533 mμ) The large open circles show the difference spectrum of the total bleaching of this fovea, the solid circles the sum of curves 1–6 The good agreement between these last two sets of measurements shows that all these difference spectra represent pigments present in the fovea originally, none of them could represent some new photosensitive product of the prior bleaching of one of the other pigments

deep red flashes yielded quite similar difference spectra, λ_{max} varying only between 560 and 568 mμ

The fourth flash, which contained wave-lengths above about 645 mμ, yielded a highly displaced difference spectrum, with λ_{max} about 546 mμ (curve 4) The fifth flash, cutting off at about 615 mμ, yielded a difference spectrum maximal at about 534 mμ (curve 5) The sixth and last flash, cutting off at about 595 mμ, yielded a smaller bleaching with λ_{max} about 526 mμ Exposure of the retina to further flashes at still shorter wave-lengths produced no measurable further bleaching.

It should be noted that curve 6 in Fig 3 displays no appreciable absorption at about 620 mμ, where curve 1 displays a strong absorption It can be expected for this reason that the filter used for exposure 1 should have bleached little if any of the pigment represented by curve 6 (Very small amounts of the latter pigment do seem to have been bleached in exposures 2 and 3, accounting for the small progressive shift of λ_{max} in each of these curves toward shorter wave-lengths) These measurements suggest that two pigments are involved, a red-sensitive pigment represented approximately by curve 1, and a green-sensitive pigment represented approximately by curve 6, the intermediate curves representing the bleaching of various proportions of both pigments

A computation based on this presumption is shown in Fig 5 The total difference spectrum obtained by adding together curves 1–6 in Fig 3 is shown as the uppermost curve in Fig 5 (curve 1) We assume that curve (1) of Fig 3 represents a red-sensitive pigment which accounts for the entire absorption in the total difference spectrum above about 625 mμ On this basis we adjust the height of curve 1 from Fig 3 so that it coincides in Fig 5 with the total difference spectrum above this wave-length (curve 2) If it is true that only two photosensitive pigments contribute to the total difference spectrum, subtracting curve 2 from the total might be expected to yield the spectrum of the other pigment This is shown in Fig 5 with open circles and a broken line (curve 1–2) It has λ_{max} about 530 mμ We have also drawn into Fig 5 the spectrum of the last bleach (curve 6) in Fig 3, giving it the same height as curve 1–2 (small closed circles and solid line) It has λ_{max} about 526 mμ

It is evident from this computation that the propositions expressed previously hold reasonably well Subtracting the spectrum of the most red-sensitive pigment from the total yields a spectrum close to that of the green-sensitive residue

In Fig. 5 the red-sensitive pigment has a maximal extinction about 1 5 times as high as the green-sensitive pigment Of five monkey foveas measured, four displayed about this proportion of red- and green-sensitive pigments, the fifth had these pigments in the reverse proportions, about 3 green- to 1 red-sensitive

The results of bleaching a monkey fovea in continuous red light (bleaching method 3 aforementioned) are shown in Fig 4 The retina was exposed for successive intervals to the focused light of a tungsten filament lamp passing through the Jena *RG* 10 filter, which transmits all wave-lengths longer than 690 mμ The difference spectra obtained in five successive exposures to this radiation are shown as curves 1–5 The first three exposures yielded very similar difference spectra, with λ_{max} about 563 mμ We take this to represent the red-sensitive pigment The fourth exposure yielded a much smaller bleaching, with λ_{max} displaced to about 557 mμ By this time most of the red-sensitive pigment had been bleached, and the green-sensitive pigment had begun to bleach Exposure 5, which was just as long as exposure 4 but at three times the intensity, caused a further bleaching with λ_{max} about 550 mμ, the curve had also broadened considerably toward shorter wave-lengths By now the red-sensitive pigment must have been almost wholly bleached, and a relatively high proportion of green-sensitive pigment was bleaching Finally the retina was exposed to the light passing through a Jena *RG* 5 filter, which transmits all wave-lengths longer than about 645 mμ This resulted in the bleaching shown in difference spectrum 6, with λ_{max} about 533 mμ, which we take to represent approximately the spectrum of the green-sensitive pigment

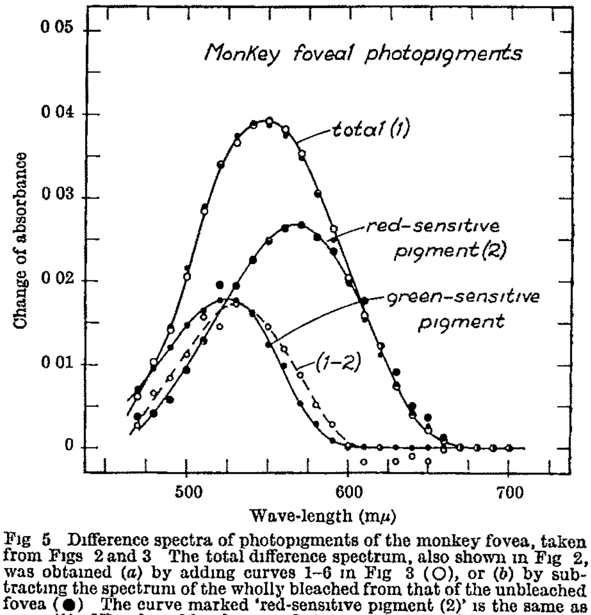

Fig 5 Difference spectra of photopigments of the monkey fovea, taken from Figs 2 and 3 The total difference spectrum, also shown in Fig 2, was obtained (*a*) by adding curves 1–6 in Fig 3 (○), or (*b*) by subtracting the spectrum of the wholly bleached from that of the unbleached fovea (●) The curve marked 'red-sensitive pigment (2)' is the same as curve (1) of Fig 3, and has been made to coincide in height here with the total difference spectrum in the red region in which the red-sensitive pigment alone absorbs light appreciably Subtracting curve (2) from (1) might then represent the difference spectrum of the green-sensitive pigment (broken line, 1–2), this has λ_{max} about 530 mμ It is to be compared with the difference spectrum obtained directly as curve 6 of Fig 3, which has λ_{max} about 525 mμ

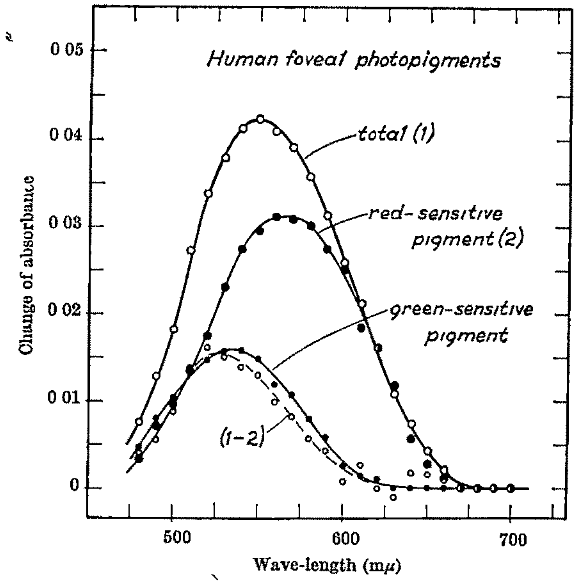

Fig 6. Difference spectrum of photopigments of the human central fovea. The difference spectrum of a red-sensitive pigment (curve 2, λ_{max} 565 mμ), obtained by differential bleaching in deep red light, accounts for virtually all the absorption at wave-lengths longer than about 625 mμ. It is adjusted in height, therefore, so as to coincide with the total difference spectrum (1) above this wave-length. Subtracting curve 2 from 1 should then yield the difference spectrum of the green-sensitive pigment, this is shown as a broken line (1–2) with λ_{max} about 530 mμ. It is to be compared with the directly measured difference spectrum of the green-sensitive pigment, shown with a solid line (λ_{max} about 535 mμ), obtained at the end of a successive series of bleaches with red light as in Fig 3.

It has been reported that some products of the bleaching of visual pigments in the living cat are themselves photosensitive[14], and similar effects have been reported also with visual pigments in solution[15]. On some such basis one might raise the question whether the green-sensitive pigment shown in Fig 5 could itself be a product of the bleaching of the red-sensitive pigment.

Apart from the fact that retinal in all circumstances is subject to isomerization by light, the only photosensitive product of the bleaching of rhodopsin we have yet observed in our laboratory involves the appearance of a pigment with λ_{max} about 467 mμ derived from metarhodopsin II when the latter is present in high concentration[16]. The 467 mμ pigment is unstable both in light and darkness, and can be observed only under quite special conditions. Neither this nor any other light-sensitive pigments of bleaching appeared in the course of the present measurements.

The data of Figs. 4 and 5 show this to be so. The tall curve in Fig 4 shows with open circles the difference between the spectra of the unbleached and wholly bleached fovea, and with solid circles the sum of the partial difference spectra 1–6. Similarly the 'total' curve 1 in Fig 5 shows with solid circles the difference between the spectra of the unbleached and wholly bleached fovea, and with open circles the sum of the partial difference spectra 1–6 in Fig 3. If in either case any of the partial difference spectra represented a new pigment derived from a prior bleaching, the partial difference spectra could not possibly add up to that representing the total bleaching, but must on the contrary yield a larger sum. The fact that the summed-up difference spectra add almost exactly to the total difference spectrum demonstrates that all the pigments bleached in this experiment were present in the retina originally. The degree to which the difference between the spectrum of the red-sensitive pigment and the total in Fig 5 (1–2) matches the directly measured spectrum of the green-sensitive pigment tends toward the same conclusion, and the similar experiment on human foveal pigments shown in Fig 6 bears on the same point.

It is therefore plain that the pigments differentially bleached in these experiments were present in the original tissue. The observations imply the presence of two such pigments in the monkey, with λ_{max} at about 565 and 530 mμ.

Similar experiments with human foveas yielded very nearly the same results. The results of one such experiment are shown in Fig 6. It involves the same procedures as were used in Fig 3. As in the monkey, differential bleaching with deep red light revealed a first pigment with λ_{max} at about 565 mμ, and a high absorption at 625 mμ, whereas the last pigment to bleach had λ_{max} about 535 mμ and very little absorption above 625 mμ. We assume again, therefore, that the red-sensitive pigment accounts completely for the total difference spectrum above 625 mμ, and makes its spectrum coincide with the total above this wave-length. On subtracting the spectrum of the red-sensitive pigment from the total, we obtain the curve shown as a broken line in Fig 6 (1–2). This is maximal at about 530 mμ. The last spectrum measured in the course of bleaching, as already said, is maximal at about 535 mμ. Both curves are reasonably close together in shape and position, and we take them—particularly the directly measured spectrum—to represent the difference spectrum of the green-sensitive pigment.

The human fovea, like that of the monkey, appears to contain considerably more of the red-sensitive than of the green-sensitive pigment. The proportions shown in Fig 7 are about 2 : 1. This is almost exactly the ratio that Rushton has reported finding between what he calls erythrolabe and chlorolabe in the human fovea[5].

The results of differential bleaching in human and monkey foveas are summarized in Fig. 7. In this figure all curves have been brought to the same arbitrary height to facilitate comparison. The red-sensitive pigments of human and monkey foveas yield virtually identical difference spectra, maximal at about 565 mμ. Small differences are found, however, in the difference spectra of the green-sensitive pigments, the human pigment lying at about 535 mμ, the averaged monkey curves at about 527 mμ. It is possible that the monkey spectrum is pulled toward shorter wave-lengths by the presence of small amounts of rhodopsin, owing to contamination of

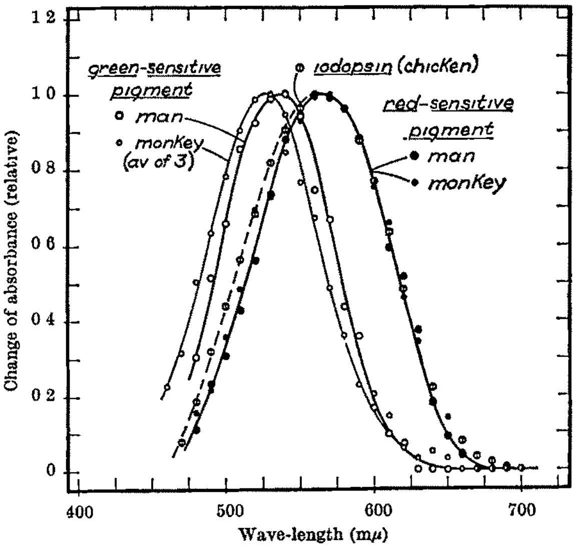

Fig 7. Difference spectra of photosensitive pigments of the central foveas of man and the rhesus monkey, brought arbitrarily to the same height for comparison. The red-sensitive pigments of human and monkey foveas are essentially identical, with λ_{max} about 565 mμ. Their difference spectra correspond closely with that of chicken iodopsin in aqueous solution, shown here with barred circles and a broken line. The difference spectrum of the human green-sensitive pigment is maximal at about 535 mμ. That of the monkey lies at slightly shorter wave-lengths (λ_{max} about 527 mμ), perhaps owing to the inclusion of a few rods and hence a small amount of rhodopsin in the measurement. The rod-free area of the monkey fovea is stated to be only 0.2 mm in diameter, the same size therefore as the area of retina measured in these experiments. The human rod-free area is about 0.5 mm in diameter, so making it easier to avoid the presence of rods in the field.

the field of measurement with a few rods. Polyak gives the diameter of the rod-free area in rhesus monkeys as 0.2 mm. If this is a reliable figure, our field of measurement should have completely filled this area, and the inclusion of a few rods in the field would have been unavoidable.

The experiments on differential bleaching, having begun with deep red light, always ended by exposing the retina to light of relatively short wave-lengths, that is, in the orange and yellow. Sometimes such light produced no further measurable bleaching. At other times it induced a very small further bleaching maximal in the neighbourhood of 440 mμ. Possibly this was due to the presence of a very small amount of a blue-sensitive pigment. We do not, however, regard our observations on this point as reliable. They were made under great difficulty since, as already noted, this region of the spectrum is very strongly absorbed by the macular pigmentation, and this is also the region in which the isomerization of retinaldehyde would yield a difference spectrum. Even if a blue-sensitive visual pigment were present in these foveas, bleaching it to retinal, which absorbs over the same region, should yield a highly distorted difference spectrum, lying further toward the red than the true absorption spectrum of the pigment.

Regeneration of cone pigments. Several times we have attempted to regenerate cone pigments in completely bleached human foveas. The cover slip of the microcell was lifted off, a droplet containing 4 μgm of 11-*cis* retinal in ethanol was stirred on its surface with 10 μl of glycerol-Ringer, and it was laid back on the retina. The tissue was then repositioned in the spectrophotometer and tested by differential bleaching with red lights as described previously. In a few such experiments enough cone pigment had regenerated to yield reasonably reliable difference spectra. The regenerated pigments never totalled more than about 0.25–0.5 of those present originally, so that no high accuracy could be achieved in such measurements, but the results showed clearly that both the red- and green-sensitive pigments regenerated. Also on re-bleaching these pigments complete difference spectra were obtained, showing clearly the emergence of retinal as a product of bleaching.

The observation that in experimental human vitamin A-deficiency cone vision deteriorates in parallel with rod vision, and that both are repaired simultaneously on administration of vitamin A, led to the conclusion some years ago that some or all of the human cone pigments, like those of the rods, are derived from vitamin A (now 'retinol')[17]. This is now demonstrated directly for two of the human colour vision pigments, together with the assurance that both pigments possess 11-*cis* retinal as chromophore. It seems probable that the human colour vision pigments are formed by joining this single chromophore to a variety of different opsins.

The red- and green-sensitive pigments of primate foveas appear from our measurements to lie at somewhat shorter wave-lengths than has commonly been supposed. We find the $\lambda_{\max}$ of the difference spectrum of the red-sensitive pigment to lie at about 565 mμ in both man and monkey, and the green-sensitive pigment to lie at about 535 mμ in man and perhaps as high—surely above 527 mμ—in the monkey. We take these difference spectra to be equivalent to true absorption spectra at wave-lengths longer than about 510 mμ, where the retinal produced by bleaching these pigments ceases to absorb appreciably.

In the case of the green-sensitive pigment the disparity with other measurements is small, perhaps negligible. Stiles's estimate of the spectral sensitivity of 'green'-cones finds it maximal at about 540 mμ[2], and Rushton gives the same value to the $\lambda_{\max}$ of the difference spectrum of the associated pigment, measured in protanopes[5]. In fact, however, Rushton's measurements have sufficient spread to agree as well with $\lambda_{\max}$ 535 as 540 mμ, so there is no clear disparity between his measurements and ours.

The red-sensitive pigment, however, raises a genuine issue, though here the disparity with data is sometimes less than with their descriptions in the literature. Stiles's original estimate of the spectral sensitivity of 'red'-cones, as also Rushton's estimate of the difference spectrum of the associated pigment, placed $\lambda_{\max}$ at about 590 mμ. Since his first measurements on his own eye, however, Stiles, working with other subjects and more varied procedures, has described a 'red'-cone mechanism, π_5, with a peculiarly broad maximum to which he assigns the value 575 mμ, not far therefore from that of our red-sensitive pigment[16]. It should be noted, therefore, that Rushton attaches little accuracy to his measurements on the red-sensitive pigment (see his discussion in ref. 19, 278). Having originally described its difference spectrum as near 590 mμ, he has recently published another such difference spectrum with $\lambda_{\max}$ near 570 mμ[20], and has also measured an action spectrum for its bleaching, with $\lambda_{\max}$ about 560 mμ (personal communication). At least some of Rushton's measurements on the red-sensitive pigment therefore are not inconsistent with ours. Weale, using methods comparable with those of Rushton, describes two human cone pigments with difference spectra which he says are maximal at about 540 and 600 mμ[21], his published measurements on the latter pigment, however, seem to us to display maxima well below 600 mμ, near 570 mμ.

It should be added that in the course of these experiments we tried hard to get the red-sensitive pigment to display a longer $\lambda_{\max}$ than 565 mμ, but without success. As already said, the first fraction of this pigment to bleach at the longest wave-lengths that were effective possessed $\lambda_{\max}$ about 565 mμ.

Fig. 7 shows that the difference spectrum of this red-sensitive pigment of primate foveas lies very close to that of chicken iodopsin in aqueous solution[22]. Indeed this pigment is iodopsin, though in this special position as one of a trio of colour vision pigments there may be an advantage in naming it accordingly. Among various sets of terms we have lately been offered for the colour vision pigments, we think those proposed by Rushton to be the most apt and beautiful. We should be glad therefore to speak of iodopsin in this special connexion as 'erythrolabe', if Rushton will accept a $\lambda_{\max}$ near 565 mμ in place of the $\lambda_{\max}$ of 590 mμ that he has attached to the latter term. Similarly our green-sensitive pigment might be called 'chlorolabe'. The difference spectrum of the blue-sensitive pigment 'cyanolabe' has yet to be measured, though we already have approximations to the associated spectral sensitivity function, maximal at about 440 mμ[2,3], undoubtedly distorted somewhat by the filtering action of the macular pigmentation.

From the point of view of the chemistry of visual pigments, it is significant that iodopsin and the red-sensitive pigment of primate foveas lie farthest toward the red of all known visual pigments having retinal (retinene$_1$) as chromophore. It is difficult enough to attempt to explain how coupling retinal, with $\lambda_{\max}$ about 380 mμ, to opsin can achieve as long a $\lambda_{\max}$ as 565 mμ, and there is some relief in knowing that there is no need at present to go further.

Another feature of our measurements of some interest is the ratio of densities of the red- and green-sensitive pigments, which we find in the human fovea to be about 2:1. This agrees well with Rushton's estimate, but in view of uncertainties surrounding his measurements of the red-sensitive pigment, that still may be held to leave the issue in doubt. It might be supposed that previous exposures to light could have displaced this ratio somewhat in our human eyes. It might be objected also that the red light used in preparing and mounting our retinas might have differentially bleached some of the red-sensitive pigment, but in that case its ratio to the green-sensitive pigment in the normal fovea should be even higher than we find.

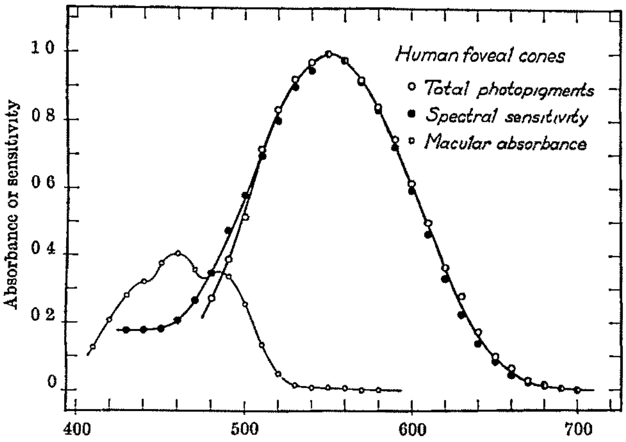

Fig 8 Comparison between the difference spectrum of the total photopigments of the human central fovea (average of 5 foveas) and the spectral sensitivity of human foveal vision, measured as though at the level of the cone outer segments To obtain the latter function, a group of representative measurements of the photopic luminosity curve was averaged, converted to a quantum basis, and corrected for the transmission of the ocular media according to Ludvigh and McCarthy, and the macular pigmentation, measured directly in the present experiments The spectrum of a human macula, as shown, was given the maximal extinction 0 405 at 460 mμ, corresponding to about 60 per cent absorption, probably close to the average in human observers The close correspondence between the difference spectrum of the total foveal photopigments and the spectral sensitivity of foveal vision above 500 mμ offers some assurance that our difference spectra of the red- and green-sensitive pigments, which, as shown in Fig 6, add up to yield the total difference spectrum, represent correctly at wave-lengths above 510 mμ the absorption spectra and proportions of these pigments in the living human eye

Some assurance with regard to this ratio and other characteristics of our measurements can be gained by comparing the difference spectrum associated with total bleaching of the fovea in our experiments with the spectral sensitivity of human foveal vision Such a comparison is shown in Fig 8

The spectral sensitivity of the human fovea is described by the well-known photopic luminosity function of the human eye This is of such great importance industrially and otherwise as to have been measured often and well In the present instance we have combined some of the most representative of such measurements to yield a composite function of high reliability[23]. This, however, represents the foveal spectral sensitivity in terms of energies incident on the cornea, whereas for comparison with the visual pigments one needs the foveal sensitivity in terms of numbers of quanta incident on the outer segments of the cones Apart from quantizing the measurements, therefore, we have corrected them for the filtering action of ocular structures light absorbed or scattered in passing through the ocular tissues as estimated by Ludvigh and McCarthy[24], and absorption by the macular pigmentation We have measured the latter repeatedly in the course of the present investigation (cf Fig 8), and will describe it in more detail elsewhere For the present computation we have assumed a macular pigment density corresponding to 60 per cent absorption at 460 mμ, close to the average value observed earlier in human subjects[10]

The spectral sensitivity of foveal cones, quantized and corrected for ocular transmission so as to be applicable at the receptor surface, is compared in Fig 8 with the average difference spectrum obtained from wholly bleaching five human foveas The two functions are in good agreement at wave-lengths longer than 510 mμ, where the difference spectrum approximates a true absorption spectrum Below 510 mμ, the spectral sensitivity curve probably continues to represent reasonably accurately the absorption spectrum of the total foveal photopigments

This comparison to a degree constitutes a searching test of the reliability of our measurements Figs 5 and 6 show that the total difference spectrum of human and monkey foveas is well accounted for by the individual difference spectra of the red- and green-sensitive pigments in the proportions shown in these figures. It is difficult to see how the total difference spectrum of the human fovea would agree with its spectral sensitivity as shown in Fig 8, were it not true that our difference spectra of the red- and green-sensitive pigments are equivalent at long wave-lengths, as we have assumed, to true absorption spectra, and that they have about the same positions and proportions in the living eye as we have found in excised retinas

This comparison bears also on a final consideration It has long been recognized that, though the spectral sensitivity of red-blind subjects (protanopes) is abnormally low in the red, and is often assumed to approximate the spectral sensitivity of the green-receptor, the spectral sensitivity of deuteranopes, which might conversely be expected to approximate that of the red-receptor, in fact lies close to that of normal trichromatic observers For this reason Konig[25] (cf also Judd[26]) supposed that the spectral sensitivity of the red-receptor must resemble the total luminosity function, and must account almost entirely for the photopic luminosity

Actually the luminosity curve of deuteranopes does depart somewhat from the normal function, displaying a measurable depression of sensitivity in the green and blue and a small displacement of maximum toward the red[27] All these relationships are made easier to understand by our observations, on one hand that the red-sensitive receptor has its peak of absorption and hence presumably its peak of sensitivity at as low a wave-length as 565 mμ, hence not far from the normal photopic luminosity maximum, and that the red-sensitive pigment dominates foveal vision

This work was supported in part by the U S National Science Foundation and the Office of Naval Research We thank the Boston Eye Bank for gifts of human eyes not needed for corneal transplantation, and Prof Frederick Hisaw of this Department for gifts of monkey eyes

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SEIZURES INDUCED BY INTRAVENTRICULAR SODIUM

By PROF GILBERT H. GLASER and GEORGE WOLF

Section of Neurology, Department of Medicine, Yale University School of Medicine,
New Haven, Conn

THE activity of cerebral neurones as characterized by membrane excitability and impulse conduction is directly dependent on the metabolic environment A critical factor is the maintenance of membrane resting potential by the distribution of sodium, mainly extraneuronally, and potassium, mainly intraneuronally Shifts of this ionic balance have been found associated with cerebral seizures, the repetitive discharging of hyperexcitable neurones. There is evidence that during and probably prior to a seizure discharge there is a decrease in the ratio of concentrations of extraneuronal to intraneuronal sodium[1,2] mainly due to an increase in the latter

The technique developed by Feldberg and Sherwood[3] of intraventricular injections in otherwise intact animals has offered an approach to this problem on a neurophysiological and behavioural level During the past ten years many drugs and certain ions have been so administered[4-6] It was found that intraventricular calcium induced a peculiar ataxia, then sleep-like state, potassium administration caused seizures with autonomic effects and circling movements, but never drinking[7] Sodium was given only as 'control', in isotonic solution, and no significant effects were noted However, Sawyer and Gernandt[8] described 'saline-induced seizures', induced by intraventricular or intracarotid hypertonic saline, in non-behavioural, acute electroencephalographic investigations in curarized rabbits The seizure activity was confined to the amygdala and hippocampus and was not produced by isotonic saline, or hypertonic glucose or urea Miller[9] has reported drinking behaviour in cats produced by intraventricular injections of hypertonic sodium chloride

The present work represents an attempt to investigate the seizure phenomena induced by increasing the sodium concentration of the intraventricular fluid by the administration of hypertonic saline, both behaviourally and by subcortical and cortical electroencephalographic recording Experiments were performed in twelve cats with chronically implanted intraventricular cannulæ and stainless steel electrodes placed stereotaxically in cerebral cortex, amygdala, hippocampus, caudate nucleus, thalamus, central grey and hypothalamus The animals were examined in a recording box in which they could move about freely while being observed through a one-way vision window After a series of experiments the brain from the animal was fixed by perfusion, the electrode and

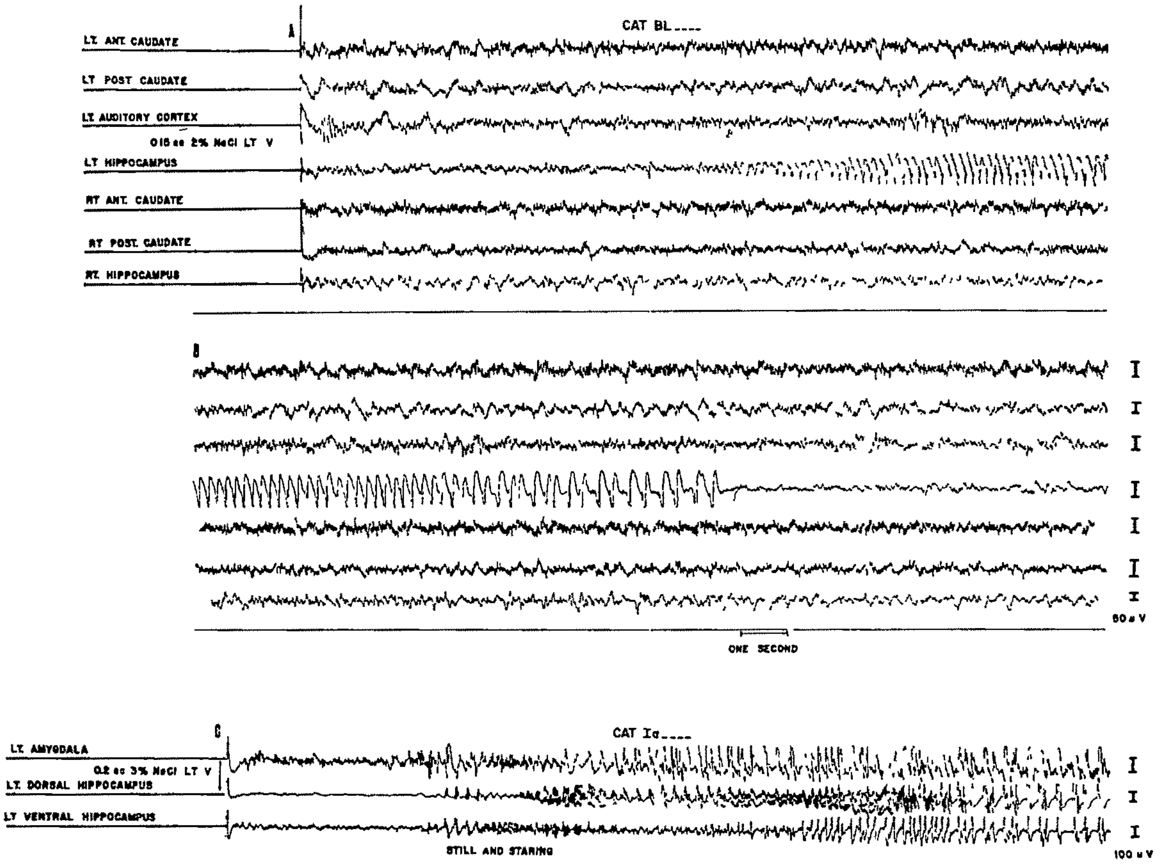

Fig 1 *A*, Electrographic seizure activity developing in left hippocampus and remaining localized therein following injection of 2 per cent sodium chloride (0 15 c c) into left lateral ventricle *B*, Continuation of discharge in left hippocampus Total duration 21 sec *C*, Electrographic seizure activity developing in left amygdala and hippocampus following injection of 3 per cent sodium chloride (0 2 c c) into left lateral ventricle During this period of discharge, which lasted for 32 sec (only the initial portion is shown here), the cat remained transfixed in a still position and stared ahead, occasionally with licking movements

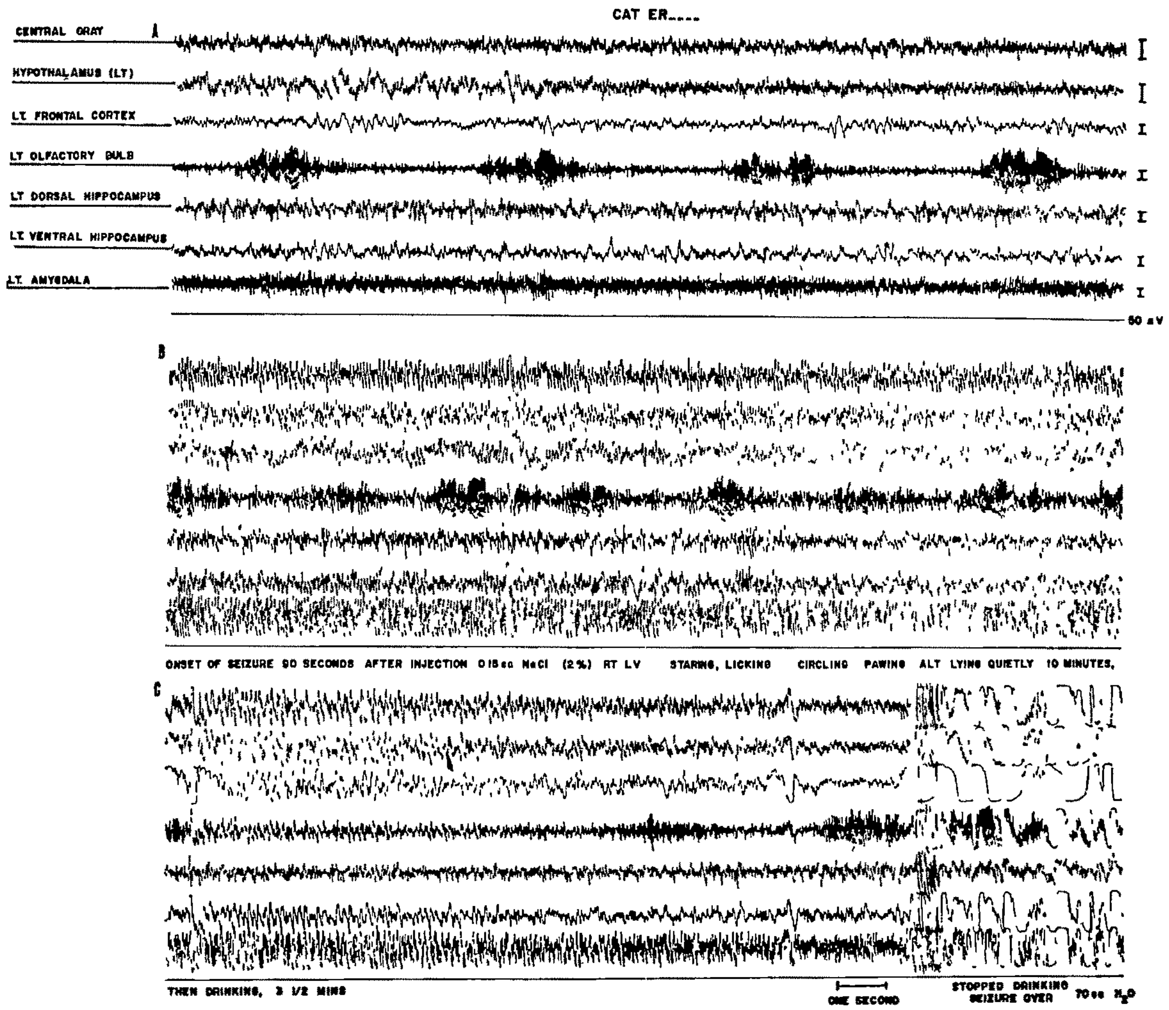

Fig 2 *A*, Control electroencephalographic recording from subcortical and cortical structures *B*, Recording during seizure induced by injection of 2 per cent sodium chloride (0 15 c c) into right lateral ventricle For a 10-min period the cat alternately circled, pawed, stared, licked lips or lay quietly *C*, Recording during the terminal 3 5-min phase of the seizure while the animal was drinking 70 c c of water

cannula placements were confirmed histologically and the tissues were evaluated for pathological changes

Control experiments included the intraventricular administration of isotonic sodium chloride solution and hypertonic mannitol and sorbitol, of the same osmolarity as the salt solutions None of these injections induced any significant behavioural or electrographic changes

The hypertonic sodium chloride injections were of 2 or 3 per cent solution in amounts of 0 15 c c or 0 2 c c These intraventricular administrations of hypertonic saline induced varying seizure phenomena in the same and different animals Seizure discharges occasionally appeared isolated to the hippocampus ipsilateral to the injection, beginning within 10 sec and lasting up to 30 sec, and were not associated with behavioural alterations At times staring and arrest of movement appeared briefly, associated with discharges involving hippocampus and amygdala (Fig 1).

Generalized seizure activity also occurred (Fig 2), probably originating in the hippocampus, then involving other subcortical structures amygdala, caudate, thalamus, hypothalamus and central grey Spread to cortex was minimal The electrographic discharges were associated with complex behavioural seizures lasting 10 or more min with staring, circling, drooling and finally heavy drinking of water

In some experiments with implantations of numerous recording electrodes in one hemisphere the ventricular cannula was placed into the opposite lateral ventricle (Fig 2) The electrographic effects of contralateral injections had a latency which varied from 10 sec to 3 min, although behavioural changes appeared within 30–60 sec In one experiment, a prolonged depression and desynchronization of caudate activity followed by slow-wave discharges occurred in the absence of changes elsewhere In another experiment a seizure discharge rapidly spread to auditory cortex and remained there for more than 1 min without any significant behavioural change The repetition of intraventricular injections of hypertonic sodium chloride usually produced a temporary refractory state Seizures could be induced two or three times in a series of experiments, then no longer for several days or weeks No significant increases in intraventricular pressure were observed There were no discernible tissue lesions other than those usually produced by implanted electrodes and cannulæ

The intraventricular injections in these experiments increased the total intraventricular fluid volume by only at most 4 per cent, which is not regarded to be significant It is regarded that the seizure phenomena observed were in some way related to the increase in the sodium concentration of the intraventricular fluid and not a change in tissue hydration since the effects were not produced by hypertonic sugar solutions The amount of sodium added by the hypertonic injection is in the range of 20–25 m mole/l , an increase of about 17 per cent This increase would occur initially, at probably a higher level, before diffusion, in the lateral ventricular region, which occupies an extensive area bathing the hippocampus, the region usually initially developing seizure discharges, which then spread to amygdala and related structures These limbic masses are known to have relatively low seizure thresholds[10] Behaviourally, the induced phenomena resembled psychomotor-temporal lobe seizures, with certain aspects of caudate and hypothalamic involvement as well

It may be postulated, therefore, that sodium diffusion occurs across the ependymal wall into glia and neurones, eventually increasing intraneuronal sodium concentration and producing thereby a state of oscillating depolarization, hyper-excitability and seizures[1] Since an increase in intraventricular potassium also induces seizures, the reciprocal relationships between these cations need further evaluation That a certain specificity of effect exists, possibly in relationship to ventricular wall osmoreceptors or specific cationic sensitive cells, is indicated by the drinking behaviour produced by sodium but not by potassium injections Further study is required of the actual distribution of these ions into cerebral tissues following intraventricular administration and the time factors involved

Of interest in relation to the work recorded here is the production of increased spinal reflex excitability by intracarotid injections of hypertonic saline, presumably mediated by supraspinal mechanisms[11]

A conclusion that a shift of sodium alone is of primary responsibility in the induction of an epileptic state in neurones would be quite unwarranted However, these experiments and the related data from the literature are quite suggestive of a pattern that does involve a major intraneuronal shift of sodium, in the important regulation of membrane stability There probably is a complex interplay between sodium, potassium, cerebral transmitters (as acetylcholine) and amino-acids[1]

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PROTECTIVE EFFECT OF DEEP-BODY HYPOTHERMIA ON X-RAY-INDUCED MITOTIC ABERRATIONS OF REGENERATING RAT LIVER

By DR. M. J. ASHWOOD-SMITH* and DR. G. F LEONG

U.S. Naval Radiological Defense Laboratory,
San Francisco, California

EARLIER investigations have demonstrated that in adult rats exposed to X-rays a high incidence of abnormal mitotic figures occurs after partial resection of hepatic tissue[1–3] This preponderance of mitotic aberrations which can be clearly identified in the anaphase and telophase stages of division has been shown to persist undiminished for long periods of time[2–4] and also after multiple episodes of induced cell proliferation[3] (repetitive partial hepatectomies) This radiation damage to the mitotic apparatus of the parenchymal cells apparently remains dormant for months after irradiation exposure without any detectable alterations in liver function[5] However, when the previously irradiated cells are stimulated to divide by partial hepatectomy, the mitotic aberrations are increased 5–7-fold Hornsey[6] has demonstrated that there is a 2 8 dose reduction factor in terms of LD_{50} when mice are irradiated under conditions of deep-body hypothermia (+1° C) Bone marrow and lymphoid tissue of mice irradiated under hypothermic conditions are protected, this protection is probably associated with the low oxygen tensions present in these tissues during irradiation[7] It is well known that radiation-induced chromosome aberrations both in plant and animal cells are reduced when the irradiation occurs under anoxic or relatively anoxic conditions[8] It is the purpose of this article to describe the protective effect of hypothermia against radiation injury with respect to growth rate, regenerative capacity of the liver, and mitotic aberrations of parenchymal cells

Rats (male Sprague–Dawley, *NRDL* strain) were divided into four groups of 10 rats per group One group acted as controls, the second group was irradiated at normal body temperature, the third group was irradiated when the rectal temperatures of the rats had been reduced to about 1° C The last group was rendered hypothermic but not irradiated All irradiations were carried out when the animals were four weeks old and the partial hepatectomies to stimulate liver regeneration were performed eleven weeks later Water and food ('Purina' laboratory chow) were supplied *ad libitum*

The closed jar method of Giaja and Andjus[9] was used to render rats hypothermic When the rectal temperature reached about 17° C and respiration ceased, the rats were taken out of the bottles and packed in ice After another 20 min the rectal temperature had fallen to about 1° C, at which temperature the rats were either irradiated or sham irradiated Resuscitation of the hypothermic rats by the technique of Andjus and Smith[10] was started immediately after irradiation These animals usually recovered within 40 min after removal from the ice bath Rats were exposed to a single whole body dose of 450-rads X-rays (250 kVp , 15 m amp , filter 0 5 mm copper and 1 mm aluminium, half-value layer 1 4 mm copper, *TSD* 40 in , dose rate 50 rads/min) The non-hypothermic rats were irradiated in 'Perspex' restrainers and received the same total dose and dose rates as the hypothermic animals which were packed in ice (radiation dose corrected for scatter)

To minimize possible variations due to the diurnal cycle of the rat[11] surgery was scheduled so that all animals would be killed in the morning hours The procedure of Higgins and Anderson[12] was used for the partial hepatectomy All animals were subjected to a single partial hepatectomy eleven weeks after they had been subjected to either hypothermia, irradiation, or a combination of both A control group which received no treatment was similarly partially hepatectomized Animals were killed by exsanguination 36 h after partial hepatectomy Histological specimens were taken from each lobe of every liver and the tissue fixed in Tellyesniczky's solution (ethyl

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* Present address Medical Research Council, Radiobiological Research Unit, Harwell, Didcot

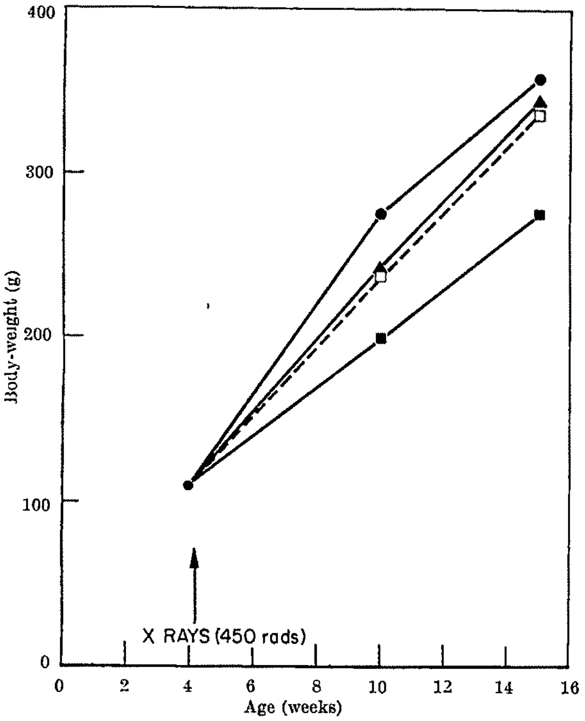

Fig. 1. Effect of hypothermia on radiation-induced body-weight changes in rats ●, Control, ▲, hypothermia, ■, radiation, □, radiation plus hypothermia

alcohol, glacial acetic acid, and formalin) for 8–12 h, dehydrated, embedded in paraffin, sectioned at 5μ, and stained with Harris's hæmatoxylin and eosin.

Mitotic aberrations and mitotic indices were determined and counted under oil immersion (×800). Although abnormalities were observed in all stages of mitoses, only those occurring during anaphase and telophase were evaluated for abnormalities from every liver. Results of counts are expressed as the ratio of abnormal figures to the total number of figures viewed.

Mitotic index is expressed as the number of mitoses per one thousand parenchymal cells counted. All mitoses from late prophase to late telophase in one hundred fields of each slide were counted, but only the parenchymal cells in every fifth field were enumerated. The reliability of this counting procedure was tested by counting all mitoses and parenchymal cells in one hundred consecutive fields on several slides from each group. With these procedures a minimum of four thousand to five thousand cells per slide was viewed.

A comparison of the body-weight changes in the four groups of animals is shown in Fig. 1. These results demonstrate that hypothermia alone or hypothermia and X-rays retard body growth of animals. A single dose of irradiation has a greater effect on the growth rate of these animals as compared with hypothermia alone. However, hypothermia all but abolished the detrimental effect of X-irradiation on the future growth of these animals.

The mitotic indices of the regenerating rat liver in all four groups are shown in Fig. 2. The mitotic index of the control group was significantly higher than that of the X-ray group, 52 mitoses per one thousand parenchymal cells as compared with 32 mitoses per one thousand parenchymal cells ($P < 0{\cdot}01$). At 36 h after partial hepatectomy no detectable differences in mitotic indices could be distinguished between hypothermic and control animals. The animals subjected to both hypothermia and X-rays did not appear to be significantly different as compared with hypothermia alone or with the control group. However, there appears to be a suggestion of a slight protective effect of the hypothermia against the X-rays when these two groups are compared ($P = 0{\cdot}05 - 0{\cdot}1$).

The percentage (± S.D.) abnormal mitoses occurring in all four groups at 36 h after partial hepatectomy is shown in Fig. 3. The control values are 7·1 per cent ± 1·7 whereas the values for the X-irradiated animals are 36·6 per cent ± 4·6. Animals subjected to hypothermia alone yielded values of approximately 9·1 per cent ± 2·5 and the animals subjected to both X-ray and hypothermia yielded values of 9·4 per cent ± 2·2. These results demonstrate that hypothermia alone does not have any effect on the incidence of abnormal mitoses, whereas the damaging effects of X-rays are completely abolished by concomitant hypothermic conditions.

There have been numerous papers published on the effect of oxygen in enhancing radiation-induced chromosome damage since the original observations of Thoday and Read[13]. The effect of temperature in modifying chromosome damage has been investigated by several workers. Sax and Enzmann[14] found that the frequency of X-ray-induced aberrations in *Tradescantia* microspores increased with decreasing irradiation temperature and that the yield of aberrations was roughly four times greater at 3° C than at 36° C. The solubility of oxygen increases with decreasing temperatures and it is quite possible that the greater number of aberrations seen at lower temperatures may have been associated, in part, with the greater number of oxygen atoms available for combination with the free radicals produced from the radiolysis of water.

Recent work has suggested that the high number of aberrations seen after irradiation at temperatures just above 0° C does not occur when oxygen is absent during irradiation. Baker and Sgourakis[15] have shown that the number of recessive lethals induced in *Drosophila* by irradiation was temperature-dependent only in the presence of oxygen, if irradiation was carried out in the presence of nitrogen then no temperature effect could be detected. A slight increase of the chromosome aberrations in *Tradescantia* with decreasing temperature has been reported by Giles[16] when the partial pressure of oxygen was kept constant. More recent work by Beatty and Beatty[17] has demonstrated that the frequency of aberrations increases with increasing temperature when microspores are irradiated under anoxic condition. A

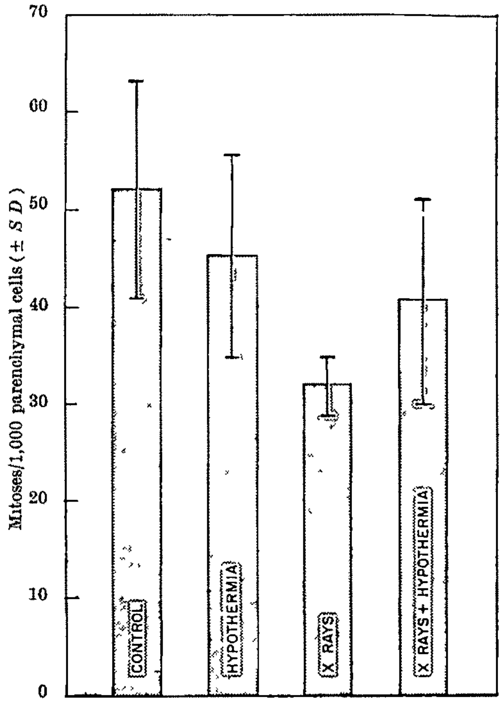

Fig. 2. Effect of hypothermia and X-rays on the mitotic index of regenerating rat liver

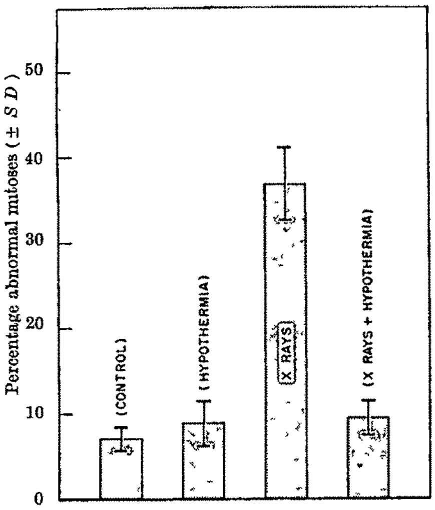

Fig 3 Protective effect of hypothermia on radiation-induced mitotic aberrations in regenerating rat liver

small decrease in the number of aberrations was noted when the temperature was lowered and irradiation performed in the presence of oxygen A slight increase in the number of aberrations is seen when Erhlich ascites tumour cells are irradiated under either anoxic or normal conditions as the irradiation temperature increases[18]

There have been several attempts to prevent radiation-induced chromosome damage by pretreatment of cells with chemicals A significant reduction in the number of aberrations induced by X-rays in *Tradescantia* has been reported by Mikaelson[19] provided that the tissues were pretreated with glutathione or cysteine Sodium hydrosulphite and BAL are also effective in reducing chromosome damage[20,21] However, the extent of the reduction was no greater than would have been expected to have occurred from the decreased oxygen tension produced by these two reducing chemicals The same degree of protection was achieved by irradiation under anoxic conditions

Weiss[7,22] has shown that oxygen tensions are very low in the tissues of mice subjected to hypothermia (rectal temperatures in the regions of +1° C) This amount of anoxia was sufficient to give considerable protection to the testes, bone marrow and lymphoid tissues of mice irradiated while hypothermic It is not surprising to find, in these present experiments, that hypothermia has protected rats from the body-weight changes induced by radiation and there is a suggestion that the induction of mitotic delay by radiation has also been slightly affected by hypothermia, however, this latter effect is not statistically significant The dramatic reduction afforded by hypothermia against radiation-induced mitotic aberrations was unexpected A reduction of 2- or 3-fold in the number of the aberrations would, perhaps, not have been unexpected in view of the probable anoxic state of the liver cells The consensus of opinion with regard to chromosome aberrations and temperature is that under anoxic conditions temperature *per se* has but a slight effect on the frequency of radiation-induced aberrations A large number of aberrations would probably have resulted during the low-temperature irradiations had oxygen tensions been within normal physiological limits There is the possibility of repair occurring during the long interval between X-ray exposure and cell proliferation and that this repair may be further enhanced by hypothermia However, this explanation does not seem likely since others[2,4] have shown that these abnormal mitotic figures do not diminish over long periods of time Although the present results do not allow distinction between temperature effects and oxygen effects it would seem unlikely that low temperature is the predominant protective factor The most reasonable suggestion seems to be that in liver parenchymal cells in interphase most of the observable mitotic aberrations induced by X-rays are oxygen-dependent and can be prevented by deep-body hypothermia

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EFFECT OF THE INTRODUCTION OF AN IRRELEVANT STIMULUS ON RUNWAY PERFORMANCE OF THE HIPPOCAMPECTOMIZED RAT

By WARREN O. WICKELGREN* and PROF R. L. ISAACSON

Department of Psychology, University of Michigan, Ann Arbor

IT is now evident that rats with hippocampal destruction have difficulty in terminating certain kinds of responses This is evidenced by greater resistance to extinction of such animals in a shuttlebox avoidance problem[1] and in the straight runway[2] Furthermore, hippocampectomized animals have difficulty in forming a passive avoidance response[3], and one interpretation of this observation is that these animals have an inability to stop making the dominant response in this situation

In experiments testing the ability to make passive avoidance responses, however, the animal's approach response is penalized by a noxious stimulus, usually an electrical shock The question arises as to the effects of introducing an affectively neutral, and task-irrelevant, stimulus while animals with hippocampal lesions are performing a well-established response Would the introduction of this kind of stimulus interrupt the ongoing behaviour patterns of the hippocampectomized animals

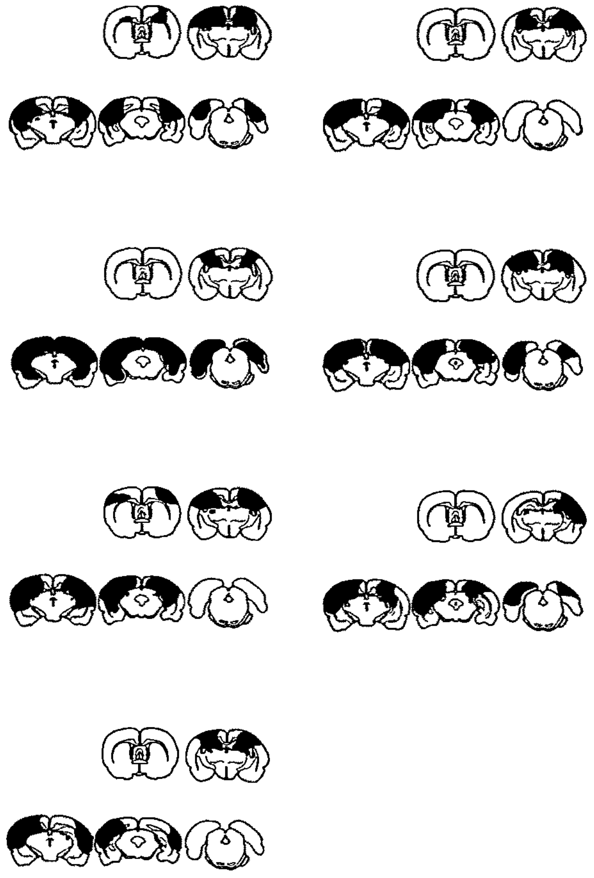

Fig 1 Diagrams of the extent of damage of all animals receiving hippocampal lesions. Thalamic gliosis indicated by stippled areas

to the same extent it would in control animals? In this experiment we attempted to assay the effects of adding an irrelevant stimulus without noxious qualities to a simple, well-established response in hippocampally ablated animals and in two control groups One control group received extensive neocortical damage and the other control group received only sham operations

The subjects were 19 male hooded rats of the Sprague–Dawley strain which were approximately 120 days old at the time of operation Seven animals received bilateral hippocampal lesions, 5 received neocortical lesions, and 7 received sham operations in which no brain tissue was damaged

The operative procedure was similar to that previously described except that the animals were not held in a stereotaxic instrument[1] The sham operated animals received all the surgical procedures used with the brain-lesioned groups except that no brain tissue was damaged After the surgery, the animals were kept in group cages for 2 days and during these post-operative days received 'Tetracyn' (tetracycline) in their drinking water Then, the animals were placed in individual cages for the remainder of the experiment

The apparatus was a straight-alley runway constructed of ¼-in plywood, painted black, with internal dimensions 54 in × 4 in , and 6 in high One guillotine door was located 11 in down the runway, providing a startbox, and a second door was located 21 in from the end of the runway and was used to prevent retracing Response latencies were measured from the raising of the startbox door and were stopped when the animal crossed a photoelectric cell unit located 5 in down the runway from the start door

Thirty days after undergoing surgery, all animals were placed on a deprivation schedule in which they were fed 15 g of 'Purina' laboratory chow, which kept all the animals at about 85 per cent normal body-weight Each subject was weighed and handled for about 5 min before being fed its daily ration Preliminary training was begun 9 days after the beginning of the deprivation period and consisted of giving each animal a 5-min period in which to explore the runway on each of two successive days The time taken to traverse the runway was recorded with no attempt made to prevent retracing After each daily preliminary trial, the subjects were placed in a small box which contained the food cup with two 45-mg Noyes Co food pellets and several drops of water The animal remained there until it had eaten the pellets and was then removed while two other pellets and drops of water were placed in the food cup This procedure was continued until every animal had been placed in the box and eaten the pellets 5 times

Acquisition consisted of 10 trials a day for 7 days with a reward of 2 food pellets at the end of the runway on each trial Each animal was allowed 10 sec to eat the pellets and was then replaced in its holding cage while the latency and running times were recorded The inter-trial interval varied between 30 and 45 sec A small fan in the experimental room emitted a constant noise and was used to mask other sounds All animals were run between 7 and 11 p m in two squads

On the eighth and ninth days of training, wooden strips on which sandpaper had been stapled were placed over the regular floor of the runway The startbox flooring remained the same, so that when the startbox door was opened there was a slight step up to the coarse, lighter-coloured flooring Ten rewarded trials were given on the days on which the sandpaper floors were added

After the experiment, the animals were killed and intracardially perfused with 10 per cent formalin The brains were removed, dehydrated, and embedded in paraffin Sections were cut at 21-μ intervals through the lesions Every tenth section was retained and stained with thionin Histological examination of the sections indicated that about 75 per cent of the hippocampal formation had been destroyed in most of the hippocampally ablated animals Minor injury to the thalamus was found occasionally In general, neocortical destruction was greater in the neodecorticate control group than in the hippocampally lesioned group as a result of our attempts to equate the total amount of brain tissue destroyed between the two

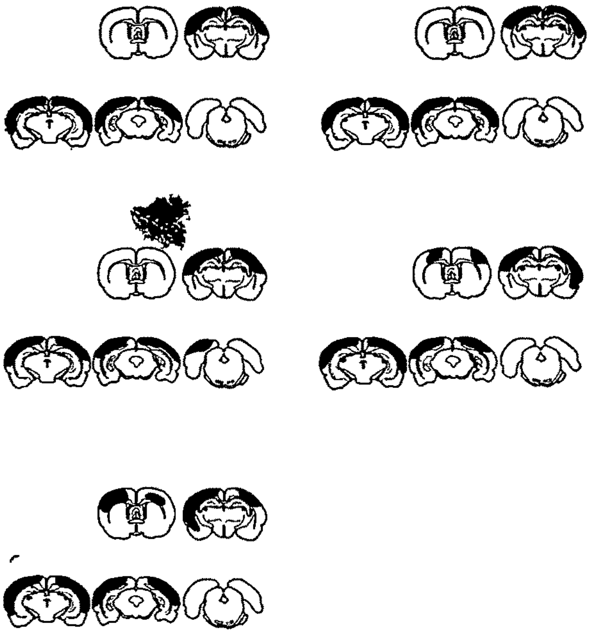

Fig 2 Diagrams of the extent of damage of all animals receiving neocortical lesions Thalamic gliosis indicated by stippled areas

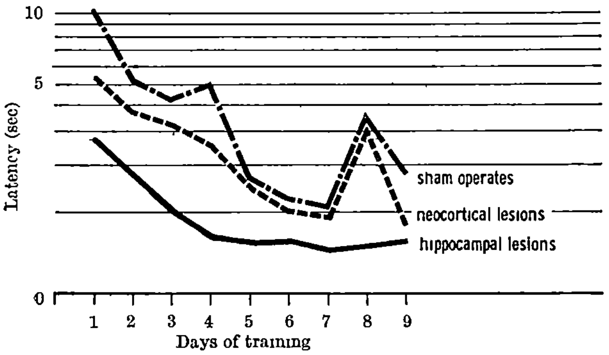

Fig. 3. Mean latency scores on the three groups of animals plotted on a logarithmic scale. Irrelevant stimuli were added to the runway on the trials given on days 8 and 9

brain-lesioned groups. In several hippocampectomized and neodecorticate animals gliosis was found in certain thalamic nuclei. The gliosis appeared to be a result of surgically induced infarction and was seen in the lateral and medial geniculate nuclei and to a much less extent the anterior and lateral nuclei. The degree of thalamic gliosis was found to be unrelated to the behavioural effects of the lesions. Figs. 1 and 2 present the extent of the lesions at 5 levels through the brains of the hippocampectomized and neodecorticated animals, determined by projecting the mounted brain sections on to standard drawings of a normal brain at that level.

The results of the experiment were clear cut. Because of the magnitude of the effect, it was felt that raw response latencies would best serve to illustrate the results.

Fig. 3 summarizes the results of the experiment in graphic form. The two control groups of animals showed a considerable increase in latencies when the irrelevant stimuli were introduced but the hippocampectomized animals did not reveal any discernible change. Due to the variability of the control groups, the differences between the groups during acquisition training do not reach significance at the 0.05-level, although the likelihood of such a consistently superior performance by the hippocampectomized rats over the first seven acquisition days occurring through chance alone is quite remote. Difference scores between the 7th acquisition day and the 1st day on which the irrelevant stimulus was presented (8th training day) for each animal yielded an F-ratio significant beyond the 0.05-level; t tests indicated a significant ($P < 0.02$) difference between hippocampal lesioned animals and those in the control groups on the 1st distraction day.

The results of this experiment fit in well with recent work in which hippocampally lesioned animals were found to perseverate an old response in the face of altered conditions. The hippocampally lesioned rats also seemed to acquire the response more rapidly than did the control groups and this agrees with other investigations in which hippocampal ablation facilitated certain kinds of learning[1].

These results extend our knowledge of the behaviour of hippocampectomized rats to show that the introduction of a novel but affectively neutral stimulus, which is irrelevant to the performance of the previously acquired running response, fails to inhibit running behaviour. Earlier reports had noted the diminution of effects of presentation of noxious stimuli in passive avoidance situations, and also a diminution of effects produced by stimuli which had been associated with noxious stimuli[4]. The explanation for this behavioural change is not unambiguous. These results would be expected on the bases of 'response inhibition theories' like those presented by McCleary and Kaada, which would suggest that animals with certain subcortical damages may notice and respond to changes in environmental stimuli, but cannot, because of the lesion, stop making the dominant response[5]. However, other types of explanations could account for our results. If the S's were merely deficient in abilities required to attend to, or orient toward, novel stimuli, this would be adequate to account for the present results. Along these lines, Lissak and Grastyan have reported evidence which links changes in the electrical activity of the hippocampus to periods when the animal is exhibiting the orienting reflex described by Pavlov[6]. It should also be mentioned that the functions of the orienting (investigatory) reflex are to cause the animal to orient toward the source of stimulus change and, at the same time, to inhibit conflicting response tendencies. Whether lesions in the hippocampus interfere with one or both of these behavioural functions remains to be established.

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* Now at Yale University, New Haven, Conn.

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EFFECT OF ACTINOMYCIN D ON INTERFERON PRODUCTION BY 'ACTIVE' AND 'INACTIVE' CHIKUNGUNYA VIRUS IN CHICK CELLS

By Dr. G. E. GIFFORD* and Dr. E. HELLER†
National Institute for Medical Research, Mill Hill, London, N.W.7

HELLER[1] has examined the effect of actinomycin *D* on the growth of Chikungunya virus in chick cells and on production of interferon resulting from such infection. He found that actinomycin markedly enhanced the growth of the virus and that interferon production was inhibited. On the basis of his kinetic investigations it seemed likely that the inhibition of interferon production by actinomycin was responsible for the enhancement of virus growth. However, it was also possible that the enhanced virus growth occurring as a result of treatment with actinomycin may have been responsible for the inhibition of interferon production in a manner analogous to the 'inverse interference' phenomenon described by Lindenmann[2]. This could occur if the production of interferon and virus by the same cell were not compatible phenomena.

We have compared the effect of actinomycin *D* on interferon production by stock preparations of Chikun-

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* Present address: Department of Microbiology, University of Florida College of Medicine, Gainesville.

† Present address: Department of Preventive Medicine, Hebrew University-Hadassah Medical School, Jerusalem.

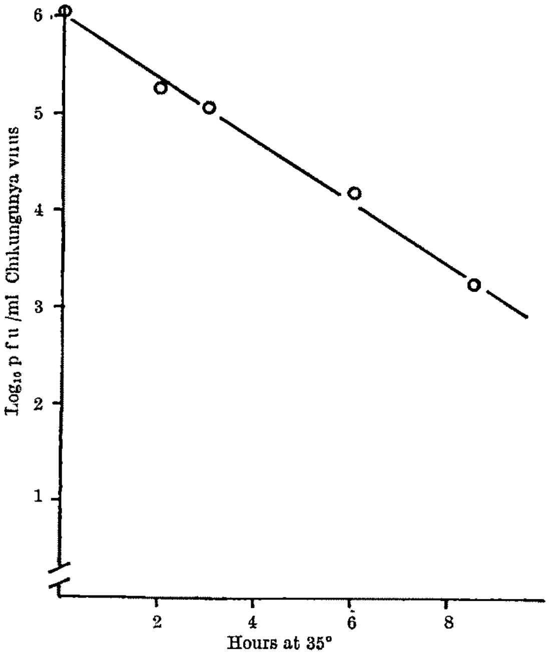

Fig 1 Rate of inactivation of Chikungunya virus at 35°

gunya virus and by preparations which have lost their plaque-forming ability as a result of incubation at 35° If the stimulation of virus growth by actinomycin was responsible for the inhibition of interferon production then actinomycin would have less inhibitory effect on interferon production by 'inactive' virus

Interferon and virus were assayed in chick cells infected with vaccinia virus by the method described by Lindenmann and Gifford[3] Chikungunya virus was assayed by a plaque technique on chick cells The medium used for interferon production consisted of Gey's balanced salt solution with 0 11 per cent sodium bicarbonate, 0 25 per cent lactalbumin hydrolysate, 0 1 per cent yeast extract, and 0 1 per cent proteose peptone Interferon-containing samples were heated at 65° for 30 min This procedure inactivated the infectivity of the virus and also its interfering ability (Heller, unpublished observations) Chikungunya virus was grown in the brains of new-born mice and vaccinia virus on the chlorioallantois of embryonated eggs Both viruses were stored in glass ampoules at – 60°

The rate of inactivation of the virus at 35° was investigated since this was the temperature utilized during adsorption of the virus on to chick cells A suspension of the virus was made in the described medium and placed at 35° At intervals, samples were removed and frozen at – 60° for plaque assay As seen in Fig 1, the virus was found to be rendered incapable of producing plaques at a rate of approximately 1 log$_{10}$ every 3 h

Chikungunya virus, incubated at 35° for 23 h, was tested for its ability to induce interferon in chick cells No plaque-forming virus was found in the inoculum, but good yields of interferon were obtained A comparison of this 'inactive' virus preparation with the stock preparation of Chikungunya virus was then made in the absence and presence of actinomycin *D* to determine if production of interferon would be inhibited Cells were treated with 0 06 μg/ml of actinomycin *D* for 4 h at 35° Medium was decanted and the cells washed with fresh medium Controls were treated in the same manner except that actinomycin was omitted Cells were then exposed to either the stock preparation or to the 'inactive' virus preparation of Chikungunya virus at the same dilution so that the number of physical virus particles was the same The cultures were incubated at 37° and after 24 h the media were collected, heated at 65° for 30 min, and assayed for interferon The results are shown in Table 1 Both preparations of virus induced the production of interferon, and actinomycin treatment inhibited interferon production almost completely in both cases The 'inactive' virus produced approximately 23 per cent more interferon than the 'active' preparation

Table 1 PRODUCTION OF INTERFERON BY 'ACTIVE' AND 'INACTIVE' CHIKUNGUNYA VIRUS AND ITS INHIBITION BY ACTINOMYCIN *D*

Nature of virus preparation*	Pretreatment with actinomycin †	Total PDD_{50} units interferon produced in 24 h ‡	Inhibition (%)
'Active'	—	312	—
,	+	18	94
'Inactive'	—	385	—
,,	+	13	97

* For details see text

† Actinomycin, 0 06 μg/ml for 4 h before virus, + = added, — = not added

‡ Assayed with vaccinia virus PDD_{50} = amount of interferon which depresses the plaque count to 50 per cent of controls[3]

To test whether actinomycin-treated cells produced some substance that interfered with or inactivated interferon, interferon was added to supernatants collected from actinomycin-treated infected cultures and the mixture titrated for interferon activity Sufficient interferon to inhibit 32 per cent of vaccinia virus plaques was added to the supernatant from infected, actinomycin-treated cultures which by itself would inhibit 21 per cent of vaccinia plaques The mixture was found to inhibit 39 per cent of vaccinia plaques which would be within the expected range of inhibition expected by a mixture of the two interferon preparations These results indicate that interferon-inhibiting material was not produced by infected and actinomycin-treated cultures

These findings suggest that the enhancement of virus growth by actinomycin was not a contributing factor in its ability to inhibit interferon production, and they provide further evidence for a direct effect of the antibiotic on some cellular mechanism involved in interferon production

Henderson and Taylor[4] have reported that Mayaro virus inactivated at 56° for 2 h was incapable of producing interferon Western equine encephalomyelitis virus, inactivated by prolonged incubation at 37°, was shown to be capable of interfering with active virus[5], but Lockart[6] afterwards showed that such inactive virus did not produce measurable amounts of interferon in *L* cells Vilček[7,8] found that tick-borne encephalitis virus inactivated by incubation at 56° or by prolonged incubation at 37° did not induce interferon production It is difficult to interpret the negative results since it is possible that the treatment may have rendered the virus incapable of entering the cells so that production of interferon could not be induced It is also possible that the stability of the viral nucleic acids to such treatment may differ with respect to interferon inducibility as well as to infectivity The results reported here are the first published evidence of interferon production by inactive arboviruses, although it has previously been shown that several arboviruses exposed to cells at relatively high temperatures at which little or no multiplication of virus occurred still produced large yields of interferon[9]

We thank Mr C Gilchrist and Mrs Barbara Knight for their assistance

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MONITORING OF SEA-WATER FOR IMPORTANT RADIOISOTOPES RELEASED BY NUCLEAR REACTORS

By DR NOBORU YAMAGATA and KIYOSHI IWASHIMA
Institute of Public Health, Tokyo

IN view of possible increase in the discharge rates of radioisotopes into the sea, an analytical technique to measure very low concentrations of radioisotopes in sea-water has been developed

The conventional method of scavenging, namely, the precipitation of mixed ferric hydroxide and barium sulphate, failed to prove itself because of its very slow filtration The fact that marine manganese nodules are enriched with heavy metals and the rare earths[1] inspired us to examine commercial manganese dioxide, and it was found an effective and easily filterable scavenger for most of the radioisotopes which were reported to be present in reactor coolant water

A radioisotope, carrier-free or of high specific activity, was added to 50 ml of sea-water and then the mixture was shaken with 0 50 g reagent-grade powdered manganese dioxide for 10 min in a slightly alkaline condition (*p*H 8 2) Table 1 summarizes the results of experiments Important corrosion products except chromium, and fission products other than strontium and cæsium, were effectively picked up by 1 per cent (w/v) quantity of powdered manganese dioxide Further experiments to find the optimum quantity of scavenger showed 0 35 per cent to be the minimum for effective removal

Table 1 REMOVAL OF RADIOISOTOPES IN SEA-WATER BY SHAKING WITH POWDERED MANGANESE DIOXIDE

Radioisotope	Removed (%)
^{54}Mn	100 1 ± 0 2
$^{55\ 59}$Fe	96 1 2 3
^{60}Co	100 0 0 1
^{65}Zn	99 9 0 1
^{95}Zr-^{95}Nb	99 9 0 1
^{106}Ru-^{106}Rh	100 0 0 1
^{144}Ce	99 8 0 1
^{51}Cr	14 5 1 2
^{89}Sr	39 3 0 7
^{137}Cs	1 8 0 1

Partial permissible concentrations of cobalt-60 and iron-59 in sea-water recommended by the U S National Academy of Sciences—National Research Council are respectively 3×10^{-9} and 5×10^{-9} μc /ml for harbours[2] Since 100 l of sea-water had to be processed in order to detect referred concentrations of radioisotopes, granular manganese dioxide (20–60 mesh) was examined for its time-saving and scavenging efficiency in the filtration in a larger-scale operation Three litres of sea-water containing cobalt-60 were mechanically agitated after the addition of ten grams of granular scavenger After 105 min of agitation 98 2 per cent of the activity was removed from the liquid phase, and fixation on the scavenger still remained after standing overnight.

One hundred litres of sea-water were collected in Sagami Bay, off Enoshima, at 35°16′N , 139°28′E on July 9, 1963, to check the feasibility in full-scale operation of the proposed method To prevent loss by possible adsorption of trace activities to the container, collected waters in five polyethylene containers, 20 l each, were treated *in situ* with 70 g each of granular manganese dioxide and transported to the laboratory After 2-h agitation and settling overnight, the scavenger, 350 g altogether, was filtered off and transferred into a polyethylene assay cylinder of 3 in diameter This was placed on top of the crystal (3 in × 3 in) of sodium iodide, inside 1-in -mercury and 4-in -lead shields, and the γ-spectrum taken for 1,000 min by means of a 256-channel pulse height analyser

Recognizable photo-peaks above the background response of the detector were 0 72–0 77 MeV, corresponding to zirconium-95 and its daughter niobium-95, and 0 52 MeV, corresponding to rhodium-106, the daughter of ruthenium-106 Comparison made with spectra from adding known amounts of the radioisotopes to 350 g of scavenger showed the concentration of 1 5×10^{-9} μc zirconium-95 + niobium-95 and 4 9×10^{-9} μc ruthenium-106 per millilitre of sea-water, the latter being calculated by neglecting the presence of ^{103}Ru–^{103}Rh

Table 2 DETECTION LIMITS OF THE PROPOSED METHOD FOR CERTAIN RADIOISOTOPES WHICH MAY BE PRESENT IN SEA-WATER WHEN APPLIED TO 100-l -SAMPLE

Radioisotope	Photo-peak energy MeV	Detection limit in sea-water μc /ml	Partial permissible concentration in sea-water* μc /ml
^{60}Co	1 17	1×10^{-9}	
	1 33	2×10^{-9}	3×10^{-9}
^{59}Fe	1 10	2×10^{-9}	
	1 29	3×10^{-9}	5×10^{-9}
^{65}Zn	1 12	1×10^{-9}	2×10^{-8}
^{95}Zr	0 72	2×10^{-10}	3×10^{-8}
^{95}Nb	0 77		5×10^{-7}
^{106}Ru	0 52(^{106}Rh)	2×10^{-9}	1×10^{-8}

*For harbours, estuaries, and inshore waters within 12 miles of the coast-line Recommended by N A S -N R C [1]

Detection limits of the proposed method are summarized in Table 2 for several important radioisotopes The limits for cobalt-60 and iron-59 are from a half to one-third of the recommended partial permissible concentrations for harbours while those for the other radioisotopes are even less This would indicate the practical usefulness of the proposed method for monitoring when distinction between cobalt-60, iron-59 and zinc-65 cannot be made easily, that is, when radioisotopes exist together The detection limit for cerium-144 would lie above its permissible concentration of 1×10^{-9} μc /ml because of the high background counting rate in the spectral region of low gamma energy Cæsium-137 remains in the filtrate from the scavenger and can be determined by a molybdophosphate scavenging method followed by gamma spectrometry, a report of which is in preparation

Neither the mechanism of pickup by manganese dioxide nor the physical states of trace elements in sea-water are clearly known and investigations are in progress About 8×10^{-3} μc of cobalt-60 in a 2-l sample of water from the thermal shield of a nuclear reactor was completely removed by the proposed method, this suggests the possibility of using manganese dioxide in place of organic ion-exchange resin in purifying hotter reactor water in closed-cycle reactor systems

Sea-water samples were collected from three locations between the end of July and the beginning of August 1963 (1) the Sasebo Harbour, Kyushu District, an important port at which the United States nuclear-powered ships are expected to call, 33°08′N , 129°43′E , (2) the Inland Sea, off the Kure Harbour, an important fishing area, 34°06′N , 132°37′E , (3) Tsuruga Bay, near the expected power reactor site, 35°44′N , 136°03′E Zirconium–niobium-95 and ruthenium-106, both of which are considered to have originated from radioactive fall-out released by nuclear weapon tests, were found in the samples in the following concentrations, respectively, 1 3 and $5{\cdot}9 \times 10^{-9}$ (location 1), 1 0 and 4 7×10^{-9} (location 2) and 2 6 and 11 8×10^{-9} μc /ml (location 3) The level of ruthenium-106 in the sea-water from location 3 indicated more than the recommended permissible level of 1×10^{-8} μc /ml for harbours However, the result for ruthenium-106 could not be taken as more than a maximum estimate because it was calculated by neglecting the presence of ruthenium-103 The higher levels of the fission products in the sea-water from Tsuruga Bay may be contributed to the heavy snow-fall in winter and leaching by river of the accumulated radionuclides

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OPTICAL ACTIVITY IN OILS DERIVED FROM COAL

By CHARLES ZAHN, STANLEY H. LANGER*, BERNARD D. BLAUSTEIN
and IRVING WENDER

Pittsburgh Coal Research Center, U.S. Bureau of Mines, Pittsburgh

COAL is a product of the progressive fossilization of plant materials. Since optically active substances are widespread in living matter, it is not surprising that optically active materials have been isolated from peat moss[1], peat[2], lignite[3] and from the montan wax of brown coal[4,5]. Both positive and negative rotations have been observed in > 180° C distillates of a saturated hydrocarbon oil from a benzene extract of a Saar bituminous coal[6]. Fractions of positive rotation have been found in neutral oils from tars of Saar and Rhine coals processed above 450° C (ref. 7).

During an examination of the composition of synthetic liquid fuels from coal at the U.S. Bureau of Mines, the question arose whether some of the optically active centres in coal would survive the rigours of high-temperature, high-pressure, catalytic hydrogenation. An exploratory study of a once-through re-cycle oil (used in the hydrogenation of Rock Springs, Wyoming, coal with ammonium molybdate catalyst at 450°–525° C and at 8,000 p.s.i.g.) disclosed that many distillates were indeed still optically active[6]. This finding prompted an examination into the nature and occurrences of optically active substances in other oils, including low-temperature carbonization tars (Table 1).

Table 1. COAL PRODUCTS EXAMINED FOR OPTICAL ACTIVITY

*Hydrogenation Products**	
Rock Springs, Wyoming, coal (*hvCb*)†	once-through re-cycle oil
West Kentucky and Pittsburgh coals	petrols‡
Velva, North Dakota lignite	middle oil (*A*)§
Pittsburgh seam coal (*hvAb*)†	middle oil (*B*).
Carbonization Products‖	
Sandow, Texas, lignite	tar, U.S. Bureau of Mines (*C*)
Sandow, Texas, lignite	tar, Aluminium Co. of America (*D*).
Pittsburgh seam coal	tar, Consolidation Coal Co. (*E*)

* These oils were products of the Bureau of Mines' pilot-plant operations at 450°–525° C and about 8,000 p.s.i.g. pressure, and with mainly ferrous sulphate as catalyst, excepting the re-cycle oil, for which ammonium molybdate was the catalyst.

† Designations of coal rank: high-volatile *C* bituminous and high-volatile *A* bituminous.

‡ An equi-volume mixture of petrols obtained separately from the coals.

§ These italicized letters are used in the text to designate the products.

‖ From low-temperature carbonizations.

Optical activity was observed in almost all the distillates from the carbonization tars and in the majority of the distillates from the hydrogenation oils. No activity was observed in the tar-acid and tar-base fractions examined. Optical activity probably would diminish with increasing coalification, but oils from coals of carbon content above 84 per cent were not examined. Generally, the methods of isolating optically active concentrates included distillation, liquid chromatography on silica gel, and adduction with urea. Although the individual compounds responsible for the optical activity in the various concentrates have not yet been isolated, the experimental findings indicate that, as the separations progressed, the optical activity segregated mainly with the branched-chain–naphthenic and hydroaromatic fractions, and occasionally with cyclic olefines. We believe that the observed optical activity is due to cyclic structures.

The distillate fractions from oils *B* and *E* (Table 1) gave only positive rotations, but those from the other sources exhibited positive and negative rotations. The lowest boiling, optically active distillate observed had b.p. 119°–124° C, α_D −0·19° (tar *C*). Mass spectral analysis of this and of some successive distillates showed that alkyl*cyclo*hexenes reached a maximum concentration simultaneously with the observed maximum in optical rotation. The fraction with these maxima had b.p. 124°–131° C, α_D −0·33°. Tar *D* also yielded active olefinic material, b.p. 121°–133° C. Mass spectrometry of a chromatographic eluate (α_D^{24} − 0·51°), before and after hydrogenation, indicated the major compounds present were C_8-cyclic olefines; the fragmentation patterns of the hydrogenated sample indicated a mixture of C_5- and C_6-ring compounds.

Besides activity in hydrocarbon fractions, the neutral oil from tar *C* showed activity in a fraction strongly adsorbed by silica gel. Infra-red and ultra-violet spectrometry of the most active distillate of the ethanol-eluted material (α_D^{24} −0·22°, b.p. 187° C) indicated the presence of an appreciable concentration of a molecule containing a carbonyl group conjugated to an olefinic group.

Fig. 1 indicates the optical rotations for the successive distillates of oil *A*. Two combinations of neutral-oil vacuum distillates from oil *A* (b.p. 230°–260°, 260°–275° C/760 mm) after chromatography on silica gel and treatment of the heptane-eluted oil with urea and chromatography of the non-adducting oil afforded a combined heptane-eluted concentrate, α −0·43. Mass spectrometry of this concentrate showed it contained C_{12}–C_{16} compounds with peak-height maxima at $C_{14}H_{28}$ and $C_{14}H_{26}$ and that the mixture consisted of about 10 per cent branched-chain paraffins and about 90 per cent uncondensed C_5- and C_6-rings and 2- and 3-ring condensed naphthenes. Ultra-violet spectrometry of the concentrate indicated the absence of aromatic compounds.

The two most active concentrates came from distillates, b.p. 260°–275° C, of oil *A*. The first concentrate had $[\alpha]_D^{26}$ +1·8 (*c* 20, heptane), the second $[\alpha]_D^{26}$ +7·4 (*c* 14, heptane). These samples contained appreciable concentrations of hydroaromatic or polyalkyl-substituted aromatic material as shown by: (*a*) their liquid-chromatographic behaviour, (*b*) ultra-violet spectra containing band maxima at 268, 272, 278 mμ, (*c*) low-ionizing voltage mass spectrometry of the first concentrate, which, although showing a wide range of molecular weights, chiefly gave peak-height maxima at 204 and 218 (alkylbenzenes ?) and 202 and 216 (alkylindanes or alkyltetralins ?).

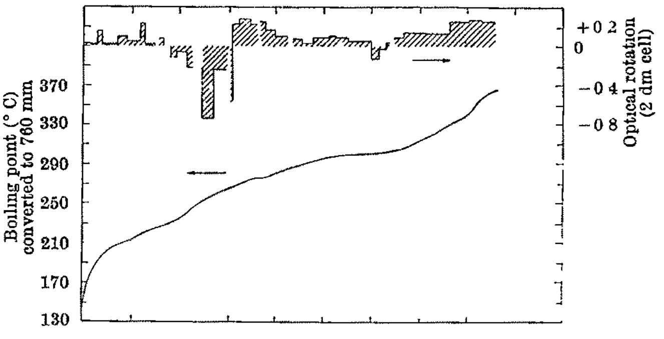

Fig. 1. Optical rotations for the neutral middle oil from hydrogenation of North Dakota lignite

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* Present address: American Cyanamid Co., Stamford, Connecticut.

Although our work has not ruled out the presence of optically active acyclic compounds in the oils, these compounds probably would have been racemized on heating with inorganic catalysts[9] or with carbon[10]

The general opinion is that both coal and petroleum resulted from biochemical and geochemical transformations of living matter Apparently, optically active naphthenic units persisted with petroleum[11] as well as with coal We observed optical rotation in coal-derived oil boiling as low as 120°, whereas it was reported[12] that activity begins only to appear in petroleum oil boiling at about 280° It must be realized, however, that this petroleum was an untreated natural product, whereas our coal-derived oils are mainly products of degradations that yielded lower-boiling active compounds

Retention of optical activity by the cyclic compounds in oils from thermal carbonization and catalytic hydrogenation of coals might be explained by degradation of the coal macromolecule into cyclic compounds possessing at least one optically active quaternary carbon atom, such compounds are probably highly resistant to racemization

Interestingly, optical activity has not been observed in lignin, in line with present-day theories of its formation and structure[13] Since the hydroxybenzene nucleus is a fundamental part of the lignin structure, the absence of optical activity in our phenolic fractions appears consistent with lignin being inactive Apparently the optically active material in coal arose from sources other than lignin, which has been proposed as a major progenitor of coal[14]

Other workers consider cellulose the primary source of coal[15] Conjecturably, cellulose may have been transformed by microbial action into products with optically active structural units that would persist in coal and survive catalytic hydrogenation. Various investigators[16] obtained coal-like products from the carbonization and catalytic hydrogenation of cellulose but none of these workers reported on observations for optical rotation However, the optically active materials in the oils from the coals probably derived mostly from the steroids[1] and the terpenes[2,3] in the coal precursors

We thank Dr R A Friedel for spectral analyses, and Mr Gus Pantages for assistance.

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DATING OF POLARITY ZONES IN THE HAWAIIAN ISLANDS

By Dr. IAN McDOUGALL and D. H. TARLING

Department of Geophysics, Australian National University, Canberra

PERIODICITY of reversal of the Earth's magnetic field is of some geophysical and geological interest Study of the polarity of the Earth's field throughout geological time provides much information relating to the origin of the field, and the stratigraphical zonation of polarities in rock sequences may offer a valuable means of geological correlation

Brunhes[1] observed that the natural remanent magnetization of some rocks has a polarity opposed to the present sense of the Earth's field, and Mercanton[2] showed that rocks of different ages from different parts of the world are magnetized in this sense These observations led to the supposition that periodic reversals of the Earth's magnetic field may have occurred during geological time Hospers[3,4] found that in a sequence of Cainozoic lavas in Iceland polarity zones occur, and that some of these zones are separated by a thin zone of rocks with intermediate directions Since then, several levels of reversal have been recognized, and it appears that reversals are frequent in the Cainozoic in which rocks magnetized in the same sense as the present Earth's field (that is, 'normal') are about as common as rocks with a direction of magnetization opposite to that of the present field (that is, 'reversed')

Self-reversal[5,6] and reversals of the Earth's magnetic field appear to be the only mechanisms able to account for the common occurrence of rocks with exactly opposed polarities It is possible to test the hypothesis of reversal of the Earth's field by comparing palæomagnetic observations from various parts of the world, as one of the consequences is that rocks of the same age, irrespective of composition and geographical location, possess the same polarity It is therefore of interest to obtain accurate dates on the reversal sequences This article attempts to give a stratigraphic table of polarity zones in the Upper Cainozoic on palæomagnetic measurements and isotopic age determinations on samples of lavas from the Hawaiian Islands A summary of the magnetic observations was given earlier[7], and a description of the dating methods and results is given elsewhere[8,9] These results are consistent with palæomagnetic and age measurements made[10] on rocks from six sites in California, but they suggest the presence of two additional polarity changes and allow more precise dating of each level of polarity change This work goes some way toward establishing a stratigraphical sequence of polarity zones during the Upper Cainozoic against which other palæomagnetic results can be compared to test the hypothesis of reversal of the Earth's field

Sampling for palæomagnetic and age measurements was undertaken separately in the Hawaiian Islands, although in a number of cases the same sample, or samples from the same outcrop, were used for both measurements Hence, there is some uncertainty in the correlation between the measured ages and polarities observed in the same formation, but it is considered that the pattern that emerges is essentially correct, particularly as the age measurements show that many of the formations (as defined by Stearns and Macdonald and summarized by Stearns[11]) were erupted over periods of 200,000 years or less

The dates were determined on whole-rock samples by the potassium–argon method. Argon was measured by isotope dilution and potassium by flame photometry. Determinations were made in duplicate in most cases, and the plus and minus figures quoted after an age indicate the range. The ages generally are precise to better than ± 3 per cent at the 95 per cent confidence-level.

A total of 164 samples were collected for palæomagnetic study from 59 sites in five of the Hawaiian Islands. The samples appear to be magnetically stable as the directions of magnetization remain constant in alternating fields[12] up to 750 œrsteds (peak), and only directions observed in samples after treatment in a magnetic field of 150 œrsteds (peak)[13] are considered here. Stability is also indicated by a high average coercivity of maximum isothermal remanence (445 œrsteds) and the consistency of results both within and between different sites in the same formation. The direction observed is thought to reflect the actual direction of the past magnetic field as 16 specimens that were heated and then cooled in the present Earth's field at Canberra (0·59 œrsteds) acquired directions parallel to the applied field. The results are listed in Table 1 and illustrated in Fig. 1.

Table 1. PALÆOMAGNETIC AND AGE DATA ON ROCKS FROM THE HAWAIIAN ISLANDS

Formation	Polarity (*N*, 'normal', *R*, 'reversed')	No. of palæomagnetic sites	K–Ar age (m.y.)	No. of samples dated, or sample No.
Hana (East Maui)	*N*	3	< 0·4	—
Kula (East Maui)	*N*	6	0·86, 0·43	2
Honomanu (East Maui)	*R*	1	> 0·86	—
Honolua (West Maui)	*R*	1	1·15 ± 0·02	4
Wailuku (West Maui)	*R*	5	1·29 ± 0·03	3
Lanai	*R*	3	—	—
East Molokai	*R*	6	1·3 –1·5	5
West Molokai	*R*	4	1·85 ± 0·01	1
Koolau (East Oahu)	*R*	8	2·2 –2·5	5
Waianae (West Oahu)	*N*	1	2·76 ± 0·02	*GA* 556
	N	1	2·84 ± 0·02	*GA* 809
	R	8	2·95 ± 0·06	*GA* 557
	N	2	3·27 ± 0·04	*GA* 810
Koloa (Kauai)	*N* and *R*	2	—	—
Napali (Kauai)	*N*	3	4·5 –5·6	3

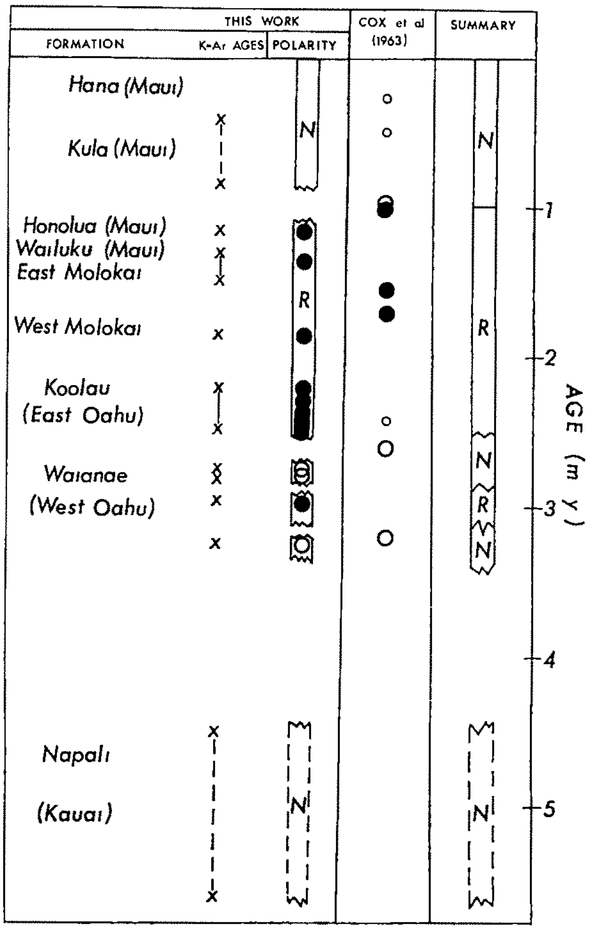

Fig. 1. Summary of palæomagnetic and age data. (Filled circles, 'reversed' polarity, open circles, 'normal' polarity.)

The polarity of the Kula and Hana Volcanic Series of East Maui is 'normal'. Potassium–argon age measurements on two samples from the Kula Series yield ages of 0·45 ± 0·02 and 0·84 ± 0·04 m.y. The older age was obtained on a sample from a lava immediately overlying the Honomanu Series, hence, this age probably closely dates the beginning of the eruption of the Kula Series. It is therefore likely that the specimens on which the magnetic measurements were made are not older than 0·84 m.y. The Hana Volcanic Series, which overlies the Kula Series, must be younger than 0·45 m.y. One site in the Honomanu Series is 'reversed', but the age of these lavas only is known to be older than 0·84 m.y.

Samples from a single site in the Honolua Series of West Maui are 'reversed'. A specimen from the same lava flow yields an age of 1·16 ± 0·02 m.y., and three other samples from the Honolua Series have measured ages agreeing at 1·15 ± 0·02 m.y. Hence, the results suggest that between 0·84 and 1·15 m.y. a change in polarity occurs.

The Wailuku Series of West Maui, underlying the Honolua Series, has 'reversed' polarity, and three samples from this formation yield ages agreeing at 1·29 ± 0·03 m.y. One of the six sites sampled for magnetic measurements has 'normal' polarity, but as the inclination is negative (–49) compared with the average of all other 'normal' sites (+35) from Hawaii this result is clearly anomalous and is not included.

The seven East Molokai sites show 'reversed' polarity, and age measurements of five samples indicate that the formation is 1·3 to 1·5 m.y. old. Two of the samples used for the age determinations are probably from the same lava flows as two of the sites sampled for magnetic measurements. The West Molokai lavas also have 'reversed' polarity, and the single specimen dated from this formation has an age of 1·85 ± 0·01 m.y.

Sites on the Island of Lanai are all 'reversed', but no age measurements were made on these rocks. As Lanai lies between Molokai and Maui it may be inferred that the age of these volcanics is probably about 1·3 m.y.; this is consistent with the 'reversed' polarity of the Lanai rocks.

Samples from five of the eight sites in the Koolau Series of East Oahu were also used for the age determinations. All the sites are 'reversed', and the ages range from 2·2 to 2·5 m.y. Two sites sampled in the Honolulu basalt are 'normal', but these were not dated, and only are known to be less than 2·2 m.y.

Two samples from the Upper Waianae Series of West Oahu yield ages of 2·76 ± 0·02 and 2·84 ± 0·02 m.y., and have 'normal' polarity. A lava of the Lower Waianae Series dated at 2·95 ± 0·06 m.y. has 'reversed' polarity, and a further sample from the same formation at a different locality has 'normal' polarity and is dated at 3·27 ± 0·04 m.y. Other sites in the Waianae Series also have 'reversed' polarity.

Three samples from the Napali Formation of Kauai[14] yield ages ranging from 4·5 to 5·6 m.y., and the polarity at three different sites is 'normal'. However, as the sites from which the samples for magnetic and age measurements were collected are widely separated, it is not possible to make precise correlations. Samples also were dated from the Makaweli Formation of Kauai, but no rocks were collected for palæomagnetic measurement. In the Koloa Series of Kauai both 'normal' and 'reversed' polarities were found. The single specimen dated from this formation is not from the same locality as any of the palæomagnetic sites, hence no correlation is possible

It may be assumed that the 'reversed' polarities observed in Hawaiian rocks are caused by reversal of the Earth's magnetic field This is supported by the observation that rocks of each polarity occur in discrete stratigraphic zones, and that in each of these zones the composition of the lavas ranges from picrite basalt to trachyte In addition, there is no evidence of self-reversal in specimens examined in the laboratory However, these observations do not refute the possibility of self-reversal, and it is necessary to compare the results from Hawaii with results from rocks of similar age in other parts of the world if the hypothesis of reversal of the Earth's field is to be substantiated

Our data are consistent with the change from 'normal' to 'reversed' polarity observed[10] in California (Fig 1) at about 1 m y, but suggest that a return to 'normal' polarity occurred between 2 5 and 2 75 m y, rather than at about 2 m y as inferred by Cox *et al* [10] They recorded 'normal' polarity in a rock dated at 2 6 ± 0 1 m y. and refer to Rutten[15], who reported 'normal' polarity in a sequence in Italy dated at about 2 4 m y These data, combined with our measurements, suggest that the change from 'reversed' back to 'normal' polarity occurred at 2·5 ± 0 1 m y The 'reversed' polarity found in a rock from the Waianae Series with a measured age of 2 95 m y indicates that the behaviour of the magnetic field is more complex than has been suggested previously. Owing to the lack of data it is not possible to state whether 'normal' polarities found in rocks dated at 3 3 and about 4 5 m y belong to the same magnetic epoch or whether a zone of 'reversed' polarity occurs during this period Our results are consistent with varying frequency of change in the polarity of the Earth's magnetic field in the Upper Cainozoic, rather than a regular change about every 1 m y [10].

Roche[16,17] showed that in France lavas of Upper Pleistocene age are normally magnetized, and that lavas of Villafranchian (Lower Pleistocene) age have 'reversed' polarity Hence, the change in polarity at about 1 m y found in the Hawaiian Islands and in California may be correlated with that found in France The stratigraphic position of the return to 'normal' polarity has not been established in France, but it is either in the lower part of the Villafranchian or in the Astian[18], although Rutten[19] suggested that it occurs at the base of the Villafranchian Clearly it is of importance to establish the position of this boundary in order that correlations may be made with greater certainty throughout the world Fourteen polarity zones were reported by Khranov[20] from sequences of Upper Cainozoic age in Russia, but at this stage it would be premature to attempt direct correlations

The evidence from Hawaii supports, but does not prove, the hypothesis of changes in polarity of the Earth's magnetic field Further study on rocks of similar age clearly is necessary to confirm the hypothesis, and to place closer limits as to the times when polarity changes occurred The present work suggests that models of the Earth's magnetic field should account for irregular periodicity of reversal

We thank E Irving for his advice

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ABSORPTION IN RADIO SOURCES OF HIGH BRIGHTNESS TEMPERATURE

By P. J. S. WILLIAMS

Mullard Radio Astronomy Observatory, Cavendish Laboratory, University of Cambridge

THE discrete radio sources have continuous spectra which generally follow a power law, $S \propto \nu^{-\alpha}$ (S = flux density, ν = frequency), and the usual graph of log S against log ν is a straight line It is generally believed that most sources are galaxies and that the radiation is synchrotron radiation from relativistic electrons, the straight radio spectrum then implies a population of relativistic electrons with an energy distribution which also follows a power law $N(E) \propto E^{-(2\alpha+1)}$ However, there are sources the spectra of which deviate significantly from a straight line, and it has been noticed[1] that these sources tend to have high surface brightness

Recently, improved measurements at 38 Mc/s have shown that, in a few sources, the flux density actually falls at low frequencies (Fig 1), and these sources also have the smallest known angular diameters and the highest known surface brightness In the case of 3*C* 147, the decrease in flux density between 81 5 Mc/s and 38 Mc/s is so rapid that no cut-off in the electron energy spectrum, however sharp, would account for it These circumstances suggest that we may be observing synchrotron self-absorption

In Fig 1, the high-frequency data are taken from Conway, Kellermann and Long's paper[1] on the spectra of 160 sources The 38-Mc/s points are taken from a survey by J E Baldwin, S Kenderdine, and me, using the instrument described in ref 2 Flux densities at 81 5 Mc/s (3*C* 48, 147, 295, 298) and 85 Mc/s (3*C* 298) have been taken from refs 3 and 4 respectively, correction factors (0 85 and 0 75 respectively) have been applied to the

Table 1

Assuming that T_e for H II region = 10,000° K
$B = 10^{-4}$ gauss

Source	Galactic latitude b	Emission measure necessary for thermal absorption cm^{-6} × parsecs	θ_p necessary for synchrotron absorption	θ_0
3*C*				
48	29°	2,450	0 4″	1 0″
119	04°	2,450	0 3″	1 5″
147	10°	5,000	0 2″	2 0″
295	61°	1,400	0 6″	double, components 1″ × 1 7″
298	61°	3,200	0 6″	4 0″
299	67°	1,250	0 2″	2 0″
CTA				
21		34,000	0 01″	—
102		20,000	0 01″	—

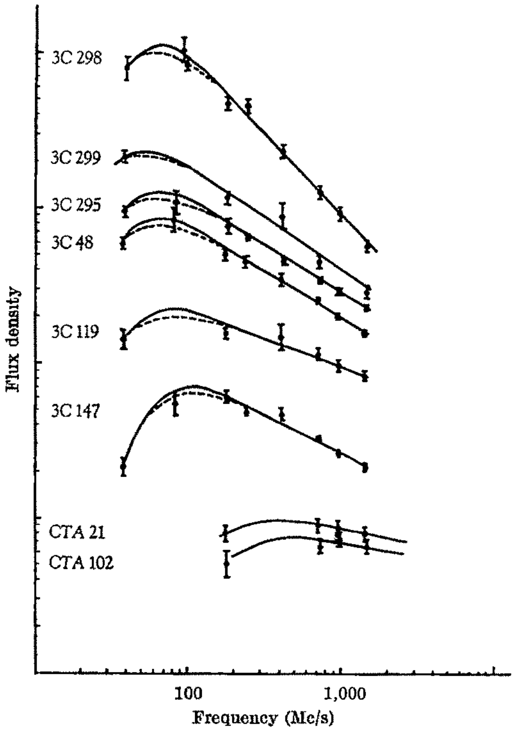

Fig 1 —, synchrotron absorption, - - -, thermal absorption

catalogue values of flux density, the factor being determined by a comparison of the flux densities of the strongest 'Class *S*' sources[1] with the values interpolated from the spectra given[1]

Two mechanisms of absorption could be held responsible for the cut-off in the spectra at low frequencies

(*a*) *Thermal Absorption.* This occurs if a layer of *H* II of appropriate optical depth, τ, lies between the source and the observer The spectrum would be

$$S = k\,\nu^{-a} \exp(-\tau) = k\,\nu^{-a} \exp(-A/\nu^2)$$

If a spectrum of this shape is scaled in both frequency and flux density to fit the observed values, we can calculate the emission measure of the *H* II layer necessary to cause the absorption The values quoted in Table 1 are $\int N_e^2\, ds$, where N_e = No of electrons/c c , and ds is distance through the layer, measured in parsecs

(*b*) *Synchrotron Self-Absorption* This occurs if the brightness temperature of emission is of the same order as the mean kinetic temperature of the relevant relativistic electrons, and so we should expect to observe this in sources of high enough brightness temperature

A detailed calculation by Le Roux[5] has shown that for a source in a uniform cube of side l, in a magnetic field of strength B,

$$S = l^2 \frac{\bar{\varepsilon}}{\chi} (1 - \exp(-\chi l))$$

where χl, the optical thickness,

$$= \frac{\mu a e^2 c l}{16\pi m_0} (\gamma + 2) \left(\frac{eB}{2\pi m_0}\right)^{(\gamma+2)/2} F'(\gamma) \nu^{-(\gamma+4)/2}$$

and $\bar{\varepsilon}$, the mean emission per unit volume,

$$= \frac{\mu a e^2 c}{8\pi r^2} \left(\frac{eB}{2\pi m_0}\right)^{(\gamma+1)/2} F''(\gamma)\, \nu^{(1-\gamma)/2}$$

If $N(E)\, dE$ = No of particles/unit vol with energies between E and $E + dE$,

$$= \text{const } E^{-\gamma}\, dE,$$

$$a = (\gamma - 1) \int_{m_0c^2}^{\infty} N(E)\, dE,$$

r = distance of the source from observer

$F'(\gamma)$ and $F''(\gamma)$ are numerical factors weakly dependent on γ If γ is determined by the spectral index at high frequency, the predicted spectrum can be scaled in flux density and frequency to fit the observed values and so indicate ν_m, the frequency at which the flux density is greatest

If synchrotron self-absorption is taking place, the predicted angular diameter of the source is then given by

$$\theta_p^2 = \frac{S_{400}}{(400\ 10^6)^{(1-\gamma)/2}} \left(\frac{eB}{2\pi m_0^3}\right)^{1/2} \frac{(\gamma + 2)\,(1 + z)^{1/2}}{\nu_m^{(\gamma + 4)/2}}$$

The 'observed' angular diameter, θ_0, is largely based on a single interferometer measurement at a spacing of 61,100 λ (ref 6), assuming that the source is single with a simple Gaussian brightness distribution However, very few sources have such a simple brightness distribution, many being made up of two separate components In such a case, θ_p corresponds to the sizes of individual components, while θ_0 corresponds to the dimensions of the source as a whole, and can only be used as a generous upper limit For example, a single interferometer measurement for *3C* 295 would only give $\theta < 12''$, more detailed measurements[7] have indicated that it is made up of two components 4″ of arc apart Each component is less than 1″ of arc wide on the line joining them, but 1 7″ of arc in length perpendicular to this line These dimensions give much better agreement with θ_p

The one clear and outstanding fact is that the six sources selected from the available data on the basis of their unexpectedly low flux-densities at 38 Mc/s all belong to the very small fraction of sources with angular sizes < 5″ of arc and exceptionally high surface brightness The high surface brightness suggests that synchrotron self-absorption may occur, but it is also possible that a source of such small size may lie entirely within the associated galaxy so that thermal absorption may take place

For both mechanisms, the observations can be fitted by calculated spectra representing reasonable physical conditions It is unlikely that improved spectral measurements will be able to distinguish between the two proposed mechanisms as the predicted spectra are based on very simple models, and will not apply in detail to a real source

In the case of *3C* 295, however, the two components are separated by more than 16 kparsecs, and it is probable that the radio emission occurs too far outside the galaxy to be absorbed appreciably by ionized hydrogen In this case, at least, synchrotron self-absorption seems the more probable mechanism

The two sources *CTA* 21 and *CTA* 102 are also included because they, too, have spectra with a maximum, but at much higher frequencies If these maxima are to be explained by synchrotron self-absorption, the sources must have linear dimensions of less than 150 parsecs (again assuming $B = 10^{-4}$ gauss, but making no assumptions about their distances)

While the known upper limits on angular diameters are consistent with synchrotron self-absorption, it is clearly desirable that more detailed measurements of brightness distribution should be made, if possible, so that a quantitative comparison can be made

I thank H van der Laan and P A G Scheuer for useful discussion of the synchrotron absorption mechanism

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LETTERS TO THE EDITOR

PHYSICS

Ionization Coefficients in Helium at High Pressures

In recent years there has been considerable effort devoted to the study of the ionization processes which give rise to ionization growth in different gases. Of particular interest from the fundamental point of view is the growth of ionization in helium, because in this case quantum mechanical methods may be more easily applied than in the more complex case of diatomic gases. Consequently, direct comparison may be made between experimental data on ionization coefficients, for example, and the quantum mechanical computations. Despite its simple atomic structure, however, the existence of high-energy metastable states of the helium atom, as well as of the helium molecule, results in the possibility of highly complex ionization processes occurring in this gas[1-3]. Moreover, experimentally, helium is a difficult gas on which to make observations because it has the highest ionization potential (24.6 eV) of any known gas. Most common impurities have ionization potentials (<16 eV) appreciably lower than the ionization potential of helium, and are thus preferentially ionized by direct electron impact even when present in only small quantities. Furthermore, ionization of the impurity atoms and molecules can be produced by collisions of the second kind with helium atoms in the high-energy metastable states, and by high-energy photons produced in the gas by the transition of excited helium states (which all have energies >20 eV) to the ground-state. The effects of impurities, even when present in quantities as low as 1 part in 10^6, become increasingly important as the parameter E/p (E, the electric field, p, the gas pressure) decreases, because, as E/p decreases, the mean electron energy decreases and the ratio of ionized impurity atoms to ionized helium atoms then increases. These considerable experimental difficulties probably explain why there are so far no published data on the measurement of ionization growth in helium at high gas pressures and low values of E/p.

It is the purpose of this note to report preliminary measurements on pre-breakdown ionization currents in uniform fields in helium over the range $3 < E/p < 5$ V cm^{-1} mm Hg^{-1} for pressures between 150 and 560 mm mercury. The measurements were carried out using a specially designed parallel plate ionization chamber with electrode separation variable from 0 to 1.5 cm which will be described elsewhere. The apparatus was initially evacuated to a pressure of 8×10^{-8} mm mercury with a rate of rise of pressure about 4×10^{-8} mm mercury min^{-1} into a volume of 13 l. The only high-pressure samples of helium available to us at the beginning of the work were those supplied in high-pressure cylinders by the British Oxygen Co. (analysis of the gas: 99.5 per cent helium, 0.5 per cent neon, 10–20 v.p.m. oxygen and 10 v.p.m. nitrogen). This gas was purified by circulation at high pressure over an activated charcoal trap at liquid air temperatures, heated copper oxide and liquid air traps, and stored in a glass reservoir. The gas was admitted to the ionization chamber through two further traps at liquid air temperature and a high-impedance copper trap previously outgassed and which was designed to eliminate any trace of grease present in the gas from the circulating system. The ionization chamber itself was completely isolated from grease and vapours by the use of all metal taps and a bellows monometer for the measurement of gas pressure.

Initially, the samples of helium prepared in this way gave values of ionization coefficients which were not reproducible from sample to sample, but during the course of experiments over some months, which involved repeatedly filling the system with purified helium and subsequent evacuation (some 100 times in all), reproducible samples were obtained. The values of the first Townsend ionization coefficient obtained when the gas was in this state were the lowest for the whole series of measurements, being a factor of about 50 less than the initial values found. This result showed that the gas sample was reproducible and is consistent with the view that the samples were the purest that had been obtained by this procedure.

A typical log I, d curve when the gas was in this state is shown in Fig. 1. The curve was analysed using the procedure developed in this Department[4] and the values

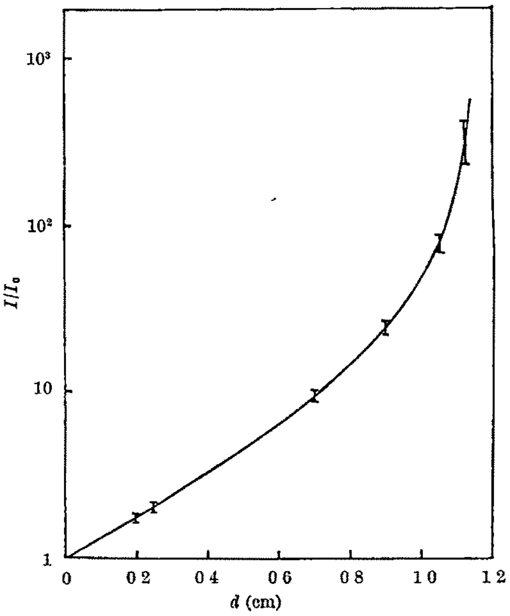

Fig. 1. Graph of log (I/I_0), d for helium at a pressure of 451.8 mm mercury and $E/p_0 = 4.00$ V (cm mm mercury)$^{-1}$. Full line gives the Townsend curve calculated for $\alpha = 2.83$ cm^{-1} and $\omega/\alpha = 0.04$.

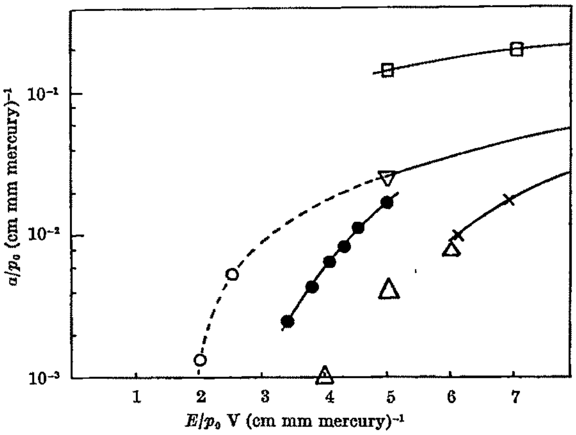

Fig. 2. Comparison of present experimental values of α/p_0 over high-pressure range 150–560 mm mercury with previous work at low values of E/p.

Experiment: Present investigation, —●—, ref. 7, —□—, ref. 8, —▽—, ref. 9, —×—. *Theory*: ref. 6, △, ref. 5, ○.

of the primary and secondary ionization coefficients obtained are shown on the figure. It is interesting to note that the experimentally observed growth is in agreement (well within the experimental error) with the generalized Townsend equation of growth with constant values of the coefficients α and ω/α.

The values of the primary ionization coefficient obtained from the measurements over the range of very low values of E/p from 3 to 5 V cm^{-1} mm Hg^{-1} are shown in Fig. 2. It was found that over a range of gas pressures of 150–560 mm mercury the similarity relationship $\alpha/p = f(E/p)$ was obeyed. Comparison of the present results with previous theoretically computed values[5,6] and experimental values[7–9] of α/p at higher values of E/p and lower pressures is made in Fig. 2, and it can be seen that the present results lie between the extrapolation of the experimental curves of Townsend and McCallum and those of Davies, Llewellyn-Jones and Morgan. Further work is in progress in attempts to obtain samples of gas at high pressures of higher purity and on the effects of known small amounts of impurities on the values of the ionization coefficients in helium.

J. Dutton
F. Llewellyn-Jones
D. B. Rees

Department of Physics,
University College of Swansea, Wales.

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Effect of Capacitor Arrangement on the Symmetry of Linear Pinch Discharges

All applications of linear pinch devices require that the current distribution in the discharge be symmetric about the axis of symmetry of the electrodes. Symmetry is not difficult to achieve when the energy supplied to the discharge is much greater than the minimum energy required for the discharge. However, certain applications, such as plasma accelerators for space-vehicles, require that the energy supplied be near the minimum value. When operating near the minimum energy condition, factors which may be unimportant for high-energy operation can have a marked influence on the discharge symmetry.

The effect of capacitor arrangement on the symmetry of a linear pinch discharge during the phase of the discharge when the current distribution was moving toward the axis was investigated using a pinch plasma accelerator[1]. Nine 10-μF capacitors, rated at 3 kV, were connected to 8-in. diameter aluminium electrodes by means of a 16-in. diameter parallel-plate radial transmission line. The discharge was initiated by briefly opening a solenoid valve which admitted a pulse of nitrogen into the previously evacuated interelectrode space. Four magnetic probes were inserted into the interelectrode space at the same radius at 90° intervals about the axis of symmetry of the electrodes. Probe signals were recorded simultaneously with the set of four probes positioned at various radii. The symmetry of the discharge was determined by comparing the magnitude and time of arrival of the signals.

When the nine capacitors were connected to the transmission line plates symmetrically, the discharge was found to be symmetric at capacitor voltages greater than 2.75 kV. The capacitors were then arranged asymmetrically by connecting them to the transmission line plates within a 270° angle, the other quadrant being vacant. For this case, symmetric discharges could not be obtained at 3 kV. This result indicates that when the capacitors are not arranged symmetrically, the current may not be distributed uniformly enough throughout the transmission line plates for a symmetric discharge to occur. This non-uniform current distribution is undoubtedly due to the tendency of the current to take the lowest impedance path to the electrodes. A similar effect probably also occurs with discharges in other geometries such as the coaxial plasma gun.

Donald P. Duclos
Leonard Aronowitz
Frederic P. Fessenden

Power Conversion Division,
Republic Aviation Corporation,
Farmingdale, New York.

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Electron Spin Resonance Evidence of Radiation-induced Electrons trapped in Organic Glasses at 77° K

It has been suggested in the radiation chemistry of the simple aliphatic alcohols, ethanol[1] and methanol[2,3], that γ-irradiation in the liquid state leads to the formation of positive and negative charged species:

$$2CH_3CH_2OH \rightarrow (CH_3CH_2OH)^+ + (CH_3CH_2OH)^-$$

The negative species, the solvated electrons, were shown to be involved in the chemical changes observed from their reactions with certain specific solutes: H$^+$, chloroacetate, 2-chloroethanol, diphenyl, naphthalene. More recently, various organic glasses[4] were irradiated at 77° K and in the case of ethanol[4,5] the absorption band formed at 5400 Å was accounted as due to solvated electrons. We present electron spin resonance evidence of radiation-induced electrons trapped or solvated in glasses at 77° K in the radiolysis of alcohols.

A conventional electron spin resonance spectrometer (Strand Laboratories, Inc., Model 601 *AX*) was used with a cylindrical reflexion cavity operating in the *T E* 011 mode, resonant frequency of the cavity 9,500 Mc/sec. All the alcohols were irradiated in thin 'Spectrosil' silica tubes in the absence of oxygen. Various alcohols have already been studied[6,7] by electron spin resonance, and some of the effects to be described here (ultra-violet irradiation and visible bleaching of unbuffered alcohols) were observed by these authors[6,7] without assigning an interpretation to them.

On γ-irradiation of, say, ethanol as a glass at 77° K it is known to turn to a dark purple colour and give a five-line paramagnetic resonance line. The intensity ratios of the lines are not binomial (1 : 4 : 6 : 4 : 1) at 77° K, the centre line appearing definitely more intense. The dark purple colour centres can be bleached by various means: (1) by thermal bleaching, when the temperature of the glass is increased to about 115° K, (2) by exposure at 77° K of the γ-irradiated glass to light in the visible range: the colour centres are rapidly bleached (2 min) by a white table-lamp light. Furthermore, on adding initially certain solutes such as acids, I$_2$, diphenyl, naphthalene, and 2-chloroethanol the purple colour is not formed on irradiation. All these solutes are known to react efficiently with electrons. The interesting observation is that following the foregoing methods when the irradiated glasses are colourless the centre line is attenuated while the five-line spectrum persists (in some cases becomes even stronger). It is therefore clear that the purple colour is associated with the single paramagnetic resonance line, which is nearly centred on the basic five-line spectrum of the $CH_3\dot{C}HOH$ radical. It was suggested[6] that this single line may be due to the positive ion of ethanol. However, the results obtained, particularly in the presence of various additives, are inconsistent with this suggestion. We

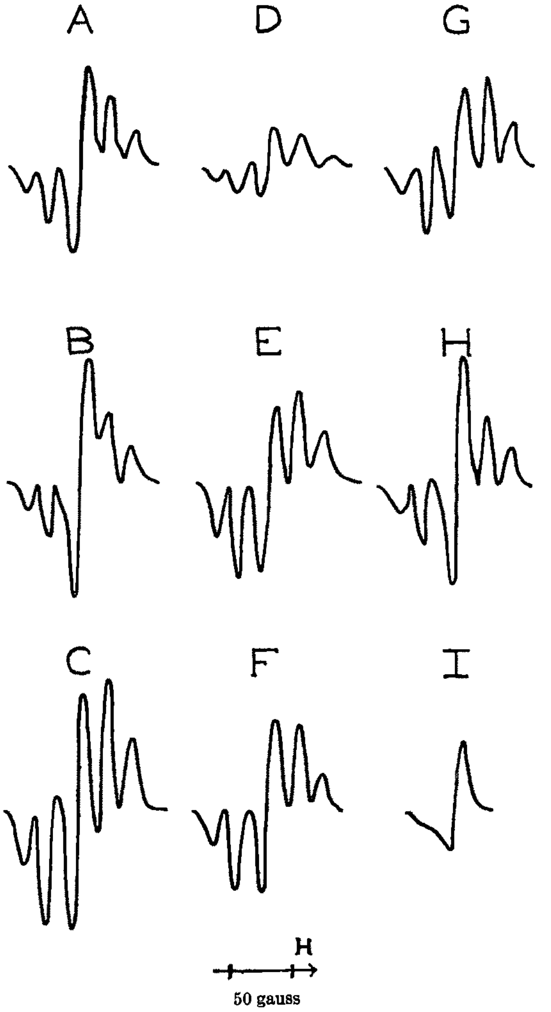

Fig 1 Electron spin resonance spectrum of γ-irradiated ethanol glasses at 77° K Total dose, 0 3 Mrad The spectra given were always observed at 77° K, and the same scale used (*A*) unbuffered ethanol, (*B*) 1 0 N sodium hydroxide in ethanol, (*C*) 1 0 N sulphuric acid in ethanol, (*D*) unbuffered ethanol warmed to 115° K, (*E*) 1 0 N sodium hydroxide after exposure to visible light, resulting in a bleached glass, (*F*) 0 1 M iodine in ethanol, (*G*) unbuffered ethanol after bleaching on exposure to visible light, (*H*) exposure of (*G*) to 2537 Å ultra-violet light, (*I*) warming (*G*) to 115° K until the five-line spectrum disappears

ascribe this line which is superimposed on the other lines to the electron trapped in the matrix at 77° K

The spectra in Fig 1 show some of the results referred to here On warming the neutral glass (*A*) to 115° K the centre line is reduced (*D*) and so is the total number of spins Presence of 1 N sulphuric acid (*C*) inhibits the formation of colour centres, reduces the centre line, and increases the intensity of the $CH_3\dot{C}HOH$ quintet as compared with (*A*) On addition of 1 N sodium hydroxide (*B*) the centre line increases, and the purple colour becomes even stronger Exposure to visible light results in rapid bleaching of the neutral (*G*) and alkaline (*E*) glasses, and a reduction of the centre line There is no change in the colour and electron spin resonance spectrum of the 1 N sulphuric acid glass (*C*) to visible light Subsequent to exposure to visible light, γ-irradiated neutral, acid, and alkaline ethanol, when exposed to ultra-violet light (2537 Å mercury lamp), result (*H*) in a decrease in the intensity of the basic five-line spectrum and an increase of another centre line superimposed on the 5-lines This new single line is relatively stable, and on warming to 115° K the $CH_3\dot{C}HOH$ radical disappears after a short period of time leaving the single line (*I*) which is seen to be disymmetric, and which seems to be due to the $CH_3CH_2\dot{O}$ radical It is to be noted that (*I*) is stable to visible light Full details and the suggested mechanism of formation of this radical will be given elsewhere

The presence of 0 1 M iodine (*F*) suppresses the colour of ethanol glass, attenuates the centre line, and reduces through secondary reactions the number of $CH_3\dot{C}HOH$ radicals stabilized at 77° K Presence of 1 0 M 2-chloroethanol also suppresses the colour and reduces the centre line In the presence of 0 1 M diphenyl the purple colour is not formed on irradiation, instead a blue-green colour is observed which is thought to be due to the mono-negative diphenyl radical ion[8]. The diphenyl ion gives under these conditions at 77° K a single centre line which is distinctly different from the centre line due to the electron Furthermore, the line of the diphenyl radical ion is relatively more stable to visible light

In conclusion, the electron spin resonance centre line obtained on irradiation of ethanol at 77° K is interpreted as evidence of radiation-induced electrons which are ejected from the solvent and trapped in these glasses The *G*-values (± 30 per cent) of the total paramagnetic species, that is, $G\ CH_3\dot{C}HOH + G(e)^-$, is 8 in unbuffered and 1 in N sodium hydroxide, and 10 in N sulphuric acid ethanol glasses Ge^- is 2, 3, and nil in the unbuffered, alkaline, and acidic ethanol, respectively

Electron spin resonance evidence of electrons was also obtained on irradiation of methanol and isopropanol Full details on all these alcohols, the various solutes used, and the photolysis of hydrogen peroxide in alcohol glasses will be published elsewhere

C Chachaty
E Hayon

Service de Chimie-Physique,
Centre d'Études Nucléaires de Saclay
(Seine et Oise)

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Surface Preparation of Electrodes for Studies of the Electrical Properties of Dielectrics

One of the major difficulties in the measurement of conduction currents and of the breakdown strength of homogeneous dielectrics is that of obtaining reproducible results This communication shows that to obtain such results mechanical polishing of the electrodes is to be preferred to the use of electrolytic polishing It has already been shown[1] that in liquid dielectrics the scatter in breakdown values is smaller for mechanically polished than for electrolytically polished electrodes

In the presence of an externally applied electric field, the overall behaviour of a dielectric medium is greatly affected by conditions at the electrode/dielectric interfaces, for example, oxide layers, adsorbed gases and vapours and surface roughness The idea that asperities on the electrode surfaces can cause field emission into a dielectric at applied fields well below the theoretically required value is well known[2,3]

In the case of liquid insulants it is extremely difficult to determine the conditions at the interfaces[4], but it is generally accepted that space charges will build up at the electrode surfaces due to the inevitable presence there of an effectively insulating layer The densities of the space charge concentrations will depend on the availability of ions in the bulk liquid and on the ability of the surfaces to neutralize them The intensification of the field at the cathode is further increased if there are protrusions or other irregularities on the surface[5,6]

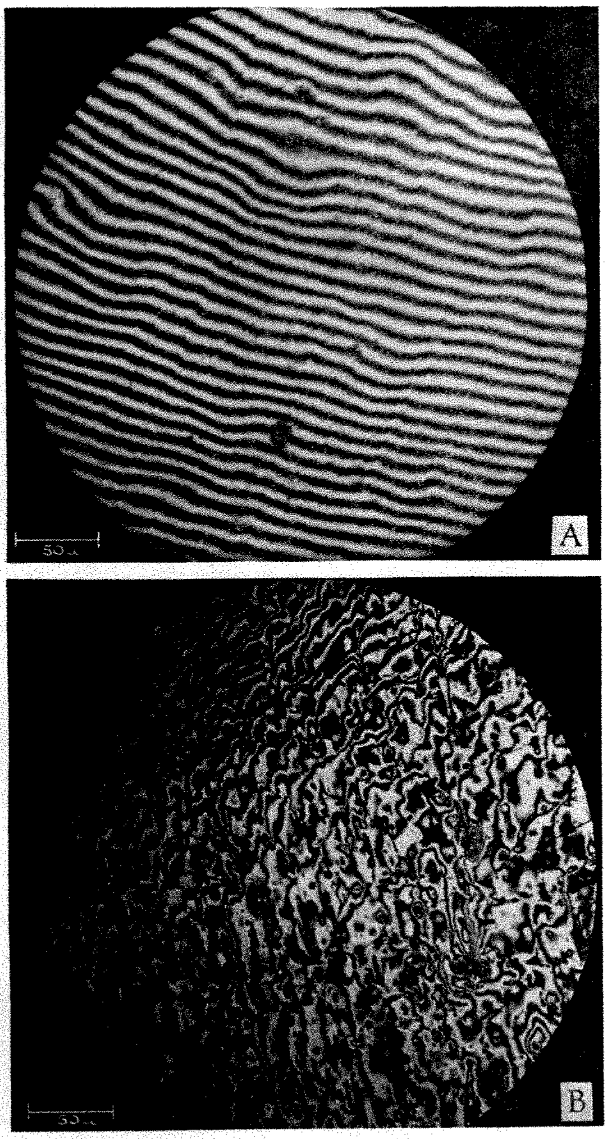

Fig. 1. Interferograms of polished magnesium surfaces. *A*, mechanically polished to 0·25μ diamond paste finish; *B*, electrolytically polished in a solution of three parts orthophosphoric acid and five parts ethyl alcohol

A first step, therefore, towards achieving reproducible results in measurements on liquid dielectrics is to obtain consistent electrode surfaces. Under normal experimental conditions it is not possible to control the oxidation of a surface but a method of producing a constant surface smoothness may be used.

Recent investigations[7], using electrode separations of up to 2 mm, have shown that electron emission from asperities can cause electrical breakdown in high vacuum. In longer gaps the removal by electrostatic forces of aggregates of material or impurities from the electrode surfaces ('clumps') is believed to initiate breakdown[8]. Therefore, to obtain possible improvements in the voltage holding of a given vacuum gap it is important to know what type of electrode preparation gives the smoothest finish.

For the foregoing reasons, an investigation was made of the two main methods of polishing electrode surfaces to determine the most suitable way of producing a consistently smooth surface. Fig. 1*A* is a typical interferogram of a mechanically polished magnesium surface and Fig. 1*B* is an interferogram of an electrolytically polished surface. The pattern produced by the electrolytically polished surface is very complex, showing that the surface is rough. Mechanical polishing produces a distinct and simple pattern which can be readily interpreted. In the example shown in Fig. 1*A* the maximum depth of a depression is $1{\cdot}4 \times 10^{-4}$ mm, the maximum height of a high spot is 2×10^{-4} mm and the scratches are 2×10^{-5} mm wide. Mechanical polishing also gives a reproducible surface finish which cannot be obtained by electrolytic polishing. In addition to producing a surface which may be even less permeable to electrons than natural oxide films[9], electrolytic polishing may give rise to a highly irregular surface with pits where impurity inclusions are preferentially dissolved and protrusions where these are less soluble than the bulk metal. The microgeometry of an electrolytically polished surface is therefore much more sensitive to the inhomogeneities of the metal than is a mechanically polished one.

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R. HAWLEY
A. A. ZAKY
M. E. ZEIN ELDINE

Corona Group,
C. A. Parsons and Co. Ltd.,
Newcastle upon Tyne, 6.

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Phantasies on a Natural Unity of Mass

THE 'artificial' unity of mass has not been solved for more than half a century and its substitution by a 'natural unity' cannot be considered as forthcoming.

However, it would seem that the day is not far off when a material can be identified, probably in crystalline form, which will be apt to define mass unity better than the actual microcrystalline league.

In so far as the choice of the physical status of the standard material is concerned, it should be noted that the use of a gas (for example, hydrogen) instead of a solid would present two insuperable drawbacks.

The first is that, if the pressure were not too high, the container would have to have considerable dimensions and a mass comparable with that of the gas contained inside.

The second drawback is that counting the atoms (for example, previously ionized), if made one after the other, with the quickest technical means (for example, with a counter capable of 10^7 atoms per sec) would require a longer time than the estimated age of the universe. The atoms to be counted are indeed in this case 3×10^{26} for each kg, while the number of seconds in two thousand millions of years is about 6×10^{16}.

A liquid would show other drawbacks.

A crystal, scaled so to result outside in a form of cube with faces parallel to the reticular planes—the number K of total elements being the same—in comparison with a gas offers the advantage to limit the number of elements to be counted down to three times the cubic root $(=3\sqrt{K})$. Therefore it gives the possibility of making the count in a reasonable time.

In this case the element or elements constituting the material should be mononuclide, with impurities not exceeding about 10^{10} and with an amount of dislocations or other defects also not greater than this percentage. Germanium, which seems to be satisfactory so far as impurities and dislocations are concerned, is not naturally a mononuclide; hence it could be used with difficulty, at least until simpler methods can be found for the separation of isotopes. Therefore, other elements or composite materials should be taken into consideration for the time being.

So far as the new definition is concerned, monocrystals should be first prepared with mass very near to 1 kg^2 and it should be compared with the standard of Sèvres, by means of the conventional balances and trying to reduce the comparison errors within one part in 10^9. (In the Italian National Metrology Commission, to avoid the known confusion between mass kilogram (kg) and kilogram-weight (kgp), a new name 'bes' has been suggested instead of 'kgp' by Prof. Polvani, now president of the Consiglio Nazionale delle Ricerche.

Secondly, using at first methods also partially destructive, as perhaps the electron microscope, or better, the field emission microscope, the number of atoms aligned on each of the crystallographic axis should be counted. Alternatively, the distance between two reticular planes should be measured with an error not exceeding one part in 10^{10}, and also the outer dimensions of the crystal (supposed cubic, for example) should be measured with the same accuracy.

From this moment onwards we can say: "The kg is the mass of K atoms of . . . ". The numerical count of the atoms will also give a highly accurate value for the Avogadro's number N, the knowledge of which—so far as is known—is now limited to one pair of parts in 10^5.

Afterwards, the uncertainty in determination of the K value can be artificially eliminated: the round figure obtained in this way could be assumed as the new unit of mass.

The procedure outlined here is practically similar to that followed for the unity of length, when the 'optical meter' has replaced the metallic m meter of Sèvres.

C. Egidi

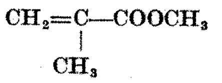
Radio Department, National Electrotechnical Institute,
'Galileo Ferraris', Torino, Italy.

GEOLOGY

Use of Polymethyl Methacrylate in preparing Thin Sections of Recent Sediments

In the examination of Recent sediments it is necessary to identify accurately the mineral grains and lithic fragments which make up the deposit. Compositional properties such as the quantity and type of quartz, feldspar, calcite and the lithic, or rock, suite are keys to the proper interpretation of source and dispersal of a sediment. Such properties as diagenetic alteration of grains, mineral inclusions and specific rock types can be determined only by optical study of specially prepared petrographic thin-sections.

Polymethyl methacrylate, an acrylic compound with the structural formula:



$$CH_2{=}\underset{\displaystyle CH_3}{\underset{|}{C}}{-}COOCH_3$$

was selected as the matrix mounting medium because molecular cross-linkages act as three-dimensional tie bars, thus giving great strength to the matrix. The polymerization can, also, be carried out quickly and without heat at room temperature. Furthermore, when polymethyl methacrylate is used and mixed properly, there is only 4 per cent, or less, volume contraction of the medium, and there is no effect from external heat or caustic chemicals on the polymerized product.

Polymethyl methacrylate is obtainable from Claudius Ash, Sons and Co., Ltd., 26–40 Broadwick St., London, W.1, where it is marketed under the trade name 'Stellon', a denture repair material.

The step-by-step procedure for making a petrographic slide of Recent sediment using polymethyl methacrylate (hereafter termed 'Stellon') is as follows:

(1) Assemble the following tools and supplies: (*a*) microscope slides, frosted, 1½ in. × 3 in.; (*b*) wire, flat, approx. ⅛ in. by 1/16 in., for use in shaping a rectangular mould to hold the medium; (*c*) crucible, about 1½ in. diam., ¼ in. deep, for mixing the acrylic; (*d*) spatula, stainless steel, with a blade about 1 in. long and ¼ in. wide; (*e*) 'Plasticine', ¼ lb., to be used in positioning the wire mould and to prevent leakage of the slurry; (*f*) petroleum jelly ('Vaseline'); (*g*) one 'Stellon' kit, consisting of 4 oz. of powder and 4 oz. of fluid; the clear type, non-pigmented powder must be used.

(2) Using the specified flat wire, make a rectangular mould about 1⅛ in. by ¾ in. in size. The mould is positioned on the frosted glass slide using banked-up 'Plasticine' to prevent leakage and air bubbles from forming prior to hardening. A thin smear of petroleum jelly over the glass will prevent subsequent sticking.

(3) Mix, in crucible, 2 parts dry sediment to 1½ parts 'Stellon' powder. (Commencing with this step, the process is continuous and must be carried to completion once begun.)

(4) Add 'Stellon' liquid to a slight excess of liquid over powder and spatulate for 25 sec and no longer.

(5) Pour mixed sediment slurry into the wire mould and then spatulate it slightly to even out any grain accumulation. The slurry will commence polymerization immediately (at a room temperature of 68° F ± 10°) and will be completely hardened in 1 h. It is most important that the slurry is not disturbed or moved during the hardening period.

(6) Remove the hardened wafer by tapping the wire frame with the spatula handle and mark sample number on rough surface of wafer with waterproof Indian-ink.

(7) Using 500 grit grinding powder, grind down the smooth side of the wafer until an adequate surface has been cut into the grains. (It is important that the wafer be ground sufficiently to provide a representative plane, or slice, through the artificial rock.)

(8) Clean wafer of grit in tap water, taking care not to polish ground side with the drying towel. Simply blot dry.

(9) For mounting, mix in a crucible, 1 part 'Stellon' powder to 1 part 'Stellon' liquid plus a very slight excess liquid. Spatulate for 30 sec. Place 2 drops of 'Stellon' liquid on polished side of wafer. Immediately pour slurry on to frosted side of glass slide. Shake excess liquid from wafer and gently lower wafer on to slide, using care not to entrap air bubbles. Apply pressure to wafer and move it back and forth to remove surplus slurry and any air bubbles. A small weight may be placed on the wafer to hold it firmly during a 1-h cementing period. Mark sample number on the glass slide.

(10) Grind the slide-mounted wafer as would be done with a normal rock. Place slide in water for 10 min and lift the very thin acrylic wafer off and wash it in tap water. At this time, the wafer may be trimmed with a pair of scissors.

(11) Number a clean glass slide with a diamond stylus and attach the thin wafer to it. Position the wafer with a spot of 'Durafix' in one corner.

(12) Place slide on 95° F hot-plate and cover-slip on 130° F hot-plate.

(13) Lift wafer and place a small amount of filtered Canada balsam beneath. Press wafer down and cover with another small portion of balsam. Overlay with cover slip, position and allow slide to cool 1 h. Remove surplus balsam with a hot knife and clean with methyl alcohol.

Slides prepared by the foregoing technique (Fig. 1) are chemically and physically stable; the polymerized polymethyl methacrylate does not discolour with time or with changes in temperature. We have made petrographic slides of unconsolidated sediments which ranged in size from argillaceous silts to mixtures of coarse sand and pebbles. The optical properties of the various minerals are in no way altered or obliterated. The refractive index of 'Stellon' is slightly less than that of quartz, and we suggest that the refractive index be checked for

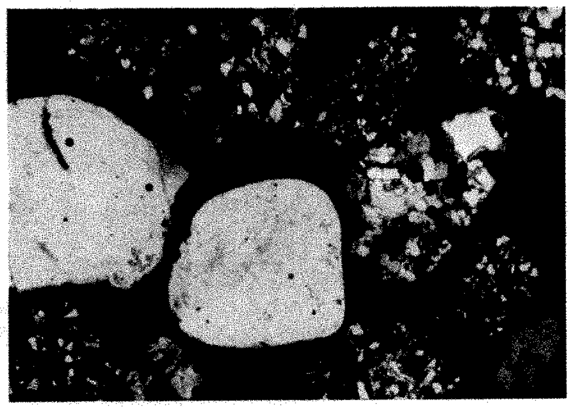


Fig. 1. Photomicrograph of lithic-quartz sand from Cardigan Bay, about two miles south-west of Borth. Note that the types are readily determined as a mixture of arenite and vacuole quartz grains. The large quartz grain in centre field is about 1 mm in diameter (crossed nicols)

each new batch of 'Stellon' in case of slight changes in compounding by the manufacturer.

This technique has been developed during the course of research into the composition of Irish Sea sediments. The investigation is directed by Prof. Alan Wood and is supported by a grant from the Department of Scientific and Industrial Research.

J. R. Moore III
S. H. Garraway

Department of Geology,
University College of Wales,
Aberystwyth.

A New Fossil (*Papillomembrana* sp.) from the Upper Precambrian of Norway

A new fossil was recently found during investigations of the rocks of the Sparagmite group in Southern Norway.

The body of the fossil has a thin outer membrane, which, in thin section, is opaque; presumably the substance is carbonaceous. Its most conspicuous features are the dense papilla-like protuberances of the membrane. They are thin-walled, hollow and about 40–45μ long and 8–10μ thick. They are usually somewhat swollen at their apices, and some carry hooks and small spines. The bases are generally expanded, and the voids of the protuberances seem to open into the body cavity.

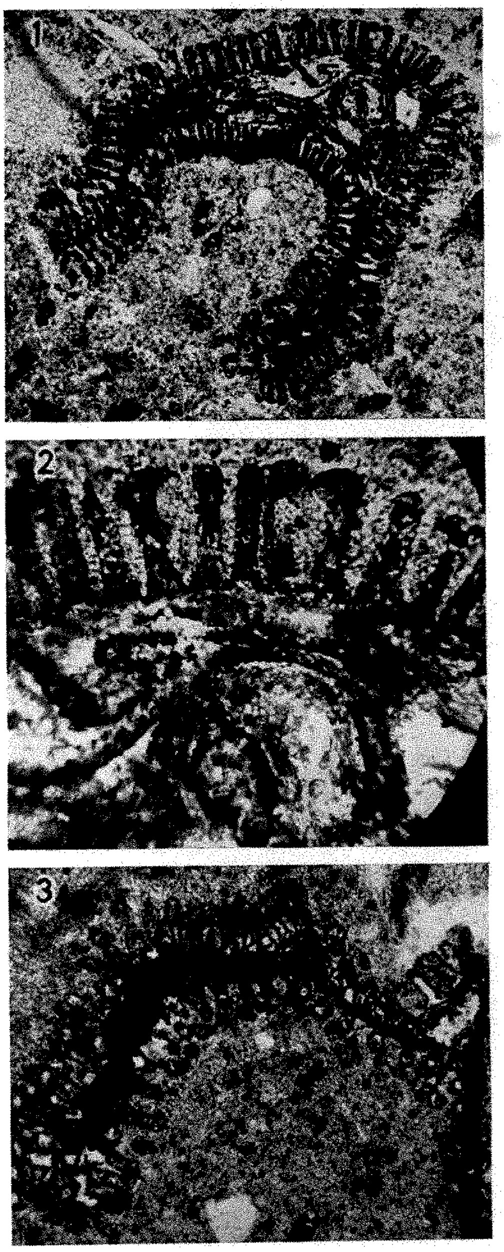
In the two best preserved specimens (Figs. 1 and 2), structures are visible in the interior of the body which appear to be a detached, irregularly folded membrane. Besides this membrane, there are irregular lumps of a yellowish-brown substance, presumably organic. This substance is not found outside the fossil. The original shape of the fossil is not known; all five specimens that have been observed are compressed. The sections indicate that it could have been cylindrical or spherical, and about 0·4–0·5 mm in diam. The fossil has been named *Papillomembrana compta* gen. nov. sp. n.

The affinities of the fossil are uncertain. It has a rather complex structure, considering its great age; similar structures, so far as I know, have not been reported from such old beds before.

Papillomembrana has some superficial resemblance to the dasycladacean algae. The body cavity might correspond to the thallus, and the papillæ to the primary branches. Most dasycladaceans are incrusted with lime, but specimens with a carbonaceous skeleton are known. *Papillomembrana* is only slightly smaller than *Vermiporella* and other small dasycladaceans. The internal structures are, however, different from anything found in this group and recall an organism with a double wall. Much further work is necessary to ascertain the systematic position of *Papillomembrana*.

In addition to the specimens of *Papillomembrana*, there are some other less well-defined fossils in the same rock. They consist of thin-shelled, hollow carbonaceous spherical bodies with a diameter of 25–100μ, and thin carbonaceous films which in some cases have spines and other outgrowths on their upper side. These spines are, however, considerably smaller than those *Papillomembrana*. They resemble some of the structures described and named by Timofeev from beds of 'early' Precambrian age[1].

The fossils are found in a black mudstone pebble in the basal part of the Biri Conglomerate Member of the Brøttum Formation. This formation, which is the oldest one in the Sparagmite Group, is of Esmarkian age ('earliest' Precambrian). The mudstone, which is highly carbonaceous and remarkably free of clastic material coarser than clay, is found as rare pebbles in the typical section of the conglomerate along road 50 at the western side of Biskopåsen in Ringsaker (120 km north of Oslo).



Figs. 1–3. *Papillomembrana compta* gen. nov., sp. n. (1) The holotype, median section (× c. 104). (2) Detail of the holotype, showing the papillæ, and part of the structures in the interior (× c. 385). (3) A completely compressed specimen, showing hollow papillæ. All figures from a thin section of a pebble in the Biri Conglomerate member of the Brøttum Formation (Esmarkian) at Biskopåsen, Ringsaker. The specimens belong to Paleontologisk Museum, Oslo, Reg. No. 73173

The stratigraphy and general geology of the area are described, and references given by Holtedahl[2]. The stratigraphic position of the various units of the Sparagmite group is shown in Table 1.

Table 1

Chronostratigraphical units		Lithostratigraphical units	
Cambrian	1 *a*	(Fossiliferous shales, etc.)	
	Eo– Cambrian	Ringsaker Quartzite formation Vardal Sparagmite formation Ekre Shale formation Moelv Congl. (tillite) formation Moelv Sparagmite formation	Sparagmite group
Precambrian	Esmarkian	Biri formation (dark shales with limestone members) Brøttum formation (greywackes and dark shales with conglomerates and shale—limestone members in the upper part) (base unknown)	
	(Riphean 800–900 million y.)	Crystalline basement complex	

The imbrication of the pebbles found in some beds of the Biri Conglomerate indicate a transport from a southerly direction. The pebbles of unmetamorphic sediments are concentrated in the basal part of the conglomerate. It might be assumed that when the relief was sharpened by a slight movement along the fault lines bordering the Sparagmite basin, the sediments on the outer margins of the basin were eroded and deposited at the base of the conglomerate. It can therefore be assumed that the mudstones in which *Papillomembrana* are found correspond to the lower part of the Brøttum Formation, as its lateral facies outside the basin proper, or that they correspond to Esmarkian beds older than the Brøttum Formation. At present, Cambrian beds rest directly on the Precambrian crystalline basement to the south of the Sparagmite basin, and all traces of the mudstones and other Esmarkian rocks were therefore probably removed by erosion prior to the deposition of the Cambrian. Radioisotope age of the Precambrian basement farther south[3] indicate that it is comparatively young, 8–900 million years, and these give a probable maximum age for *Papillomembrana*.

I thank Prof. O. Holtedahl, Prof. O. A. Høeg and Dr. S. Manum for discussions about these fossils. It was Prof. O. Holtedahl who, in 1943, encouraged me—then a very young student—to look for fossils in the Sparagmite group.

N. SPJELDNÆS

Institutt for Geologi,
Blindern, Oslo.

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CRYSTALLOGRAPHY

Structure of 10,10-Dibromoanthrone

As part of a programme devoted to the examination of electron lability in various classes of organic compounds, we have determined the crystal and molecular structure of a halogen derivative of anthrone: 10,10-dibromoanthrone.

The compound was prepared by the reaction of bromine with anthrone in carbon disulphide. The crystalline reaction products appear as light lemon-coloured rods having a rhombus-shaped cross-section. They are not stable in air at room temperature for extended periods. However, they do keep well under refrigeration, and we have been able to draw excellent crystals from a batch kept in the refrigerator over a 12-month period.

Two specimens were used for the collection of the data. They were very much alike in shape and size having lengths along the rod axis of about 0·6 mm. and an average cross-sectional 'diameter' of about 0·1 mm. $h0l$ and $0kl$ data were obtained from one crystal using molybdenum radiation on the precession camera. The other crystal was used for multiple-film equi-inclination Weissenberg photographs (copper $K\alpha$-radiation) of levels $l = 0$ to $l = 10$. About 49 per cent (1,122 out of 2,297 possible) of the available reflexions were recorded on film, and intensities were measured by visual comparison with a graded standard. Lorentz and polarization factors were applied to all of the data, and in addition, the Weissenberg data were corrected for absorption effects. The various sets of Weissenberg data were scaled together using commonly occurring reflexions observed on the precession camera films.

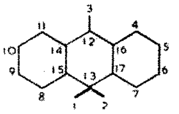
The unit cell was found to be monoclinic (b-axis unique) with $a = 9{\cdot}09 \pm 0{\cdot}02$ Å, $b = 10{\cdot}52 \pm 0{\cdot}03$ Å, $c = 15{\cdot}64 \pm 0{\cdot}04$ Å and $\beta = 125° \; 10' \pm 5'$. The observed symmetry extinctions give unambiguous assignments of space group $P\,2_1/c$, No. 14. The density calculated for $Z = 4$ is 1·91 g/c.c. and that determined in n-heptane with the Berman balance is 2·00 g/c.c. Linear absorption coefficients for copper $K\alpha$- and molybdenum $K\alpha$-radiation are 90·486 cm.$^{-1}$ and 75·44 cm.$^{-1}$ respectively.

A three-dimensional 'cold-atom' Patterson function was calculated, and analysis of Harker-related bromine–bromine interactions led to location of the heavy atoms.

Three least-squares refinement cycles were made on the bromine positions using the set of 485 of the strongest reflexions. The R-factor dropped to 26 per cent and a 3-D Fourier was calculated using bromine phases only. Of the fourteen carbon atoms and one oxygen atom omitted from the structure factor calculations, eleven carbon atoms and the oxygen atom appeared clearly in the Fourier as well as the bromine atoms. The positions of the three missing carbon atoms were postulated from chemical considerations and the resulting seventeen-atom structure refined rapidly in four cycles from 32 to 14 per cent. Structure factors and least-squares refinement were computed now for 1,122 observed reflexions and in three cycles the R-factor dropped from 40 to 17·5 per cent. Individual isotropic temperature factors for all atoms were varied for all refinement cycles which included the complete contents of the asymmetric unit. Table 1 lists fractional co-ordinates and temperature parameters for a complete molecule. Fig. 1 is a crude sketch of the structure viewed along c. The circles represent bromine atoms and the long lines represent the carbon planes seen edge-on. Fig. 2 is a drawing of the structure projected on to (010).

Our earliest conceptions of the conformation of this molecule were based, in large part, on the stereochemistry of a molecule built of model atoms designed to exhibit correct van der Waals' volumes. In such a model, the anthrone system assumed a puckered form. In contrast, our results show that the ring system is close to being planar. The sum of the bond angles about the carbonyl

Table 1



Atom No.	Atom type	*X*	*Y*	*Z*	β
1	Bromine	0·795	0·696	0·369	5·37
2	,,	0·682	0·410	0·297	5·85
3	Oxygen	0·861	0·632	0·038	8·11
4	Carbon	0·520	0·680	0·985	5·83
5	,,	0·343	0·699	0·970	9·12
6	,,	0·315	0·676	0·041	6·94
7	,,	0·441	0·634	0·134	4·97
8	,,	0·084	0·503	0·386	6·30
9	,,	0·248	0·466	0·392	5·39
10	,,	0·273	0·484	0·318	6·18
11	,,	0·132	0·529	0·219	6·22
12	,,	0·837	0·613	0·108	5·65
13	,,	0·765	0·565	0·265	4·20
14	,,	0·970	0·560	0·212	4·24
15	,,	0·933	0·538	0·275	4·82
16	,,	0·646	0·637	0·086	5·17
17	,,	0·603	0·617	0·152	5·84

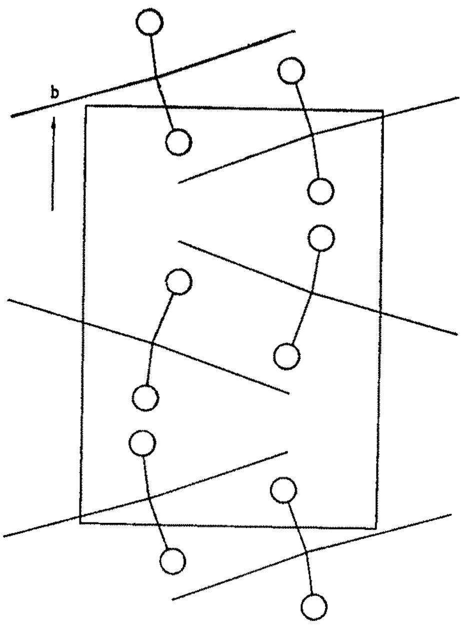


Fig 1 View of the structure along the c-axis

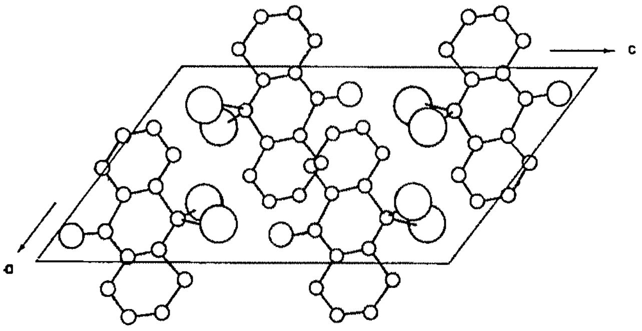


Fig 2 Projection of the structure on to (010)

carbon atom is 359 9°, and the sums of the bond angles about the carbon atoms which are shared by ring systems are 359 7°, 358 9°, 356 3° and 359 9°

The plane containing the bromine atoms and the tetrahedral carbon atom to which they are bonded is approximately perpendicular to the plane containing the tetrahedral carbon atom and the two carbon atoms in the ortho positions in the centre ring The angle between the two planes is 92 1° Our first model also implied that the bromine atoms were placed unsymmetrically above and below the ring system The calculated interatomic distances between the bromine atoms and the carbon atom para to the tetrahedral carbon atom are 4 384 Å and 4 475 Å Accordingly, the molecular structure is a close approximation to that produced by two intersecting mirror planes One of these contains the ring system The other contains the two bromine atoms and the carbon atom to which they are bonded

The intramolecular bromine–bromine distance is 3 169 Å, in very close agreement with the value of 3 17 Å found in CBr_4[1]. The shortest intermolecular bromine–bromine distance is 4 203 Å The closest intermolecular carbon–carbon distance is 3·556 Å Unusual carbon–carbon distances of 1 610 Å and 1 575 Å are found between C_{13}–C_{17} and C_{12}–C_{16} respectively Also there are some short carbon–carbon distances of 1 238 Å, 1 310 Å, 1 296 Å and 1 326 Å in the ring systems Fourier and difference Fourier syntheses indicate anisotropy in the thermal motion of the atoms, and the decision as to the validity of these distances will depend on least squares refinement using anisotropic thermal parameters

The molecular packing leaves infinite channels extending in the *a* direction between the parallel planes of centro-symmetrically related molecules There do not appear to be any discrete large holes or cavities in the structure

We thank Miss A Pauline Krukonis for estimation of the 3-*D* intensity data and Dr T Mukherjee for preparation of this compound

JERRY SILVERMAN
N F YANNONI

Crystallography Section,
Energetics Branch,
Space Physics Laboratory,
Air Force Cambridge Research Laboratories,
L G Hanscom Field,
Bedford, Mass

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CHEMISTRY

Preparation of Very High Specific Activity Iododeoxyuridine, Iodouridine and Iodouracil, labelled with Iodine-125, -131 or -132

As part of a contribution to a study of the relative merits of radioactive substances used for brain tumour[1] localization we were asked to prepare ^{131}I-5-iododeoxyuridine of very high specific activity (of the order of 100 µc./µg) and in a solution suitable for injection into rats

Two methods of synthesizing the unlabelled compound have been reported by Prusoff[2] In the first of these, equal weights of deoxyuridine and iodine are refluxed together with chloroform and nitric acid, and the product is separated by crystallization This method was successfully used to prepare ^{131}I-labelled samples of low specific activity, using many milligrams of iodine carrier In the second method, equal weights of deoxyuridine and iodine are heated in a solution of sodium hydroxide, and the product is separated from the reactants by elution from an anion-exchange column with 0 1 N formic acid This method was also utilized in preparing low specific activity samples, but the resulting solutions were too toxic to be used *in vivo*

There are obvious practical limitations to the specific activity which may be achieved if the product is to be isolated by crystallization, so we first sought an alternative method of isolation Using approximately 4 mg each of deoxyuridine and low specific activity iododeoxyuridine (prepared by the first of the methods outlined here and purified by several recrystallizations from hot water) dissolved in 2 ml of 0 1 N sodium hydroxide, we found that the two compounds could be separated from one another by elution from an anion-exchange column with 0 01 M citric acid The column was of 'Amberlite *CG*-400 resin', 100–200 mesh, previously conditioned with 0 1 M hydrochloric acid and washed with water, its dimensions were 22 cm × 0 5 cm diam

The elution curve so obtained is shown in Fig 1*a* The two compounds were identified by their ultra-violet absorption spectra (deoxyuridine has an absorption peak at 262 mµ and iododeoxyuridine at 288 mµ, in 0 01 M citric acid) and the iodinated compound by its activity measured in a well-type scintillation counter The eluate containing the iododeoxyuridine was itself suitable for use *in vivo*, but if required could readily be buffered and made isotonic with normal saline by adding the appropriate weights of sodium phosphate and sodium citrate

In order to attain the required high specific activity we next carried out the iodination with carrier-free ^{131}I-iodide, so that the specific activity of the product would be limited only by the presence of traces of inactive iodine contaminating the reactants or the glassware which were used This was achieved by refluxing together 16 mg deoxyuridine, 0 4 ml 1 N nitric acid, 0 2 ml chloroform,

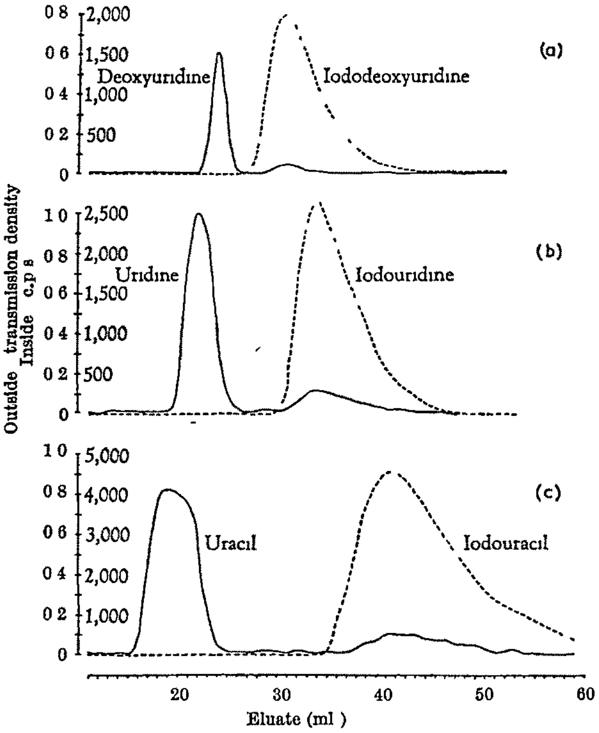

Fig 1 Elution of (*a*) deoxyuridine and ^{131}I-5-iododeoxyuridine, (*b*) uridine and ^{131}I-5-iodouridine, and (*c*) uracil and ^{131}I-5-iodouracil from column of 'Amberlite *CG*-400' resin with 0 01 M citric acid at room temperature - - - -, Activity, ——, transmission density at 262 mμ

and 0 1 ml carrier-free ^{131}I-iodide solution containing about 2 5 mc iodine-131 (This solution was supplied by the Radiochemical Centre, Amersham, Code *IBS*–3, and was essentially 0 02 N sodium hydroxide) After refluxing for 2 h on a water-bath, the aqueous phase was separated, made just alkaline with 0 5 M sodium hydroxide, and placed on an anion-exchange column prepared as described here The column was washed with 35 ml 0 01 sodium hydroxide, which carried off less than 1 per cent of the initial ^{131}I-iodine activity, presumably that which was still present as iodide

On eluting with 0 01 M citric acid, the deoxyuridine was first removed and identified as before Next, at the stage at which iododeoxyuridine was expected to be eluted, about 50 per cent of the iodine-131, measured this time by means of a re-entrant ionization chamber, came off the column in a single peak in some 10 ml of eluate No inactive iododeoxyuridine could be detected by ultra-violet absorption spectrometry, the detection limit being about 1 μg/ml The specific activity of the iodinated product was thus at least 100 μc /μg (probably in fact much higher) while the specific activity of the eluted solution was more than 100 μc /ml This was adequate for the subsequent *in vivo* studies, but samples of higher specific activity could obviously be obtained by collecting only the fractions under the peak of the elution curve, or by using more ^{131}I-iodide in the synthesis The ^{131}I-iodine which remained after elution with citric acid was believed to be present in a high oxidation state, being only removed from the column with difficulty As a check on the identity of the ^{131}I-iodinated product, in a later experiment we spiked the reaction mixture with inactive iododeoxyuridine just before starting to separate the products The activity peak in the elution curve then corresponded exactly with the peak for ultra-violet absorption at 288 mμ

Attempts to carry out the iodination under alkaline conditions, as in the second of the methods reported by Prusoff, were completely unsuccessful with carrier-free iodine, none of the activity being eluted from the anion-exchange column by citric acid

Prusoff, Holmes and Welch[3] have described syntheses of low specific activity ^{131}I-5-iodouridine and ^{131}I-5-iodouracil We have also prepared these compounds, but in high specific activity, using the method described here for iododeoxyuridine Calibration elution curves for the separation of uridine from iodouridine, and of uracil from iodouracil, are shown in Fig 1, curves *b* and *c* For these two iodinated compounds, as for ^{131}I-5-iododeoxyuridine, the specific activity attainable is limited only by the presence of traces of inactive iodine in the reactants or on glassware, and by the quantity of ^{131}I-iodine which can conveniently be handled

Myers and Vanderleeden[4] have shown that for many biological studies the physical properties of iodine-125 make it more attractive than iodine-131 as a radioactive tracer With this in mind, we have used the iodination and separation methods already described to prepare ^{125}I-labelled iodouracil, iodouridine and iododeoxyuridine Since at present iodine-125 is not available in carrier-free form, each product was necessarily of relatively low specific activity, but because of the longer half-life of iodine-125 (57 4 d, compared with 8 05 d for iodine-131) the shelf-life was also longer In contrast, carrier-free iodine-132 (half-life 2 3 h) may readily be obtained (by milking its parent tellurium-132 supplied in suitable form by the Radiochemical Centre, Amersham, Code *TMC* 1) We have been able to prepare the same iodinated compounds labelled with this isotope, again in high specific activity, using the same methods as before but reducing the refluxing time to 30 min

It is worth noting that carrier-free ^{131}I-iodine has also been used recently by Reith and Tampion[5] in preparing very high specific activity samples of ^{131}I-labelled tyrosines

The samples of iodine-131 and iodine-132 (tellurium-132) used in our work were obtained from the Radiochemical Centre, Amersham; the iodine-125 was made on the Medical Research Council cyclotron, but may also be obtained from the Radiochemical Centre The deoxyuridine, uridine and uracil were obtained from L Light and Co , Ltd

We wish to thank the director of the Medical Research Council Cyclotron Unit, Mr D D Vonberg, for his interest and for permission to publish this work

D J SILVESTER
N D WHITE

Medical Research Council Cyclotron Unit,
Hammersmith Hospital,
London, W 12

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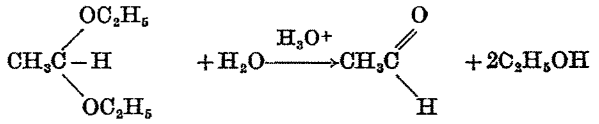
Radiation-induced Hydrolysis of Acetal: Evidence for the Reaction of H_3O^+ Ions in Spurs in the Radiolysis of Water

IT has recently been experimentally demonstrated that in water close to *p*H 7 (ref 1) and at *p*H 4 (ref 2), the reducing radical formed during radiolysis bears a unit negative charge. This suggests that an ejected electron escapes from its parent H_2O^+ ion It follows that H_2O^+ ions should temporarily exist in a spur The H_2O^+ ions, or their hydrolysis products, $H_3O^+(H_2O^+ + H_2O \rightarrow H_3O^+ + OH)$, might cause a suitable solute to 'see' a spur as a volume which temporarily has a lower *p*H than the body of the solution The purpose of this communication is to present some experimental results which we interpret as evidence for this effect

We have irradiated aerated aqueous solutions of diethyl acetal with 50-kVp X-rays A brief description of the thermal hydrolysis of acetal will explain our choice of solute. Acetal is stable in solutions of $pH \geq 7$. It is extremely sensitive to hydrolysis catalysed by H_3O^+ but not by undissociated acid molecules[3], for example undissociated acetic acid. The hydrolysis kinetics are first order with respect to H_3O^+ and acetal. Acetaldehyde (AcH) and ethanol are formed.



$$CH_3C(OC_2H_5)_2H + H_2O \xrightarrow{H_3O^+} CH_3C(=O)H + 2C_2H_5OH$$

Solutions were buffered at *p*H 7 using a potassium dihydrogen phosphate–sodium hydroxide buffer. The Fricke dosimeter, using $G(Fe^{+3}) = 14·5$ for 50-kVp X-rays, was utilized for dose-rate measurements. After irradiation it was observed that acetaldehyde and ethanol had been formed. Acetaldehyde and ethanol were identified by their retention times on a 2·5 m gas chromatographic column packed with a mixture of 40 g of diglycerol on 100 g of ground firebrick. The diglycerol retained 0·5 ml samples of water while the hydrolysis products and acetal were eluted by a flow of nitrogen. Acetaldehyde was also identified by its broad absorption peak at 2750 Å (ethanol and acetal do not absorb at this wave-length and a blank compensated for the small absorption of potassium dihydrogen phosphate). Quantitative analysis for acetaldehyde was carried out by measuring the optical absorption of irradiated solutions at 2750 Å, using an extinction coefficient of 8·5 l mole^{-1} cm^{-1}. The amount of acetal remaining after irradiation was then determined by completely hydrolysing the solution by adding a few drops of concentrated sulphuric acid and measuring the total acetaldehyde thus formed.

For doses up to about 5×10^{23} eV l^{-1} and at an initial acetal concentration of 0·14 moles l^{-1}, *G*(AcH) was constant and equal to *G*(–acetal) within 10 per cent. Beyond this dose, oxidation of ethanol and reduction of acetaldehyde became too significant to permit interpretation of the results. *G*(ethanol) was not determined. A plot of *G*(AcH) against initial acetal concentration increased from the origin and became constant at $G(\text{AcH}) = 2·0 \pm 0·2$ and at an initial acetal concentration equal to 4×10^{-2} molar (Fig. 1). These results refer only to irradiations of less than 5×10^{23} eV l^{-1} dose. Neutral buffered solutions, irradiated at a dose rate of $4·5 \times 10^{19}$ eV l^{-1} sec^{-1}, formed acetaldehyde at a rate comparable with that observed in thermal hydrolysis at *p*H 4. When solutions ($pH \sim 6·8$) were not buffered, an unirradiated solution hydrolysed to a small extent, but radiolysis produced much more acetaldehyde in the same period of time.

These results are interpreted as hydrolysis catalysed by H_3O^+ ions formed in spurs, in competition with both neutralization of H_3O^+ and dilution of H_3O^+ by spur expansion. We assume the rate constant for attack by a proton on an acetal molecule to form an ion-molecule to be at least 10^9 l mole^{-1} sec^{-1} because this type of reaction is usually diffusion controlled. If this were true, acetal would be easily capable of reacting with H_3O^+ during the early stages of the lifetime of the spur. Admittedly, a gap exists in our argument since we must assume that the next stage in the hydrolysis reaction proceeds at a considerably greater rate than deprotonation.

In a spur, $G(H_3O^+)$ should equal *G*(OH). Based on an estimated average initial linear energy transfer in the solution of 0·15 eV/Å, *G*(OH) for neutral water should be between 1·4 and 2·0 (refs. 4 and 5). Decomposition of the ion-molecule formed from H_3O^+ and acetal should be a relatively slow step compared with spur expansion due to diffusion. Thus the charge distribution in the spur should approach that in the body of the solution during the time required for one acetal molecule to hydrolyse. It is therefore not unreasonable that one H_3O^+ ion only catalyses, on the average, the decomposition of about one acetal molecule, that is, that *G*(AcH) is of the same magnitude as $G(H_3O^+)$.

If a free radical mechanism were operative, one would expect *G*(AcH) to reach a limiting value at a much lower initial concentration of acetal than observed. For example, most solutes scavenge the radical yield of H and OH and start to compete for precursors of H_2 and H_2O_2 at about 10^{-6} molar (ref. 6).

For an irradiated solution to have an 'apparent *p*H' of four, the *p*H in a spur must be much lower. In a 0·04 molar acetal solution, a spherical volume with a radius of 30 Å contains three acetal molecules. If a spur of the same volume contained three H_3O^+ ions, the *p*H in the spur would be 1·4.

We believe this is the first time a reaction in radiation chemistry has been interpreted as hydrolysis catalysed by H_3O^+ ions formed during the primary radiolytic processes in water. However, observations have been made by others in the radiolysis of carbohydrate solutions which might be explained by acid-catalysed hydrolysis. For example, radiolysis of dextran solutions yields products which can also be produced by thermal, acid-catalysed hydrolysis[7]. Radiolysis of aqueous ethyl acetate solutions has also yielded the hydrolysis products ethanol and acetic acid[8].

It is possible that a *p*H effect such as we have described contributes to biological damage caused by ionizing radiation.

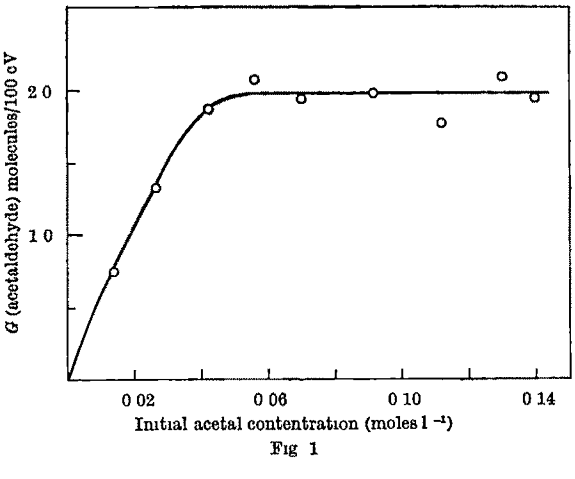


Fig. 1

D. R. SMITH
W. H. STEVENS

Atomic Energy of Canada, Ltd.,
Chalk River,
Ontario, Canada.

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Action of Polar Reagents on Nylon 66

VARIOUS workers have reported on the action of lithium bromide and similar salts on simple amides and fibrous proteins and have offered contrary explanations as to the mechanism of interaction; it is therefore instructive to consider the effect of these reagents on polyamides.

In the investigation recorded here, nylon 66 films, *c.* 10µ thick and partially oriented by cold drawing, and nylon 66

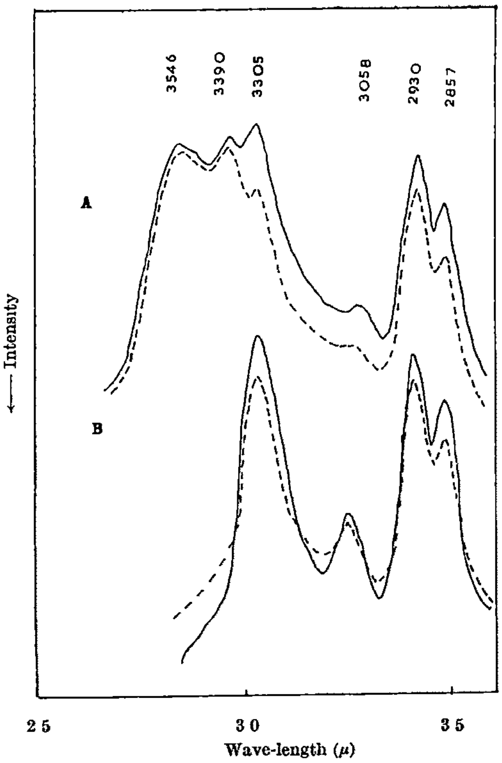


Fig 1 Infra-red spectra of nylon 66 *A*, Treated in magnesium perchlorate, 80° C, 12 h, *B*, untreated film – – –, Parallel, ——, perpendicular to direction of stretching

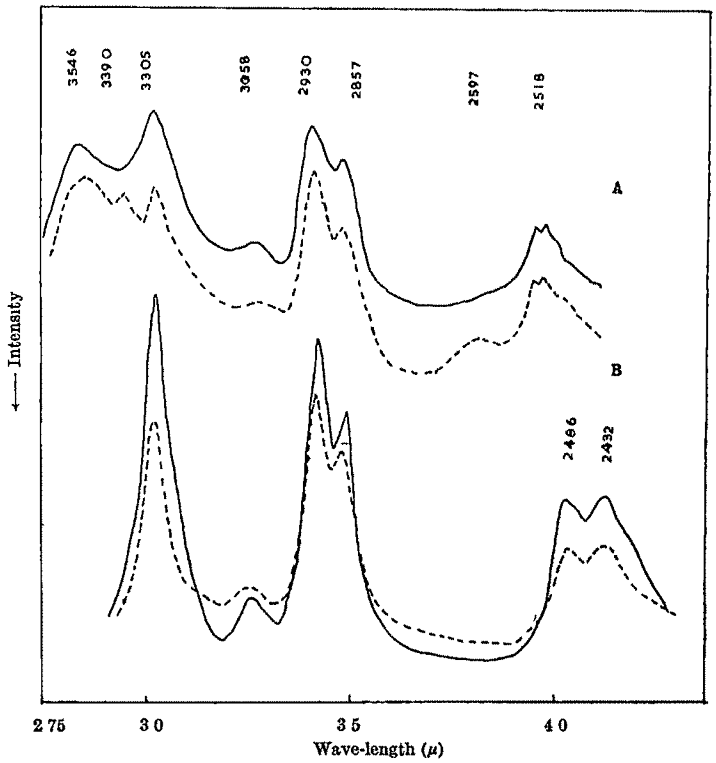


Fig 2 Infra-red spectra of nylon-66 *A*, Treated in magnesium perchlorate, and deuterated, *B*, untreated film, deuterated – – –, Parallel, ——, perpendicular to direction of stretching

filaments, 7 5 denier, commercially drawn, were treated with aqueous solutions of lithium bromide (100 g/100 ml water), magnesium perchlorate (100 g/100 ml water) and lithium perchlorate (50 g/100 ml water) for 12–24 h at temperatures ranging from 20° to 100° C Deuteration was carried out by exposing the films to deuterium oxide vapour at 20° C The films were examined on a Grubb Parsons double-beam infra-red spectrometer, using polarized radiation, and the X-ray diffraction patterns and stress-strain behaviour of the filaments were examined Auxiliary experiments were carried out to study the effect of the reagents on polyacrylonitrile fibres and on *N*-ethylacetamide, and the effect of antimony trichloride on nylon 66

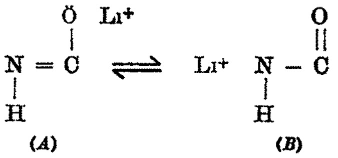
The spectral evidence is illustrated in Figs 1 and 2 A strong interaction between the polar reagents and the peptide group is indicated a new peak appears at 3,390 cm^{-1} and, apart from the broadening of amide bands in the 1,400–1,600 cm^{-1} region, no significant change in spectra is noticed On deuteration, the 3,390 cm^{-1} peak disappears and a band appears at 2,518 cm^{-1} Keeping in view the results obtained by other workers[1–4], it is suggested that the peak at 3,390 cm^{-1} is a new N–H band, for the following reasons·

(1) With the appearance of the new band, the intensity of the original N–H band at 3,300 cm^{-1} gradually diminishes

(2) X-ray evidence is that the interaction is confined to the less-crystalline regions, and this is confirmed by the spectral changes when the treated films are deuterated The broad band due to free water molecules and the new peak disappear rapidly compared with the residual peak at 3,300 cm^{-1}, but on re-hydrogenation the O–D band disappears even during the scanning period while little change is noted in the 2,518 cm^{-1} band this indicates that the peak at 3,390 cm^{-1} is probably not a simple O–H one

(3) The new peak shows slight parallel dichroism and this does not favour its being due to sorbed water

Considering the two possible resonance structures of the peptide group[5]



$$\underset{\underset{H}{|}}{N} = \overset{\overset{\ddot{O}\;\; Li^+}{|}}{C} \rightleftharpoons Li^+ \;\; \underset{\underset{H}{|}}{N} - \overset{\overset{O}{\|}}{C}$$

(*A*) (*B*)

and assuming that a consistent part is played by the ions in an ion-dipole interaction, one may reason out the most probable configuration of electrophilic ions with respect to the ligand[6]

(1) The marked change in the 3μ region (NH stretching mode) and the absence of change in the amide I band (essentially the $C=O$ stretching vibration[7]) do not favour co-ordination as in (*A*) It may be objected that the broadening of the amide bands in the presence of water[4] would mask any effects in this region, but when nylon 66 is dissolved in molten antimony trichloride, the presence of an N–H peak at 3,400 cm^{-1}, amide I band at 1,680 cm^{-1} and amide II band at 1,605 cm^{-1} indicates that reagents which attack $C=O$ groups[1] can bring about a more radical change in the spectrum than is reported here We suggest that degradation of molecular chains, resulting in a large number of $CONH_2$ groups, may be responsible for these changes in the amide I and II bands

(2) Polyacrylonitrile fibres dissolve in aqueous solutions of magnesium perchlorate and lithium bromide one would think that the ions would interact with the cyanide groups and, therefore, that the nitrogen atom would play an important part

(3) Co-ordination through the nitrogen atom, as shown in (*B*), generally results in lowering of the N–H stretching frequency[8], unless the ligand is highly associated by hydrogen bonding, when the frequency may be raised[9] Hence the increased frequency observed here may well result from an ionic interaction as shown in (*B*)

(4) Nylon filaments treated in the foregoing aqueous solutions show a definite decrease in initial modulus and insignificant molecular glide prior to rupture, when stretched If greater rotational freedom is imparted to the

chains by the single-bonded (B) structure, there will be a decrease in the initial modulus[10] and at the same time a closer alignment of the polyethylenic segments will be possible and this will eliminate the gliding of molecules

(5) When lithium bromide is dissolved in N-ethylacetamide there is some sharpening of the broad band in the nuclear magnetic resonance spectrum, although the viscosity of the solution is higher than that of the pure solvent This may be attributed, very tentatively, to a decrease in the effective delocalization of the lone-pair electrons of the nitrogen atom[11]

It is concluded that the probability of an ion-dipole interaction at the nitrogen atom[9,12] in polyamides, when they are treated with polar reagents, may not be ruled out

G Sarda
N Peacock

Fibre Science Research Laboratory,
Department of Textile Technology,
Royal College of Science and Technology *, Glasgow

* Now the University of Strathclyde

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Reaction Kinetics of the Atomic Oxygen-Graphite System

A RECENT communication by Marsh *et al* [1] reported the existence of a small activation energy for the reaction of graphitic carbon with atomic oxygen at temperatures below 350° C Investigations carried out in this laboratory provide a quantitative measure of the activation energy and suggest reasons for the poor agreement with earlier reports[2,3] which indicate a zero activation energy

The high-frequency electrodeless discharge system used in these studies consisted of a 13 56 Mc/s oscillator capable of supplying 250 W to the gas and a 4-cm diameter borosilicate glass reaction tube[4] Oxygen was admitted to the system at a rate of 30 c c /min S T.P and maintained at a pressure of 1 3 mm mercury The rate investigation used spectroscopic grade graphite rods (National Carbon Co , No 3829) 6 4 mm long and 6 1 mm in diameter, with pyrolytic carbon and activated charcoal used in supplementary experiments The oxidation rate was determined from the loss in weight of the carbon rod after a 15-min exposure to the oxygen plasma

As the oxidation of carbon with oxygen is a surface reaction, the temperature at the surface of the specimen is considered to be the significant parameter This temperature was obtained from measurements of the infra-red radiation in the 1 2–2 5μ range emitted from the carbon rods The temperature measuring device, consisting of an optical telescope, a Ge filter, and PbS semiconductor detector (Infrascope Radiation Thermometer, Applied Systems Corp , Palo Alto, Calif , 1963), was calibrated with a source of known temperature and similar emissivity

The relationship between oxidation rate and surface temperature over the range 215°–300° C for a graphite rod immersed in the plasma is shown in Fig 1 The Arrhenius equation, $k = Ce^{-E_a/RT}$, fits the data well and yields a value of 6 5 kcal/mole as the activation energy, E_a A major source of heat is the exothermic oxidation reaction As the average sample temperature, which is considerably lower than the surface temperature, does not increase in direct proportion to surface temperature, use of the average sample temperature will lead to a low value of the activation energy

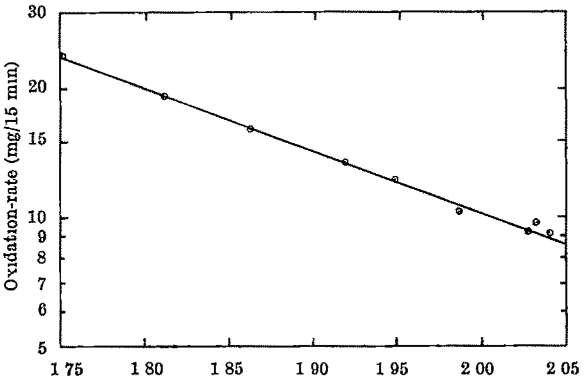

Fig 1 Oxidation rate of spectroscopic grade graphite rods in electrically activated oxygen as a function of surface temperature

Several other factors strongly affect activation energy. Metallic impurities are known to lower the requisite activation energy[5] and increase the oxidation rate of graphite[6] For example, the measured activation energy of a spectroscopic graphite rod wetted with 0 01 N cupric chloride is 12 per cent lower than that of a pure rod Preliminary experiments indicate that in addition to neutral atomic oxygen (3_{P_g} and 1_{D_g}) ionic species such as $O_2+(2_{\pi_g})$ and negatively charged species of oxygen also contribute to the overall reaction rate in the radiofrequency excited plasma Applying a d c potential of -25 V to the carbon specimen reduces oxidation rate to 50 per cent of the rate of a comparable sample at 0 to $+200$ V d c Measurements of atomic oxygen concentration made with differential thermocouple recombination probes[7] do not correlate well with the observed oxidation rates The apparent discrepancies in activation energy may be due, in part, to different proportions of active species of oxygen produced by the various discharge systems

C E Gleit
W D Holland
R C Wrigley

Tracerlab,
A Division of Laboratory for Electronics,
Richmond, California

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Thermal Stability of Polynuclear Aromatic Hydrocarbons and the Polarographic Half-wave Reduction Potential

WE have noted a relation of the thermal stability of certain polynuclear aromatic hydrocarbons to the polarographic half-wave potential In attempting to find some theoretical justification for this relation, it was found that Bergman[1] had related the $E_{1/2}$ to the 'p' ultra-violet band as determined by Clar[2] Also, Basu[3] has related the $E_{1/2}$ to the lowest unoccupied energy orbital Thermal stabilities were determined by Madison[4] by pyrolysis

Table 1 THERMAL STABILITY AND THE $E_{1/2}$, '*p*' BAND FREQUENCY, AND ENERGY OF LOWEST UNOCCUPIED ORBITAL

Compound	Class	$E_{1/2}$ (V[1])	'*p*' Band cm^{-1} (ref 1)	Energy[3] of lowest unoccupied orbital	Moles[4] of gas produced/ moles of charge	Temp (°C)	Time (min)
Naphthalene	I	−1 98	35,090	0 618	2 × 10^{-3}	500	90
Phenanthrene	I	−1 95	34,190	0 605	2 9 × 10^{-3}	500	90
Chrysene	I	−1 80	31,350	0 520	4 6 × 10^{-3}	475	60
Dibenz (*a,h*)-anthracene	I	−1 545	28,490	0 473	15 × 10^{-3}	475	60
Benz (*a*)-anthracene	I	−1 53	27,860	0 452	22 × 10^{-3}	475	60
Anthracene	I	−1 46	26,700	0 414	37 × 10^{-3}	475	60
Naphthacene	I	−1 135	21,230	0 294	414 × 10^{-3}	475	30
Triphenylene*	—	−1 97	35,210	0 684	2 4 × 10^{-3}	475	60
					6 9 × 10^{-3}	500	90
Pyrene	II	−1 61	29,990	0 445	1 1 × 10^{-3}	475	60
Benz (*a*)-pyrene	II	−1 36	26,010		10 × 10^{-3}	475	50
Biphenyl	III	−2 075	40,000		% decomposed = 0 1 (ref 6)	900°F	60
p-Terphenyl	III	−1 905	36,230		% decomposed = 0 28(ref 6)	900°F	60
Fluorene	IV	−2 65 (ref 5)			133 × 10^{-3}	475†	60
Acenaphthene	IV	−2 58 (ref 5)			128 × 10^{-3}	465†	30

* Note that although triphenylene ought to be related structurally to Group I, its $E_{1/2}$ does not plot linearly with the '*p*' band frequency. It would, however, be valid to compare a triphenylene anellation series

† Note that temperatures and times nullify apparent reversal

followed by mass spectrometric analysis of the gas phase products. Table 1 presents the composite data· there the compounds are grouped into classes on the basis of Bergman's classification The groups have theoretical justification in so far as that compounds of the same class plot linearly with respect to $E_{1/2}$ versus '*p*' band frequency Further, comparisons are valid· (*a*) if the compounds are planar molecules of the same class; and/or (*b*) if the compounds are members of an anellation series (for example, naphthalene, anthracene, naphthacene)

It is interesting to note that "the $E_{1/2}$ for an aromatic hydrocarbon measures the energy required to place one or more electrons in the lowest unoccupied orbital"[1] and that "the first electronically excited state of any polyene is derived from the corresponding ground state by the promotion of one electron from the highest occupied level to the lowest unoccupied level"[7] It would appear, then, that the thermal stability of a polynuclear aromatic hydrocarbon is governed by the energy required to promote electrons to the lowest unoccupied orbitals, and the higher that energy, the most stable the molecule This is further confirmed by the higher frequency and more negative reduction potential.

H F MARTIN
B J GUDZINOWICZ

Monsanto Research Corporation,
Boston Laboratories,
Everett 49, Mass

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BIOCHEMISTRY

A Direct Microbiological Assay for Urine Phenylalanine in Phenylketonuric Screening

INCREASED amounts of both phenylalanine and orthohydroxyphenylacetic acid are found in the urine of phenylketonuric (PKU) infants at age 5–8 days, frequently several days before phenylpyruvic acid can be detected in the urine[1]. Determination of these substances in the urine by paper chromatography is not suited to large-scale screening programmes The direct microbiological assay procedure described here is simple enough to be used as a screening test

Direct microbiological assays were used to detect increased amounts of valine, leucine, and isoleucine in urine specimens, dried on filter paper, obtained from mentally retarded children[2] This principle was extended to measurement of phenylalanine in urine specimens collected in a similar manner from infants and mentally retarded children. The standard phenylalanine assay with *Leuconostoc mesenteroides* was utilized together with a commercially prepared media (Difco 'Phenylalanine Assay Broth')[3] As preliminary controls, we used specimens from 100 infants obtained in the Cincinnati PKU testing programme

For each specimen, ½-in paper squares were cut and placed in clean, dry bacteriological culture tubes (13 mm × 100 mm) Uniformity in size of the paper squares from urine or standards was essential to obtain reproducibility of results The culture medium (Difco 'Phenylalanine Assay Broth') was prepared by suspending 5 25 g of dried broth plus 0 0064 g of phenol red in 100 ml. distilled water and heating to boiling as recommended by the manufacturer The addition of phenol red to the media provided a quicker and more accurate method for reading the specimens After 2 ml of the broth was placed in each tube, they were covered with aluminium caps and autoclaved for 15 min at 15 lb pressure, 121° C, and allowed to cool before use Three 10-ml and one 5-ml pipettes and 50 ml of 0 9 per cent saline were sterilized at the same time

Stock solutions of phenylalanine were prepared to contain 20 μg/ml , 40 μg/ml , 120 μg/ml , 200 μg/ml , and 400 μg/ml The standards were also dried on filter paper and ½ in squares cut, placed in tubes and the broth added

Leuconostoc mesenteroides was maintained on Difco 'Yeast Agar' The inoculum was prepared by making a transfer from the agar stab to a tube containing 10 ml Difco 'Micro Inoculum Broth' and incubating for 24 h at 30° C The inoculum was centrifuged for 10 min at medium speed, the cells washed twice in 10 ml sterile saline and resuspended in 5 ml sterile saline Sterile unknowns and standard were inoculated immediately with a loopful of the cell suspension *Leuconostoc mesenteroides* produces acid and the colour of the phenol red indicator changed with increasing amounts of phenylalanine and increasing growth of the bacteria (Table 1)

Table 1

Amount of phenylalanine (μg/ml)	Turbidity	Colour
20	− − −	Red
40	±	Red
80	+	Yellow-orange
120	+ +	Yellow-orange
200	+ + +	Yellow
400	+ + + +	Yellow

Specimens from infants used as controls all gave negative tests for phenylpyruvic acid using ferric chloride Phenylalanine was also measured by paper chromatography in all the specimens from control infants Specimens from 3 of 100 infants were positive at the 80–120 μg/ml. level (microbiological assay), increased amounts of phenylalanine were detected on the paper chromatograms, together with excessive amounts of other amino-acids No other metabolites characteristic of PKU were found

Filter paper specimens obtained from 1,000 mentally retarded children living at home (age 6–18 years) were then tested Seven specimens were positive at the 200–400 μg/ml level Approximately 200 additional specimens were positive in the range 40–120 μg/ml level Confirming tests were carried out on all specimens showing any growth Total amino-acids were determined by two-dimensional chromatography and orthohydroxyphenylacetic acid and phenylalanine by one-dimensional chromatography[4] of urine eluted from the test specimens Table 2 shows the results of the confirming tests on the 7 strongly positive specimens The excretion of other than phenylalanine was in the normal range Orthohydroxyphenylacetic acid was demonstrated in each of the specimens The diagnosis of phenylketonuria was confirmed by determination of serum phenylalanine In 5 of these children, phenylketonuria had not been recognized previously

Table 2 CONFIRMING TESTS ON URINE SPECIMENS SHOWING STRONGLY POSITIVE TEST FOR PHENYLALANINE BY MICROBIOLOGICAL ASSAY

Specimen No	Phenylalanine		Orthohydroxyphenylacetic acid
	Microbiological assay	Paper chromatography*	Paper chromatography*
B-30	+ + + +	350	160
B-30 *A*	+ + + +	280	125
G-18	+ + + +	310	88
L-47	+ + +	200	90
S-9	+ + + +	420	65
T-4	+ + + +	430	90
Y-90	+ + +	230	100

+ + +, 200 μg/ml , + + + +, 400 μg/ml. * μg/ml

The 200 additional specimens were negative for orthohydroxyphenylacetic acid, however, the excretion of phenylalanine was found by paper chromatography within the range determined by microbiological assay Among these specimens the highest value found by chromatography for urine phenylalanine was 100 μg/ml There appeared to be a positive correlation between elevated urine phenylalanine in the range 70–110 μg/ml and generalized mild aminoaciduria

Approximately 95 per cent of non-phenylketonuric children excreted less than 73 μg/ml[5] The minimum phenylalanine excretion observed in 44 untreated phenylketonuric patients was 100 μg/ml In this work 10 per cent of the urine specimens from retarded children were retested because of phenylalanine concentration over 40 μg/ml. Among these 4 8 per cent contained phenylalanine in the range 70–120 μg/ml and an additional 0 7 per cent contained phenylalanine in excess of 200 μg/ml

The direct microbiological assay for phenylalanine appears to be a simple and rapid screening method for phenylalanine It is useful as a supplementary test for PKU as well as for screening for aminoaciduria

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CAROLYN SCHEEL
HELEN K. BERRY

Children's Hospital Research Foundation,
College of Medicine,
University of Cincinnati

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Inhibition of Pyridine Nucleotide Transhydrogenase by Dicumarol

WE have recently described the hormonal sensitivity of a transhydrogenase reaction in mitochondria (reaction I)[1].

$$NADPH_2 + AcPyAD \rightarrow NAD + AcPyADH_2 \qquad I$$

Reaction I is stimulated by diethylstilbœstrol and œstradiol and thus differs from the transhydrogenase of Kaplan[2]. This may reflect a difference in the method of preparation which preserves its hormonal sensitivity[3] In further studies of this reaction, the reverse direction (reaction II) has been examined.

$$NADH_2 + NADP \rightarrow NAD + NADPH_2 \qquad II$$

In this report, the inhibitory effect of dicumarol on reactions I and II will be described

The enzyme was prepared as follows Livers were removed from male rats and placed on ice A 10 per cent homogenate (w/v) in 0 25 M sucrose was prepared in a glass homogenizer with a 'Teflon' pestle. After centrifuging for 10 min at 700 *g*, the pellet was discarded and the supernatant centrifuged at 7,000 *g* for 15 min This pellet was washed twice in sucrose, suspended in 0 03 M phosphate (*p*H 7 0), and centrifuged for 20 min at 20,000 *g* The pellet was suspended in phosphate so that 1 ml represented 2 g of liver. The mitochondrial suspension was subjected to ultrasound for 45 sec, an equal volume of phosphate was added and this suspension was centrifuged at 25,000 *g* for 20 min. The supernatant containing mitochondrial fragments was then used in the following experiments

The inhibitory effect of dicumarol on the rate of reaction I is shown in Fig 1 Simultaneous reactions were followed for 5-sec intervals in four cuvettes with a 1-cm light-path at 375 mμ by means of a Gilford model 2000 multiple sample absorbance recorder The reaction mixture contained in a volume of 1 ml , phosphate buffer (*p*H 7 5) 50 μmoles, Mg^{++} 1 μmole, enzyme 10 μl , and where indicated $NADPH_2$ 0 23 μmoles, 3 AcPyAD 0 55 μmoles, and dicumarol 0·1 μmole This concentration of dicumarol (10^{-4} M) was found to be a potent inhibitor of reaction I. A similar effect was seen when $NADPH_2$ was replaced by $NADH_2$. The effect of dicumarol on the reverse reaction (II) was examined As Danielson and Ernster have reported[4], this reaction is accelerated in the presence of Mg^{++} and ATP (Fig 2) The reaction was followed at

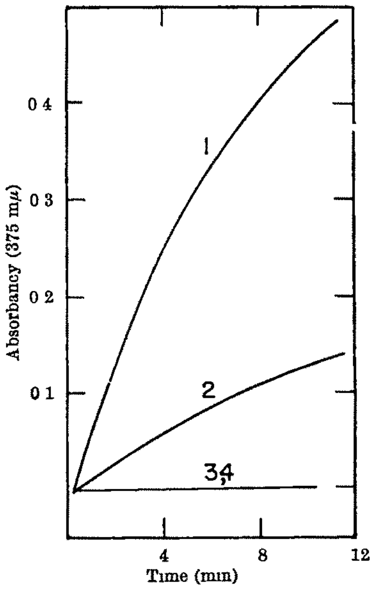

Fig 1 Inhibition of reaction I by dicumarol Four simultaneous reactions were followed sequentially for 5-sec intervals Cuvette 1 contained buffer, $NADPH_2$, AcPyAD, Mg^{++} and enzyme Cuvette 2 contained, in addition to reaction mixture I, dicumarol Cuvette 3 lacked $NADPH_2$, cuvette 4 lacked AcPyAD See text for concentrations of the components

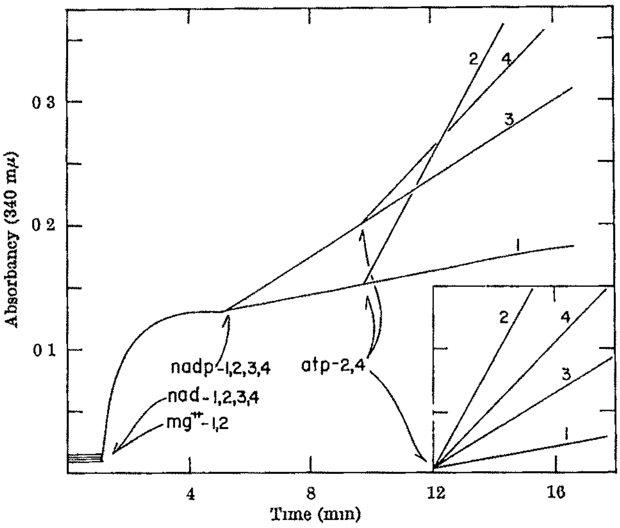

Fig 2 Effect of Mg^{++} and ATP on reaction II Four cuvettes were used as in Fig 1 All cuvettes contained buffer, potassium cyanide, alcohol dehydrogenase, ethanol and enzyme Cuvettes 1 and 2 contained Mg^{++} NAD added to all cuvettes was promptly reduced NADP was added to all cuvettes ATP was added to cuvettes 2 and 4 The rates from the time of ATP addition are shown in the inset to demonstrate their different slopes

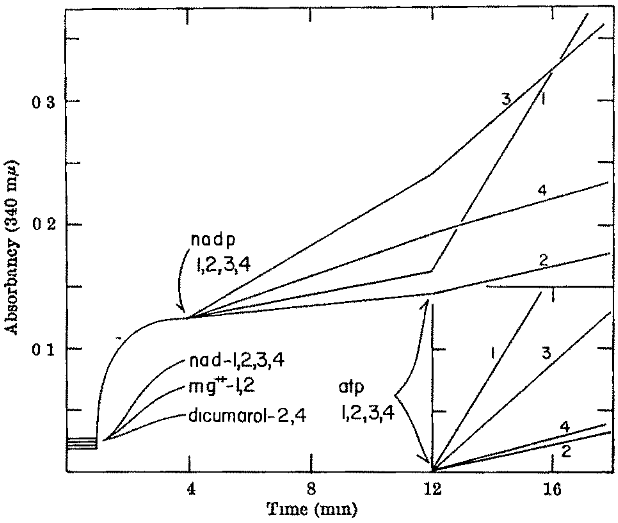

Fig 3 Inhibition of reaction II by dicumarol Four cuvettes were followed as in Fig 1 All cuvettes contained buffer, potassium cyanide, alcohol dehydrogenase and ethanol Cuvettes 1 and 2 contained Mg^{++}, cuvettes 2 and 4 contained dicumarol NAD added to all cuvettes was reduced NADP was added to all cuvettes and at 12 min, ATP was added to all cuvettes The curves from the point of addition of ATP have been placed in the inset to demonstrate the inhibition produced by dicumarol in cuvettes 2 and 4

340 mμ in cuvettes with a light-path of 1 cm which contained in a volume of 1 ml, *tris* buffer (*p*H 8) 50 μmoles, sucrose 250 μmoles, yeast alcohol dehydrogenase 0 33 mg, potassium cyanide 1 μmole, ethanol 5 μl, NAD 0 033 μmoles, NADP 0 23 μmoles, and where indicated Mg^{++} 6 μmoles, dicumarol 0 1 μmole, and ATP 2 μmoles Fig 2 illustrates the stimulatory effect of Mg^{++} and ATP Mg^{++} in the absence of ATP inhibits reaction II while ATP has only a slight effect on the rate in the absence of Mg^{++} The effect of dicumarol on reaction II is shown in Fig 3 It retards the rate of transhydrogenation whether or not Mg^{++} and ATP are present However, the inhibition is best seen after these additions

That both reaction I and reaction II are inhibited by dicumarol supports the suggestion by Hommes and Estabrook[5] that the enzymes catalysing these reactions are similar This suggestion was made on the basis of their sensitivity to triiodothyronine and high-temperature coefficients The sensitivity to dicumarol also suggests a relationship between transhydrogenase and D-T diaphorase This diaphorase, which is located predominantly in the soluble cytoplasm, is found to a small extent (5 per cent) in mitochondria and is inhibited by dicumarol[6] D-T diaphorase was present in the enzyme preparation used in these experiments and, when dichlorophenolindophenol was the acceptor, its reduction using NADH$_2$ (D) and NADPH$_2$ (T) was inhibited by dicumarol D-T diaphorase is inhibited by triiodothyronine[7], which again points to its similarity to the transhydrogenase(s) which triiodothyronine inhibits[5,8] Since D-T diaphorase differs in its intracellular distribution from transhydrogenase and can be prepared free of transhydrogenase activity, it must be considered as a separate enzymatic entity It is conjectured, however, on the basis of their sensitivity to triiodothyronine and dicumarol, that there may be a common component in these three reactions, I, II, and D-T diaphorase

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JOSEPH MCGUIRE
Section of Dermatology, Department of Medicine,
Yale University School of Medicine,
New Haven, Conn
LEROY PESCH
Rutgers Medical School, Rutgers, the State University,
New Brunswick, New Jersey
HELEN FANNING
Yale University School of Medicine,
New Haven, Conn

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Circular Dichroic Studies of Synthetic Polypeptides

RECENTLY in solutions of α-helical polypeptides and proteins a Cotton effect was observed, having an inflexion point at 225 mμ and which has been shown to be conformation-dependent[1-3] This Cotton effect probably plays a major part in determining the shape of the rotatory dispersion curve in the visible region and therefore is responsible for the Moffitt term, which has been the empirical factor in estimates of the helical contents of polypeptides[4]. We wish to report here a sensitive method for studying the conformation of polypeptides by using the circular dichroism (C D) measurements[10] in the ultra-violet region of this Cotton effect at 225 mμ The surface of the C D bands (a measure of the rotational strength) can be correlated with the amount of helical content, while the sign of C D bands might be related to the absolute conformation, that is, the sense of helix

In order to elucidate the influence of the amino-acid residues on the sense of twist of helix the following L- and D-polypeptides were prepared polypeptides such as poly-glutamic acid, poly-lysine, and poly L- and D-aspartic acid, which in the side-chain contains a chromophore (carboxyl group) beyond the β-carbon[5]

The poly-glutamic acid and poly-L-lysine were prepared according to Idelson *et al*[6] Poly-L-aspartic acid was prepared from poly benzyl-L-aspartate (mol wt = 90,000) debenzylated with anhydrous hydrogen bromide in a mixture of benzene and chloroform Elementary analysis of a sample dried in vacuum at 100° for 16 h gave the proper results of poly aspartic acid In view of the possible formation of β-peptide linkage, one sample was checked following the chemical method of Kovacs *et al.*[7]. The results indicated about 7 per cent of β-peptide linkages. However, ultra-violet analysis showed the absence of succinimide rings and no detectable benzyl groups.

The results shown in Fig. 1 indicate that the helical form of poly-L-glutamic acid (*p*H 4) and of poly-L-lysine (*p*H 11) exhibit a negative circular dichroism curve, whereas the enantiomorph poly-D-glutamic acid in identical conditions yields a positive C.D. curve. Similarly a negative circular dichroic curve was observed for whale myoglobin and therefore indicating a right-handed helix[8]. Our results indicate that the maxima of these bands are centred at about 225 mμ, confirming previous optical rotatory dispersion data on the Cotton effect[1–3]. Approximate estimation of rotational strength yields the following values: poly-D-glutamic acid, about $+125$ in 10^{-40} C.G.S. units; poly-L-glutamic acid, about -125×10^{-40} C.G.S. units; poly-L-lysine, about -134×10^{-40} (assuming a Gaussian curve[9]). These values are in good agreement with Moffitt's theoretical prediction[4] (about 120×10^{-40}) but at variance with previously reported values for poly-L-glutamic acid[9]. The disordered form of these polypeptides (at neutral *p*H) causes the disappearance of the circular dichroic absorption band at 225 mμ, confirming the previous observations[1–3,9].

The poly-L-aspartic acid at *p*H 4·3 exhibits a negative C.D. curve centred also at 225 mμ (Fig. 2), but of smaller intensity with strength of about -35 in 10^{-40} C.G.S. units, while the poly-D-aspartic is characterized by a positive band of approximately the same absolute value of rotational strength. At neutral and alkaline *p*H, a very pronounced decrease of this C.D. curve was observed, indicating the transformation from ordered to random coil form. This behaviour is fully reversible, that is, on lowering the *p*H (from 12 to 4) the same C.D. curve reappears. The optical rotatory dispersion data of poly-L-aspartic acid at *p*H 4·2 obey the Moffitt equation and yield the b_0 value of -160, which also indicates the existence of a helical conformation, whereas at alkaline *p*H the b_0 vanishes and the dispersion follows the one-term Drude equation. The helical form of poly-aspartic acid is very sensitive to the presence of interfering substances; for example, acetate buffer 0·3 M causes a sharp decrease of the intensity of the C.D. band and a pronounced shift of the maximum from 225 mμ to 235 mμ (Fig. 2), indicating that probably another form of macromolecular conformation of poly-aspartic acid takes place. This demonstrates the advantage of circular dichroism[10] studies, which permit resolution of the optical rotatory contribution of each individual optically active transition. Work is in progress in order to gain a more complete characterization of poly-aspartic acid.

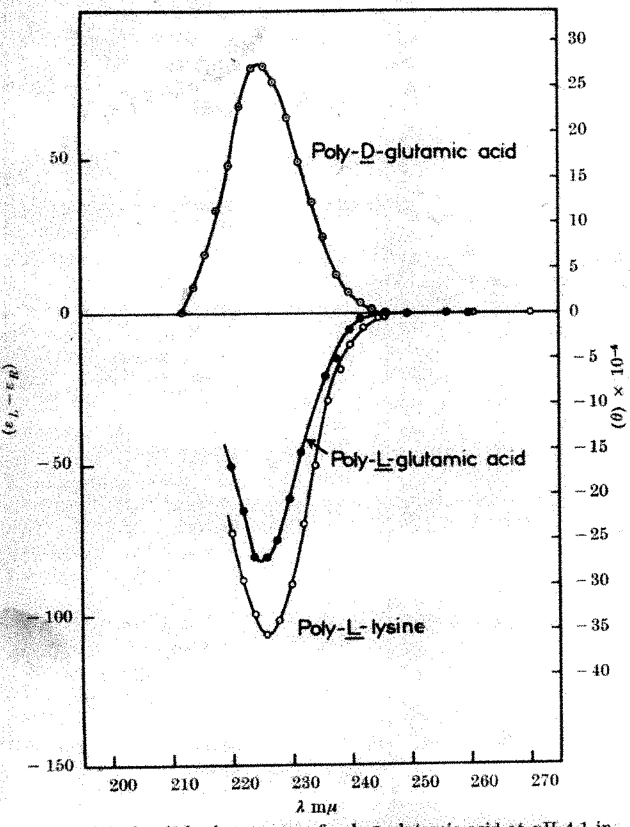

Fig. 1. Circular dichroism curves of poly-L-glutamic acid at *p*H 4·1 in 0·15 M NaCl, 0·1 acetate buffer; poly-D-glutamic acid in the same conditions; poly-L-lysine 0·2 M NaCl, *p*H 11. Measurements were carried out on a Russel–Jouan dichrograph. The apparatus is equipped with the sensitivity setting of 3, 2, 1·5 and 0·46

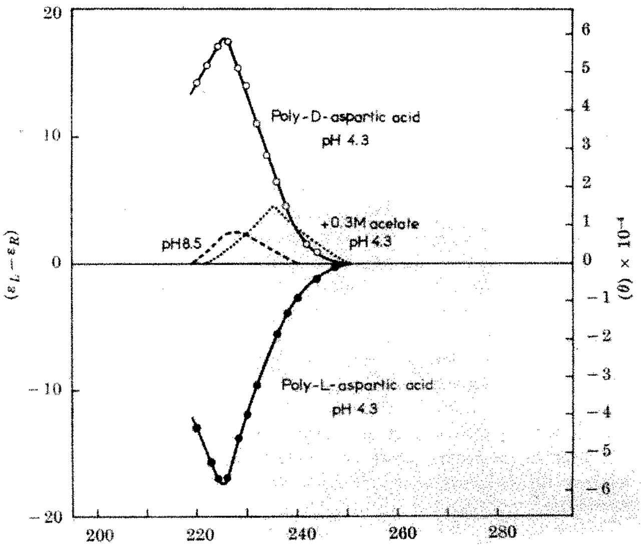

Fig. 2. Circular dichroism curves of poly-L-aspartic acid at *p*H 4·3 (–) and *p*H 8·5 (– – –) in 0·2 M NaCl

All these results indicate that poly-L-aspartic acid, like other L-polypeptides, forms a right-handed helix whereas the D-poly-amino-acid exists as a left-handed helix. Some differences observed for poly-L-aspartic-acid expressed in the relatively low rotational strength value, low value of $-b_0$, reflect less organized helical structure as compared with its homologue poly-L-glutamic acid. It appears that the rotational strength value of C.D. band at 225 mμ may be used as a measure of the amount of helical content in polypeptides.

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J. Brahms
G. Spach

Centre de Recherches sur les Macromolécules,
Strasbourg, France.

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A Helical Structure in Ribonucleoprotein Bodies of *Entamoeba invadens*

THERE have been conflicting reports in the literature about the chemical nature and origin of chromatoid and related bodies found in the cytoplasm of certain protozoa[1-8] and in the spermatogenic cells of certain crustaceans[9], insects[10-11], cyclostomes[9], reptiles[12], birds[9], and mammals[13-15]. Of special importance is the recent work of Barker[1,16]. This investigator has shown in his cytochemical and electron microscope studies that chromatoid bodies in *Entamoeba invadens*, a parasite of snakes, are composed of ribonucleic acid and protein and that chromatoid bodies arise by a process of aggregation of small groups of 250–300 Å units which form polycrystalline masses in precysts and early cysts. The purposes of this report are to show: (*A*) that the chromatoid bodies are also found in axenically grown trophozoites, where there is no cyst formation, and (*B*) that the ribonucleoproteins within the chromatoid bodies are arranged in a helical pattern.

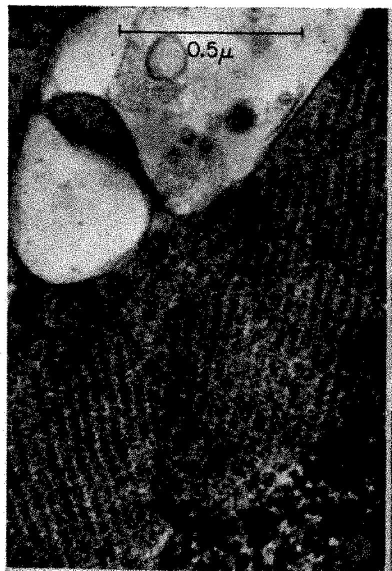
Trophozoites of the PZ strain of *E. invadens*, grown axenically in Diamond's medium[17], were fixed for 1 h at 0° C in 1 per cent OsO_4 buffered with veronal acetate at *p*H 7·4. The trophozoites were then dehydrated in ascending concentrations of ethyl alcohol with occasional gentle centrifugation and embedded in cross-linked methacrylate[18]. Polymerization was carried out at 50° C for 24 h. Thin sections, cut with glass knives in a Porter–Blum microtome, were stretched by the application of xylol, mounted on 'Formvar' and carbon-coated grids, and stained with Karnovsky's stain[19]. Electron micrographs were taken with a Philips electron microscope model Em-100 at original magnifications 9,700–13,400 and enlarged further photographically.

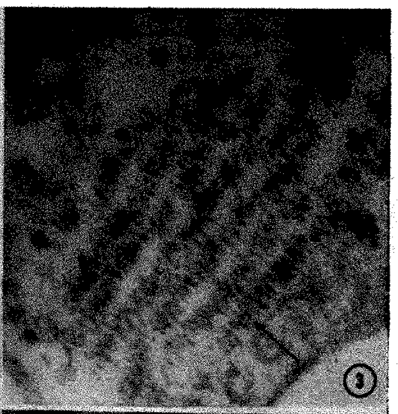
The detailed description of electron microscope studies of trophozoites of *E. invadens* will be published elsewhere; this report deals exclusively with results of studies on 'chromatoid bodies'. Two to six chromatoid bodies of varying size from 1·0 × 0·55μ to 2·9 × 0·7μ may be seen in a single trophozoite. They are scattered throughout the cytoplasm and in electron micrographs appear to be composed of bands or lamellæ arranged in parallel arrays (Figs. 1 and 2). The number of lamellæ varies from 9 to 34 depending on the size of the chromatoid body. The width of individual lamellæ ranges from 250 to 350 Å and the distance between two adjacent lamellæ is 100 to 150 Å. At higher magnification (Figs. 1, 2 and 3) the lamellæ appear to be composed of coiled fibrils which seem to form a closely packed helix. In some places the helix is particularly well defined.

This helical structure in a ribonucleoprotein body is of particular interest. Since Watson and Crick's[20] successful approach to the structure of DNA, similar procedures have been adopted by various workers to study the structure of RNA. X-ray diffraction studies of a complex of polyinosinic and polycytidylic acids gave a crystalline diffraction pattern resembling that of DNA and a non-crystalline pattern like that of RNA[21]. This suggested that RNA might have a structure like DNA[22]. Similar conclusions were also drawn from X-ray studies of soluble RNA[23] and from molecular model-building[24]. Recently, X-ray diffraction studies on crystalline yeast transfer RNA have given conclusive evidence that RNA molecules are helical in structure[22]. In another report[25], the structure of RNA has been shown to be helical in nature based on X-ray diffraction studies of ribosomes extracted from *E. coli*, *Drosophila* larvæ, rat liver and rabbit reticulocytes. Barker[16] proposed the hypothesis that the chromatoid body represents a crystalline store of ribosomes and his preliminary results of analytical centrifugation of the particles indicated that they may have sedimentation rates of approximately 180, 160 and 100*S*.

Our study shows a helical pattern in chromatoid bodies. In places the structure is clearly defined. The dimensions of the helices in the chromatoid bodies of *E. invadens* as they appear in electron micrographs are far larger than those calculated by X-ray diffraction studies for transfer ribonucleic acid[22] and for ribosomal RNA in several organisms[25]. It is too early to speculate on the interpretation of this difference, for more detailed biochemical data on the chromatoid body are needed.

The significance of the findings described in this article resides in the fact that this is the first demonstration by electron microscopy of a helical structure in ribonucleoprotein bodies. The structure disclosed seems important enough to be reflected in terminology, particularly in view of the number of different other terms used in addition to 'chromatoid bodies', most of them (including the latter) misleading and confusing. It seems appropriate, therefore,





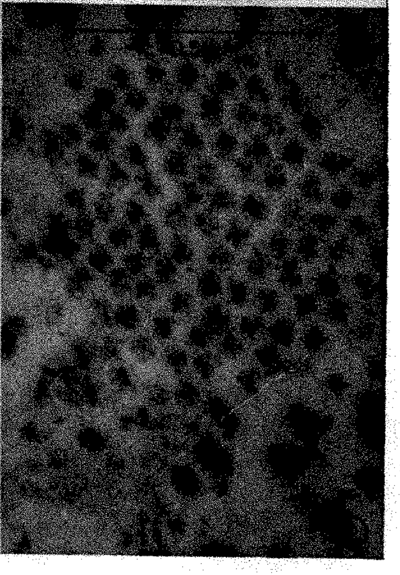


Fig. 1. Electron micrograph of 'chromatoid body' to show that in a longitudinal section it is composed of bands or lamellæ arranged in parallel arrays. The helical structure of the lamellæ can be seen at arrows

Figs. 2 and 3. Higher magnification of lamellæ. Note that the lamellæ are composed of coiled fibrils, forming a closely packed helix. The helical structure is particularly seen at arrows

Fig. 4. Cross-section through lamellæ

at this point to propose the introduction of a new, more specific term, 'helical RNP bodies', for chromatoid bodies of the same nature as those found in *E invadens* The new term combines the biochemical composition defined by Barker with the helical structure described in this article

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WASIM A SIDDIQUI
MARIA A RUDZINSKA

Rockefeller Institute,
New York, 21

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Effect of DL-Ethionine on the Level of Cystathionase in Rat Liver

WE have previously reported that the level of cystathionase ('soluble' cysteine desulphurase) of rat liver is increased when L-methionine is injected in male rats maintained on a commercial diet[1] We have recently shown that a similar increase could be obtained with rats placed on a synthetic diet (diet *B*) containing 18 per cent casein, furthermore, the level of cystathionase is higher in liver of rats maintained on a synthetic diet (diet *C*) containing 60 per cent casein, and we have observed that addition of a definite quantity of DL-methionine to diet *B* increases the enzyme-level[2] These results indicate that some correlation could be established between dietary protein and the level of enzyme activity, and suggest that 'adaptation' of cystathionase is mediated, at least in part, by the content of methionine in the diet As the increase in enzyme activity found on varying methionine intakes could be the reflexion of alteration of the content of specific protein in the liver, in the investigation described here we have determined the effect of addition of DL-ethionine (*S*-ethyl/analogue of methionine) to the diets on the level of hepatic cystathionase

We used Wistar rats from the Centre de Sélection des Animaux de Laboratoire du C N R S, with mean body-weight of 80 g, the preparation of the supernatant fraction of the liver, the conditions of determination of hydrogen sulphide formation and the composition of the diets have been described earlier[2-4] The experiments were conducted as follows all rats were placed for 8 days on the diet *B*, then they were divided in two groups, the first group was maintained on diet *B*, while the second received diet *B* containing 2 5 per cent DL-ethionine After 8 days, rats were weighed and killed, the weight of liver and the activity of cystathionase were determined The contents of protein in supernatant are measured according to the method of Lowry *et al* [5]

Other experiments were similarly carried out with rats routinely maintained in diet *C* and diet *C*+2 5 per cent ethionine, furthermore, as from the results of Farber and Corban[6] ethionine inhibits hepatic protein synthesis more effectively in female than in male rats, some experiments were performed on female rats All results are expressed as μmoles hydrogen sulphide/μg protein/h

Table 1 shows the results obtained in these assays From the figures it is evident that DL-ethionine increases the level of enzyme Such an increase is rather surprising and the stimulation of cystathionase by DL-ethionine merits further comments The increase of activity was observed in male rats maintained on diets *B* and *C*, but in this last case the increase did not start before 10 days, while it is faster in rats placed on diet *B* Furthermore, we have observed no difference between male and female rats, ethionine is equally effective for the alteration of enzymatic activity Moreover, while methionine by itself is not a substrate for cystathionase, some products formed from methionine, like cystathionine, homoserine and cysteine, are good substrates, and the response of enzyme to injections of some of these substrates is an increase of level[1] If ethionine is de-ethylated, one can suppose that cystathionine and other substrates might arise; however, it seems improbable that de-ethylation of ethionine is of considerable importance in the rat[7]

Table 1 EFFECT OF ADDITION OF DL-ETHIONINE TO SYNTHETIC DIETS ON THE LEVEL OF CYSTATHIONASE

Diet		Body weight (g)	Liver weight	Mg protein/ml supernatant	H_2S μmoles/μg protein/h
I Male rats					
B	(8 days)	133	7 4	13 2	286 ± 12
B + eth	(8 days)	85	3 0	10 4	1,383 ± 95
C	(10 days)	164	6 0	14 8	795 ± 38
C + eth	(10 days)	116	3 9	12 0	840 ± 74
C + eth	(19 days)	99	4 7	12 8	1,197 ± 168
II Female rats					
C	(8 days)	130	6 3	12 9	551 ± 97
C + eth	(8 days)	84	3 1	11 7	1,143 ± 71

Natori[8] has reported that the level of methionine-activating enzyme in female rats is almost twice that of male rats, and he observed that the ethyl group of ethionine is extensively incorporated into the liver RNA fraction It might be supposed that action of ethionine may be due to the formation of an abnormal cystathionase in which methionine is partially, at least, replaced with ethionine This new protein could have different physico-chemical properties and enzymatic activity to those of normal cystathionase

An examination of the properties of purified enzyme obtained from livers of normal rats and from livers of ethionine-treated rats is under investigation

FERNANDE CHATAGNER
ODILE TRAUTMANN

Laboratoire de Chimie biologique,
Faculté des Sciences,
Paris

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Excretion of Indolyl Acids in Phenylketonuric Monkeys

EXPERIMENTAL phenylketonuria has been induced in the rhesus monkey by feeding excess dietary phenylalanine[1,2] The phenylalanine plasma level was markedly elevated, and the urinary excretion of phenylketones and phenylacetyl glutamine were comparable with that found in children with phenylketonuria Behavioural and learning tests have demonstrated a severe degree of mental retardation in these experimental animals This report provides data on the excretion of 5-hydroxyindolyl-acetic acid and indolyl-3-acids in these monkeys and demonstrates that the role of tryptophan is similar in both the experimental disease and in phenylketonuric infants

Five normal monkeys and five experimental monkeys (*Macaca mulatta*) were studied over a one-year period Each animal was placed in a metabolic chair constructed in this laboratory and modified from that previously described[2] Only male monkeys were used and appropriate collection devices similar to those used in pediatric patients were attached to the genital area Urine samples, taken over 24-h periods, were collected in a bottle kept in ice Several crystals of thymol were added to each bottle as a preservative The urine was kept frozen at – 10° C until ready for analysis The animals received commercially modified milk either with or without added phenylalanine (3 0 g/kg/body-weight/day) and were fed *ad lib* at 4-h intervals The method of Udenfriend *et al* [3] was used for the determination of free 5-hydroxyindolylacetic acid (5-HIAA), and the method of Weissbach *et al* [4] for free indolyl-3-acids (indolyl-3-acetic, indolyl-3-lactic and indolyl-3-pyruvic acids) The control animals ranged in age from 3 to 18 months, whereas the experimental animals varied from 8 months to 24 months at the time of the urine collections

The analysis of the 24-h urine collections is shown in Table 1 The excretion of indolyl-3-acids was not significantly different between the experimental and the control groups, but the decreased excretion of 5-HIAA in the experimental group was highly significant ($P < 0\ 0025$) Although not shown in Table 1 (for reasons of brevity), no differences in base line values were found for either the indolyl-3-acids or for the 5-HIAA between the younger and older animals In addition there was no evident correlation between the total 24-h excretion of the indolyl acids and the weight of the monkey or the length of time on phenylalanine diet.

Table 1 EXCRETION OF 5-HYDROXYINDOLYL-ACETIC ACID AND INDOLYL-3-ACIDS IN NORMAL AND PHENYLKETONURIC MONKEYS PER 24 H

Group	No of animals	No of urine collections	5-HIAA (μg/day)	3-Indolyl-acids † (mg/day)
Control	5	15	304 ± 230*	1 48 ± 2 03*
Experimental	5	10	47 ± 26*	1 15 ± 1 45*

* Standard deviations are reported for differences between collections
†Total of indolyl-3-acetic, indolyl-3-lactic and indolyl-3-pyruvic

The close biochemical relationship between phenylalanine and tryptophan and tryptophan metabolites has found support in a number of observations Work in this laboratory has shown that excess phenylalanine causes an *in vitro* inhibition of tryptophan hydroxylation in rat liver[5] It was, therefore, not surprising that decreased serotonin was found in the brain and liver of rats fed excess phenylalanine[6] Since 5-HIAA is the end excretory product resulting from oxidation of serotonin, any decrease in excretion represents either an inability to form the serotonin precursor (5-hydroxytryptophan) from hydroxylation of tryptophan or an inability to decarboxylate this product Another factor which influences 5-HIAA excretion is the amount of tryptophan absorbed from the intestinal tract before it enters the blood Akedo and Christensen[7] have reported evidence of competition between certain amino-acids in their transfer across the intestine, and it is not unlikely that excess phenylalanine may likewise interfere with the intestinal absorption of tryptophan McKean *et al* [8], and Guroff and Udenfriend[9] have shown that excess phenylalanine and certain other amino-acids also inhibit transport of 5-hydroxytryptophan across the blood-brain-barrier and thus effectively decrease the formation of serotonin

An increased indolyl-3-acid excretion by human phenylketonurics has been reported by Armstrong and Robinson[10] This increase was attributed primarily to indolyl-3-lactic acid, which is not distinguished from indolyl-3-acetic acid in the methods described here The site of formation of the 5-HIAA is probably different from that at which indolyl-3-acids are formed

Following a tryptophan load test, Toda and Bessman[11] found an increased excretion of indican and indolyl-3-acetic acid and a decreased excretion of kynurenic acid, xanthurenic acid and *N*-methylnicotinamide These findings are understandable, since the former compounds arise largely from intestinal putrefaction of unabsorbed tryptophan whereas the latter products arise mainly from tissue metabolism of absorbed tryptophan These observations would seem to offer further evidence for decreased absorption of tryptophan followed by absorption of its putrefactive products from the lower intestine[4] Since a milk diet modifies the intestinal microflora, the effect of an excess phenylalanine milk diet on excretion of indolyl-3-acids was probably an added factor in our experimental monkeys The effect of diet on intestinal flora could also be why all phenylketonuric children do not excrete increased amounts of indolyl-3-acids Bessman and Toda[12], on the basis of indole tolerance tests, suggest that the increased excretion of indican and indolyl-3-acids in phenylketonuric children is due to a more general derangement of indole metabolism However, another explanation may be that these compounds are increased mainly as a result of decreased tryptophan absorption whereas the decreased excretion of 5-HIAA is due to alterations in the activity of the hydroxylases and decarboxylases as well as to effects on the transport of amino-acids

The decreased 5-HIAA excretion found in phenylketonuric monkeys demonstrates still another biochemical similarity of the experimental condition to that found in the human disease[13] The return to normal of the excretion of 5-HIAA by phenylketonuric patients treated with a diet low in phenylalanine[14] is comparable with removal of the phenylalanine excess diet in the monkey

DALLAS E BOGGS
DAREN MCLAY
MICHAEL KAPPY
HARRY A WAISMAN

Joseph P Kennedy, jun , Laboratory,
Department of Pediatrics,
University of Wisconsin Medical School,
Madison Wis

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Partial Separation of Rat Liver Mitochondrial Amine Oxidases

ALTHOUGH the mitochondrial monoamine oxidase (monoamine O_2-oxidoreductase (deaminating), *EC* 1 4 3 4) is generally believed to possess broad substrate specificity[1], numerous results may be considered as indirect evidence of 'multiplicity' of mitochondrial amino oxidases[2–7] Nevertheless, separation of mitochondrial amine oxidases has not so far been reported to my knowledge

This communication describes an example of partial separation of rat liver mitochondrial amine oxidases attacking two different amines suitable for spectrophotometric determination of the enzymatic activity, namely, *m*-nitro-*p*-hydroxybenzylamine (PHB) (ref 8) and *p*-nitrophenylethylamine (PN) (ref 9).

Both substrates were synthesized by Dr N A Kitrossky and G A Leontyeva (Department of Organic Chemistry, Institute of Biological and Medical Chemistry) Mitochondria prepared from rat liver homogenates[10] were treated by hypotonic phosphate[11], solubilized in the presence of a non-ionic detergent '*OP*-10' (final concentration 1 25 per cent) as described earlier[12] and adjusted to *p*H 7 00 All the subsequent operations were performed at 3° The solubilized material was applied to a 24 mm × 95 mm 'Brushite' column[13,14] Stepwise elution by 0 1, 0 4, 0 5 and 0 7 M potassium-phosphate buffers, *p*H 7 00, containing 1 25 per cent (w/v) of '*OP*-10', was carried out 15-ml fractions of effluent were collected at a flow rate of 4 ml /min Protein content was determined by the method of Lowry *et al* [15] with precautions necessary when working in presence of phosphates and detergents[16] PHB oxidation was followed by measuring optical density increase (due to formation of *m*-nitro-*p*-hydroxybenzaldehyde) at 315 mμ in 10-mm cuvettes put in a thermostat at 37° (spectrophotometer *SF*–4) for 5 min The samples containing 0 2 M potassium-phosphate buffer (0 5 ml), enzyme solutions (0 2–2 0 ml), PHB (final concentration 5 3 × 10^{-4} M) were brought up to 3 0 ml with water The spectrophotometric unit (SU) was defined as a quantity of enzyme necessary to cause an increase in optical density by 0 001 in 1 min For PN oxidation assay PHB in samples described was substituted by PN (1 mg per sample) and after incubation at 37° during 2 h optical density measurements at 453 mμ in 10-mm cuvettes of 'FEKN–57' photoelectric colorimeter were taken The assay yields fairly reproducible results and proved to be very sensitive The quantity of the enzyme necessary to cause the optical density increase by 0 01 in the standard conditions was defined as a unit (un) of enzymatic activity

Table 1 PARTIAL SEPARATION OF RAT LIVER MITOCHONDRIAL AMINE OXIDASES

	Starting material	Combined effluent fractions				Recovery (%)
		1–8	9–16	17–25	26–34	
Volume (ml)	20 0	131 2	131 0	149 5	135 5	
Phosphate concentration (M/l)	0 1	0 1	0 4	0 5	0 7	
Protein content						
mg/ml	2 25	0 225	0 06	0 02	0 02	
Total	45 0	29 32	7 86	2 99	2 71	95 2
PHB-deamination						
SU/ml (max)	40 0	0 0	0 0	2 5	1 0	
Total	800 0	0	0	37 5	12 5	6 25
SU/mg of protein (max)	17 8	0 0	0 0	125 0	50 0	
PN-deamination						
Un /ml (max)	67 5	1 0	19 5	8 0	5 7	
Total	1,350 0	13 0	753 5	327 0	138 0	90 2
Un /mg of protein (max)	30 0	4 4	325 0	400 0	285 0	

Results of one of five similar experiments presented in Table 1 indicate that 0 4 M phosphate elutes up to 56 per cent of the 'PN-deaminating' enzyme applied to the column while no 'PHB-deaminating' activity could be detected in these fractions At higher phosphate concentrations both enzymatic activities are found in eluates While up to 90 per cent of initial 'PN-deaminating' activity are recovered in eluates, the recovery of 'PHB-activity' was rather low Our preliminary observations suggest the phenomenon is caused by increased lability of the later enzyme on column chromatography

Further work on separation and purification of mitochondrial amine oxidases, designated at present by a common term 'monoamine oxidase', as well as investigation of their substrate specificity and effect of inhibitors are in progress and will be published elsewhere in Russian

V Z GORKIN

Institute of Biological and Medical Chemistry,
Academy of Medical Sciences of the U S S R ,
Moscow

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A Pentose identified in the Carbohydrate Group of Spongin

Bergmann and Feeney[1] have isolated a new thymine pentoside from a sponge (*Cryptotethia*) Isolation and identification of the carbohydrate fragment of this nucleoside have been unsuccessful, but the high dextrorotatory power of this nucleoside suggests that it is a xylofuranoside rather than the as yet unknown thymine nucleoside

Previously we had examined[2] the carbohydrate group of a scleroprotein (spongin) and identified the sugar (galactose) by paper chromatography In the work recorded here we have identified a different sugar (xylose) in the carbohydrate group from *Euspongia officinalis*

Euspongia officinalis were cut in very small pieces, mixed with 2 N hydrochloric acid and shaken in a vacuum several times to remove the calcium carbonate The material so obtained was washed with distilled water and dried in a vacuum desiccator over phosphorus pentoxide To 1 5 g dried sponge were added 150 ml water and this mixture heated for 6 h at 80° C under a reflux condenser on a water-bath, and filtered The filtrate was concentrated to 1–2 ml on the water-bath under vacuum (sample I) The same procedure was repeated using hydrolysis with

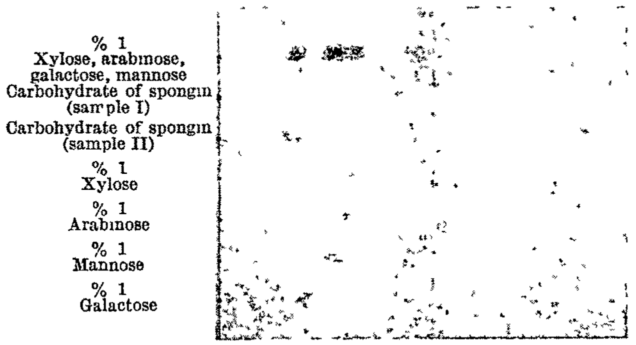

Fig 1

0 1 N sulphuric acid at 62° C, the acid neutralized with barium carbonate and the mixture filtered and concentrated (sample II) Both samples gave a positive Molisch reaction and there was no reducing action with aniline hydrogen phthalate reagent[3]

Samples I and II were further hydrolysed separately for 5 h with an equal volume of 2 N sulphuric acid and neutralized with barium carbonate, filtered and concentrated to 1–2 ml The hydrolysates were run for 30 h on paper chromatograms (Whatman No 1 paper descending technique) using the organic layer from a freshly prepared *n*-butanol–acetic acid–water mixture (4 1 5 v/v/v) (ref 4) The chromatograms were sprayed with aniline hydrogen phthalate reagent and then dried in an oven at 110° C The chromatograms showed a pale spot corresponding to authentic xylose (Fig 1)—a sugar more generally obtained from plants

S Tekman
N Öner

Institute of Biochemistry,
University of Istanbul,
Turkey

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Homarine (*N*-Methyl Picolinic Acid) in Muscles of some Australian Crustacea

During the examination of guanidino compounds in trichloroacetic acid extracts of crustacean muscles, it was observed that treatment of paper chromatograms with alkaline α-naphthol diacetyl[1] sometimes resulted in the appearances of a yellow spot in addition to the pink spots characteristic of guanidine and its mono- and di-*N*-substituted derivatives The unknown compound responsible for this yellow spot was present in muscle extracts from the marine crustacea, Moreton Bay lobster (*Thenus orientalis*), king prawn (*Penaeus plebejus*), tiger prawn (*Penaeus esculentus*), and spiny crayfish (*Jasus verreauxi*), but was absent from the corresponding extracts from the fresh-water organisms, Murray River crayfish (*Euastacus elongatus*) and fresh-water crayfish (*Cherax albidus*) This variation in distribution prompted further investigations, and this communication reports evidence for the identification of the compound as homarine[2]

Preliminary experiments with paper chromatograms of crude extracts indicated that the compound strongly absorbed ultra-violet light in the region of 270 mμ and that the formation of yellow colour was due to the alkali and α-naphthol contained in the Barritt reagent[1] Isolation of the compound was achieved by the following method 10 kg of freshly excised prawn muscle (from either *P plebejus*, or *P esculentus*) was homogenized with 10 l of methanol in a Waring blender and the mixture allowed to stand, with occasional stirring, for several days at room temperature The mixture was then filtered, the residue was re-extracted with 10 l of methanol and the methanolic extracts were combined and concentrated to approximately 500 ml by vacuum distillation. The copious precipitate which formed during this distillation was removed and discarded The remaining solution was passed several times through columns of 'Dowex-1' (OH⁻ form; 500 g) and 'Dowex-50' (H+ form, 500 g) ion-exchange resins Progress of the compound was followed by ultra-violet absorption (273 mμ) and paper chromatography of eluate samples The compound passed unretarded through the anion exchanger, but was retarded on the cation exchanger and appeared in the eluate only after the passage of three bed-volumes of distilled water This retardation was in contrast to suggestions that homarine is firmly bound to strong cation-exchangers[3,4] Eluates containing the compound were pooled and concentrated to a viscous oil by vacuum distillation This oil was dried *in vacuo* over phosphorus pentoxide and recrystallized twice from absolute ethanol to yield 4 g of white hygroscopic crystals A sample of these crystals was further purified by chromatography on a cellulose column using *n*-butanol, saturated with water, as the mobile phase Paper-chromatographic examination of the recrystallized product, using five different solvent systems, revealed only one ultra-violet-absorbing spot in each case No ninhydrin-reacting materials were detected on these chromatograms

Comparison of the ultra-violet spectrum of the product with published spectra[5] led to tentative identification as homarine This was confirmed by comparisons of other properties of the isolated compound with those of homarine synthesized by oxidation of α-picoline to α-picolinic acid and methylation of the latter by dimethylsulphate[6,7] (Table 1) The isolated and synthetic compounds also showed identical behaviours on ion-exchange resins and gave the same R_F values after ascending paper chromatography in several solvent systems Thus, R_F values at 25° C (Whatman No 1 paper) were 0 30 in *n*-butanol glacial acetic acid water (73 10 17) and 0 75 in *tert* -butanol ammonia water (60 30 10)

Table 1 COMPARISONS OF ISOLATED COMPOUND WITH HOMARINE

Compound	Ultra-violet spectra			
	λ_{max}, *p*H 7	λ_{min}, *p*H 7	λ_{max}, *p*H 1	ε at λ_{max}, *p*H 7
Isolated compound	274 mμ	241 mμ	271 mμ	6,200
Homarine, refs 4 and 5	273 5 mμ	240 mμ	270 mμ	6,325

Compound	Elementary analysis (%)*		
	C	H	N
Picrate of isolated compound (m p 153°–154° C)	42 7	2 8	15 1
Synthetic homarine picrate (m p 155°–156° C)	42 6	2 8	15 3

* Values for synthetic homarine picrate calculated for $C_{13}H_{10}O_9N_4$

Synthetic homarine, when spotted on filter paper and sprayed with alkaline α-naphthol (equal volumes of 5 N sodium hydroxide and 1 per cent α-naphthol in ethanol), also gave the yellow colour A number of other related compounds were tested with this reagent and yellow colours were obtained with *N*-methyl pyridinium iodide, pyridine betaine, α-picoline methiodide, and trigonelline (*N*-methyl nicotinic acid) Negative results were obtained with pyridine, pyridine *N*-oxide, nicotinic acid, picolinic acid, pyridoxine hydrochloride, betaine hydrochloride, and choline chloride From these limited investigations it appears that a pyridinium ring system, containing an alkyl or substituted alkyl radical as the *N*-substituent, is required for this colour reaction

One of us (K M) is grateful to Prof A H Ennor for the opportunity of working in the Department of Biochemistry, Australian National University, during part of this work

G J Leonard
K Macdonald

Department of Biochemistry,
University of Queensland,
Brisbane

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Solvent Systems for Silica-gel Column Chromatography of Indole Derivatives

An earlier paper[1] described a method for separating a number of indole derivatives and auxin-like substances in plant extracts, using silica-gel partition columns and petroleum ether: *n*-butyl alcohol solvents used with a step-wise elution technique. Further investigations, the results of which are reported here, have extended this technique to include a number of other useful solvent mixtures.

Most eluting mixtures consisted of a more polar solvent that had been saturated with 0·5 M formic acid, and a non-polar one. A few consisted only of the acid-saturated polar constituent. The column, described in detail elsewhere[1], was prepared by hydrating 8·0 g of silica gel with 5·0 ml. of 0·5 M formic acid, then slurring the hydrated silica gel with the eluting solvent and pouring into a glass tube 14 mm internal diameter. The column was about 13 cm high after it was packed by applying air pressure from a hand squeeze bulb. Approximately 5×10^{-7} moles of each indole derivative were chromatographed and collected in 3-ml. fractions on a fraction collector. When mixtures of indoles were chromatographed it was often necessary to use a step-wise elution technique in which increasing concentrations of the more polar constituent were utilized in order to obtain a satisfactory separation. Each fraction was taken to dryness in the test tube in which it was collected *in vacuo* by use of a rotating evaporator (Rotary Evapo-Mix, manufactured by Buchler Instruments, Inc., Fort Lee, New Jersey). The indoles were afterwards detected with the Gordon–Weber modification of the Salkowski reagent[2].

Most of the solvent mixtures used *n*-hexane as the non-polar phase. Hexane does not cause column cracking at elevated laboratory temperatures as does the petroleum ether, b.p. 30°–60°, used in earlier work[1]. The hexane solvent mixtures are listed in Table 1, and their chromatographic properties with seven indoles of widely varying solubility characteristics in Table 2. As a group the alcohol-hexane mixtures were the most versatile. At a given concentration they could elute a particular indole more readily than any of the other solvents. With one or two exceptions they were able to separate the seven indoles listed in Table 2. A definite inferiority of one alcohol over another one was not established, with the exception of hexyl alcohol, and possibly *n*-butyl alcohol, where their higher boiling points may be objectionable. The ester-hexane mixtures were intermediate in their ability to elute the indoles. They generally effected elution and separation of all the indoles except tryptophan and tryptamine. All but two of these esters have boiling points appreciably lower than the alcohols. This is a distinct advantage in laboratories not equipped for efficient evaporation of liquids from test tubes. Butanone-hexane was a slightly stronger eluting solvent than the esters; it could elute tryptophan and tryptamine. Chlorinated hydrocarbon-hexane mixtures were least satisfactory as a group. Methylene chloride, chloroform, and 1,2-dichloroethane in high concentrations were able to elute all the indoles of Table 2 except tryptophan and tryptamine. Carbon tetrachloride and trichloroethylene were able to efficiently elute only ethyl indoleacetate and indoleacetonitrile, and would thus appear to have little value as eluting solvents for silica-gel column chromatography of indole compounds. In no case did any of the chlorinated hydrocarbons resolve ethyl indoleacetate and indoleacetonitrile.

Table 1. Some Solvents used for the Silica-gel Column Chromatography of Indole Derivatives

n-Hexane : methylene chloride
n-Hexane : chloroform
n-Hexane : carbon tetrachloride
n-Hexane : 1,2-dichloroethane
n-Hexane : trichloroethylene

n-Hexane : 2-butanone

n-Hexane : methyl formate
n-Hexane : ethyl formate
n-Hexane : methyl acetate
n-Hexane : ethyl acetate
n-Hexane : *n*-propyl acetate
n-Hexane : methyl propionate
n-Hexane : ethyl propionate

n-Hexane : *n*-butyl alcohol
n-Hexane : *sec*-butyl alcohol
n-Hexane : isobutyl alcohol
n-Hexane : *t*-amyl alcohol
n-Hexane : *n*-hexyl alcohol

Table 2. Ability of Chromatography Solvents to elute Indole Derivatives from Silica Gel Columns

	Concentration of solvent in *n*-hexane required for elution		
	Chlorinated hydrocarbons	Esters and 2-butanone	Alcohols
Ethyl indole-3-acetate	50–100%	3–5%	0·5–1%
Indole-3-acetonitrile	50–100%	3–5%	0·5–1%
Indole-3-butyric acid	100% to not eluted	25%	5–10%
Indole-3-acetic acid	100% to not eluted	25%	5–10%
Indole-3-acetamide	100% to not eluted	50%	25–50%
Tryptophan	not eluted	100% to not eluted	100%
Tryptamine	not eluted	100% to not eluted	100%

Using the solvent systems above, the indoles were generally eluted by 50 ml. or less of solvent and came off the column as sharp peaks in volumes of about 6–15 ml. Substances said to be not eluted did not come off the column when quantities of 100–200 ml. of the solvents were used.

The choice of the non-polar constituent does, of course, markedly affect the elution pattern. Benzene with *n*-butyl alcohol, for example, eluted ethyl indoleacetate and indoleacetonitrile much more readily than did *n*-hexane. Even closely related non-polar solvents could give different results; *n*-pentane and petroleum ether (b.p. 30°–60°) with 0·2 per cent *n*-butyl alcohol were able to effect separation of ethyl indoleacetate and indoleacetonitrile, whereas *n*-hexane in similar or any other likely concentration was unable to do so.

With one or two exceptions all the solvent systems eluted the seven indoles in the same order, if indeed they eluted and separated them at all. That order was ethyl indoleacetate, indoleacetonitrile, indolebutyric acid, indoleacetic acid, indoleacetamide, tryptophan, and tryptamine.

Full details of the solvent systems used have been purposely omitted since the substances to be dealt with in a particular problem are not likely to be exactly those used here. The information given should be sufficient, however, to guide the investigator towards a wise choice for his own circumstances.

This work was carried out while the author was a member of the staff of the Department of Pomology, New York State Agricultural Experiment Station, Cornell University, Geneva.

Loyd E. Powell

Department of Pomology,
Cornell University,
Ithaca, New York.

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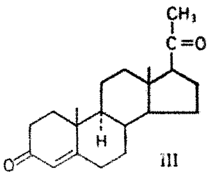
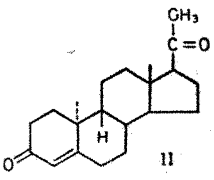
Metabolism of some Progestationally Active 9β,10α-Steroids in Man

In 1960 Reerink *et al.*[1] announced a new class of hormonally active steroids named 'retro-steroids'. This class is characterized by the β-position of the C-9-hydrogen atom and the α-position of the C-10-methyl group.

In clinical trials two members of this class, 6-dehydro-9β,10α-progesterone (generic name, 'Dydrogesterone') (I) and 9β,10α-progesterone (II), were found to exert a markedly progestational activity when administered


III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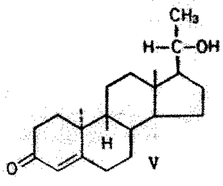
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orally[2,3]. This suggested the possibility that their intermediate metabolisms would be different from that of progesterone (III). Therefore these two compounds were labelled with tritium (at carbon atom 4) (ref. 4), in order to examine their urinary metabolites.

In four experiments each of the two labelled compounds, containing 30–60 μc. of tritium (specific activities 0·1 and 0·5 μc./mg), were administered orally to two post-menopausal women, and urine was collected for 6–12 days. After extraction with ether, the urines were hydrolysed exhaustively with β-glucuronidase ('Ketodase', Warner–Chilcott Company) and extracted with toluene (first toluene extracts). The unhydrolysed radioactive material remaining in the urines was then extracted with tetrahydrofuran and hydrolysed according to Jacobsohn and Lieberman[5]. The radioactive substances liberated were extracted with toluene (second toluene extracts).

Virtually no radioactivity was found in the ether extracts and in the spent urines. Approximately half the total urinary radioactivity was present in the first toluene extracts. Thin-layer and column chromatography of these extracts revealed that in each case approximately 90 per cent of the radioactivity could be attributed to a single compound. In the case of I this proved to be 20α-hydroxy-9β,10α-pregna-4,6-dien-3-one (IV), while in the case of II it was 20α-hydroxy-9β,10α-pregn-4-en-3-one (V).

Urinary IV was obtained in a crystalline form with the same molar radioactivity as the administered I. Its identity with authentic IV is based on its chromatographic properties and ultra-violet and infra-red absorption spectra, as well as on the determination of melting point (174°–176° C) and mixed melting point.

Urinary V was identified with authentic V on the basis of its chromatographic properties, ultra-violet and infra-red absorption spectra, and co-crystallization—to constant specific radioactivity—of the radioactive compound and the added inactive compound. Also in this case the molar radioactivity was the same as that of the administered II.

The second toluene extracts, which were obtained following perchloric acid hydrolysis, were found to be less homogeneous than the first toluene extracts. Paper chromatography indicated in each case the presence of at least ten radioactive substances. Both IV and V were found, although in smaller quantities than in the first toluene extracts; also their 20β-hydroxy-isomers were detected. In the paper chromatography systems used all the other radioactive substances had a more polar behaviour than IV and V. Work on their identification is in progress.

We are not aware of the occurrence of 20α-hydroxy-pregn-4-en-3-one in human urine after administration of progesterone. Since the principal urinary metabolites of the retro-steroids investigated are the 20α-hydroxylated forms, it seems justifiable to postulate a major difference in the metabolism of progesterone and of 9β,10α-progesterone.

We thank Dr. H. van Kamp of N. V. Philips–Duphar Laboratories for putting the authentic compounds IV and V at our disposal.

E. DICZFALUSY
K.-G. TILLINGER

Hormone Laboratory,
Department of Women's Diseases,
Karolinska Sjukhuset,
Stockholm, Sweden.

R. J. E. ESSER
A. C. HOUTMAN

N.V. Philips–Duphar Research Laboratories,
Weesp, The Netherlands.

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PHYSIOLOGY

Changes of Dehydrogenase Activity during Transneuronal Atrophy

ALTHOUGH the cytology of transneuronal atrophy in the lateral geniculate nucleus is well documented in cat[1], cytochemical studies of transneuronal atrophy are lacking. In the cat there are 3 laminæ in each nucleus. Following unilateral enucleation, the neurones of the middle lamina of the ipsilateral lateral geniculate nucleus and the first and third lamina of the contralateral lateral geniculate nucleus undergo transneuronal atrophy. The opportunity of observing normal and atrophied laminæ side by side permits an immediate appreciation of any cytochemical changes in the atrophied lamina with respect to the adjacent normal control lamina.

Twenty 10-week-old kittens underwent unilateral enucleation, and 2 months later the animals were killed and the lateral geniculate nucleus from each side removed and sectioned at 25μ thickness. Some sections were incubated with the appropriate substrate and the tetrazolium salt nitro-BT according to the technique of Nachlas *et al.*

The following enzymes were investigated: TPN-diaphorase, DPN-diaphorase, the dehydrogenase of 6-phosphogluconate, succinate, lactate-DPN and α-glycerophosphate. The remaining sections were stained with cresyl violet for routine cytology.

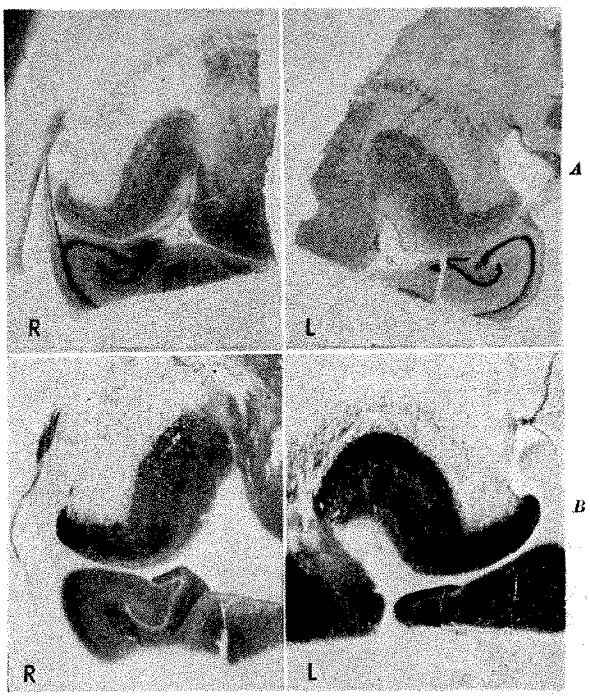

Fig. 1*A*, Parasagittal section of the right (*R*) and left (*L*) lateral geniculate nuclei following enucleation of the left eye. Cresyl violet. Noteworthy is the appearance of normal and atrophied laminæ side by side. *B*, Section adjacent to that shown in Fig. 1*A*. The substrate is succinate. Noteworthy is absence of succinic dehydrogenase activity in the atrophied laminæ

Fig. 1*A* demonstrates the transneuronal atrophy in the right and left lateral geniculate nuclei following enucleation of the left eye. Fig. 1*B* illustrates the marked decrease in succinic dehydrogenase in the atrophied laminæ as compared with the normal adjacent laminæ. A decrease in enzyme activity was characteristic for all substrates except α-glycerophosphate, with which there was no difference in enzyme activity between normal and atrophied laminæ.

Although these observations are of interest in themselves, their primary importance lies in illustrating an approach to the examination of the cytochemical correlates of transneuronal atrophy which has the advantage of permitting simultaneous observation of control (normal) and experimental (atrophied) neurones side by side.

CARL KUPFER

Howe Laboratory of Ophthalmology,
Harvard University Medical School,
Massachusetts Eye and Ear Infirmary,
Boston, Mass.

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Glycogenolysis in Aliquots of Glycogen Bodies of the Chick

PREVIOUS attempts to change the glycogen concentration in the glycogen body of birds failed[1-4] except for a few cases in which it was slightly increased[5,6]. In the experiments recorded here the effect of tissue homogenates on the polysaccharide of the glycogen body of the chick was examined. Glycogen bodies of chicks 1–4 weeks old were isolated, weighed on a Roller Smith torsion balance and homogenized in glass tissue-grinders. Ringer's solution, or isotonic sodium chloride buffered with 0.01 M phosphate to *p*H 7.4, was added to the homogenate in the proportion of 1 ml. per mg of tissue. Twenty-four aliquots of 1 ml. were then prepared from the homogenate and were divided into 4 groups of 6 each. Ringer's solution (0.2 ml.) was added to the aliquots of one group and 0.2 ml. of tissue homogenate (containing 20 mg of chick skeletal muscle, liver or brain) to the others. To the samples of the first group, in 2 series of experiments ATP, DPN+ and glucose-6-phosphate (1 mg each) were added. Two aliquots of each group were extracted[7] for glycogen immediately, two after 4 h and 2 after 24 h of incubation at room temperature (70° F.). Glycogen was assayed colorimetrically by the anthrone method[8].

In the first group of aliquots, containing only homogenate of glycogen body, 25 per cent of the polysaccharide was destroyed in the 24-h incubation (Table 1). Addition of ATP, DPN and glucose-6-phosphate (first and second series) had no effect. In the 3 other groups, to which homogenates of other tissues were added, the breakdown of glycogen was greater. In the presence of skeletal muscle homogenate more than 75 per cent of the initial glycogen was destroyed during the 24-h incubation. Brain homogenate was the least glycogenolytic of the 3 homogenates studied.

An analysis of variance of the data of Table 1 reveals that the time differences in each group and the differences between the 4 groups are statistically significant.

The results obtained indicate that hydrolysis of the glycogen body polysaccharide is increased by homogenates of other tissues (muscle, liver and brain), suggesting that it is of the same nature as that in these tissues. Bloom *et al.*[9] and Russell and Bloom[10] distinguish in liver and muscle a trichloroacetic acid (TCA) soluble fraction of glycogen (free glycogen) and a TCA insoluble fraction (bound glycogen). In the glycogen body all the glycogen is extractable with cold trichloroacetic acid[4]. This, however, does not mean that the polysaccharide in the glycogen body is different from that of liver and skeletal muscle. According to Hanson, Schwartz and Barker[11] the amount of the so-called bound glycogen (the TCA insoluble fraction) depends on the amount of protein precipitated. The glycogen body is extremely poor in tissue protein; the precipitate in a TCA extract and the amount of bound glycogen would be, consequently, negligible.

The relatively low rate of glycogen hydrolysis in aliquots of glycogen body alone could not be attributed to the lack of phosphorus, the addition of ATP, DPN and glucose-6-phosphate was without any effect. Similarly, the presence of an inhibitor would appear to be ruled out, since then muscle or liver homogenate would be expected to have no effect. The results suggest that the glycogen body is relatively poor in glycogenolytic enzymes. In favour of this hypothesis is also the fact that the cells in the glycogen body, almost entirely filled with glycogen, are poor in cytoplasm and contain only a few mitochondria[12]. This, perhaps, explains the low glycogenolytic activity in the glycogen body.

A. M. LERVOLD
J. SZEPSENWOL

Department of Anatomy,
University of Puerto Rico School of Medicine.

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Table 1. PERCENTAGE OF GLYCOGEN IN ALIQUOTS OF GLYCOGEN BODY OF THE CHICK AFTER VARIOUS HOURS OF INCUBATION WITH HOMOGENATES OF SKELETAL MUSCLE, LIVER OR BRAIN

	Glycogen body alone			Glycogen body skeletal muscle			Glycogen body liver			Glycogen body brain		
	0 h	4 h	24 h	0 h	4 h	24 h	0 h	4 h	24 h	0 h	4 h	24 h
	23.6	20.3	18.6	23.2	18.0	3.5	34.9	28.7	14.6	22.6	19.7	14.1
	25.5	22.9	19.8	25.2	21.4	3.1	33.7	30.5	12.6	21.2	16.7	13.7
	24.9	23.7	16.3	22.5	16.7	7.3	23.8	21.5	19.0	26.2	23.6	13.3
	21.5	20.2	16.2	28.7	15.6	6.7	36.2	32.3	24.2	21.2	15.8	8.2
	23.8	20.3	20.1	21.4	19.4	2.9	28.2	20.1	13.8	19.8	18.5	13.9
	21.7	20.2	16.7	23.5	16.2	5.3	23.2	19.8	9.3	19.5	19.4	14.9
	26.2	22.8	20.9	23.7	17.0	4.0	23.2	18.4	13.3	23.9	21.6	16.5
	29.6	26.1	20.9	26.8	23.3	3.3	38.4	36.3	18.8	26.1	23.7	16.5
	32.0	31.6	30.1	30.3	29.8	14.7	44.7	42.3	24.7	29.9	27.3	24.6
	25.3	22.2	18.2	22.8	16.2	2.5	26.0	20.1	6.3	24.7	21.4	5.2
Average	25.4 ±3.0	23.0 ±3.2	19.7 ±3.7	24.8 ±2.6	19.5 ±3.9	5.3 ±3.3	31.2 ±6.7	27.0 ±7.5	15.6 ±5.4	23.5 ±3.0	20.7 ±6.3	14.0 ±4.6

Inhibitory Effects of Œstrogens on Augmented Activity of Endometrial Carbonic Anhydrase caused by 4,4′-Methylenedianiline

PREVIOUS work[1] has shown that 4,4′-methylenedianiline causes an increase in the endometrial carbonic anhydrase when administered to rabbits. The mechanism under-

Table 1 EFFECTS OF ŒSTROGFNS ON ENDOMETRIAL CARBONIC ANHYDRASE INCREASE CAUSED BY 4,4′-METHYLENEDIANILINE

Series of compound	No of rabbits	Dose (mgm /animal)		Carbonic anhydrase content (E U /gm wet tissue)	% Inhibition of methylenedianiline by œstrogen
		Methylenedianiline	Œstrogen		
(1) Control	5	0	0	75 ± 11*	
(2) Methylenedianiline	9	500		321 ± 34	
(3) Methylenedianiline + Œstradiol	5	500	0 2	80 ± 4	98†
	4	500	0 02	146 ± 20	78
	4	500	0 002	291 ± 43	14
(4) Methylenedianiline + Ethynyl œstradiol	4	500	0 2	73 ± 9	(100)
	4	500	0 02	101 ± 12	90
	4	500	0 002	142 ± 19	73
(5) Methylenedianiline + Hexœstrol	4	500	0 2	80 ± 16	98
	5	500	0 02	97 ± 7	91
	4	500	0 002	188 ± 9	55

* Mean ± *S E*

† Percentage inhibition is expressed as $\frac{Rm - Re}{Rm - C} \times 100$, Rm = uterine enzyme response to methylenedianiline alone, Re = uterine enzyme response to combination of methylenedianiline and œstrogen, C = control level

lying the activating effect of methylenedianiline on the endometrial enzyme is considered to be dependent on the adrenals, presumably due to the release of progestogen arising by a block of adrenal corticosteroidogenesis Since evidence[2–4] concerning antagonisms between œstrogen and progesterone on endometrial carbonic anhydrase has been given, attempts were made to clarify how certain œstrogens affect the response of endometrial carbonic anhydrase to methylenedianiline

A modified Clauberg test[5] was used Immature albino rabbits, approximately 1 5 kg in weight, were primed with 5 μg of œstradiol once daily for seven days The animals were then subjected to treatment for 4 days with methylenedianiline and œstrogen The standard total dose of methylenedianiline was 500 mg and œstrogen was used in logarithmic spaced doses of 0 002–0 2 mg

The rabbits were then killed by breaking their necks 24 h after the last injection The uteri were removed and rinsed with cold saline The endometrium was dissected, weighed rapidly on a torsion balance and homogenized with distilled water for carbonic anhydrase analysis The carbonic anhydrase content of homogenate was then estimated by the method of Miyake and Pincus[6], the activity being expressed in terms of enzyme units/g wet weight of the endometrium[3]

The results are shown in Table 1 When a total of 500 mg of methylenedianiline was given systemically, an increase in the content of endometrial carbonic anhydrase was invariably noted However, following treatment with a combination of methylenedianiline and each œstrogen, a typical fall in the enzyme activity was observed, the extent of which depended on the dosage of œstrogen In a dose of 0 2 mg, the full effect of methylenedianiline was completely nullified by œstradiol, ethynyl œstradiol and hexœstrol However, some difference in degree of inhibition exists among each œstrogen Degree of response was directly related to the log dose of œstrogen in a nearly straight line

The results recorded here suggest that the œstrogens used are effective in inhibiting the methylenedianiline-induced endometrial carbonic anhydrase increase In previous work, treatment with cortisone effected a substantial inhibition of the endometrial carbonic anhydrase response to methylenedianiline It was suggested that the response was due to the suppression of production of ACTH by cortisone In numerous observations[7–9], the ability of œstrogen to cause hypertrophy of the adrenal cortex which is attributed to a release of ACTH in rats has been emphasized A different view was proved by Vogt's demonstration[7] showing that in the rabbit neither the adrenal histological structure nor the corticoid secretion was altered by the treatment, probably due to species difference in the response of adrenal histology to œstrogen Also, it has already been shown[4] that œstrogens themselves do not affect the activity of endometrial carbonic anhydrase In the investigation reported here it is thus possible that œstrogen-progestogen antagonism in the uterus is responsible for the ineffectiveness of methylenedianiline

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KAZUKUNI YAMASHITA
Department of Physiology,
Nagasaki University School of Medicine,
Nagasaki, Japan

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HÆMATOLOGY

The Xg Blood Groups of Negroes

SAMPLES of blood from 219 unrelated Negroes, mostly from New York or Jamaica, have been tested for the X-linked blood group antigen Xg^a The antigen is significantly less frequent in these Negroes than it is in Caucasians as the following comparison shows

	Male Xg(a+)	Female Xg(a−)	Male Xg(a+)	Female Xg(a−)
Negro	65	54	80	20
Caucasian	516	280	674	80
	χ^2 for 1 d f = 4 7		χ^2 for 1 d f = 7 5	

The number of Negroes is small because tests have mainly been confined to members of diagnostic panels and to parents of families in linkage studies The 1,550 Caucasian samples were mostly from British people, this number includes 1,008 samples previously reported[1]

Gene frequencies calculated from the figures for males and females are

	Xg^a	*Xg*
Negroes	0 550	0 450
Caucasians	0 661	0 339

Phenotype frequencies calculated from these gene frequencies are very close to those observed (Table 1)

Table 1 XG PHENOTYPE FREQUENCIES IN NEGROES AND CAUCASIANS

	Negroes				Caucasians			
	Males		Females		Males		Females	
	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs.
Xg(a+)	0 550	0 546	0 797	0 800	0 661	0 648	0 885	0 894
Xg(a−)	0 450	0 454	0 203	0 200	0 339	0 352	0 115	0 106

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June Gavin
Ruth Sanger
Patricia Tippett
R. R. Race
Medical Research Council Blood Group Research Unit,
Lister Institute,
Chelsea Bridge Road, London, S.W.1

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IMMUNOLOGY

Protective Effect of Killed Trypanosome Vaccines with Incorporated Adjuvants

Attempts to control protozoal diseases by active immunization were unsuccessful until Mulligan *et al.*[1] showed that chickens could be partially protected against sporozoite-induced *Plasmodium gallinaceum* infection by means of an inactivated sporozoite preparation. Freund *et al.*[2,3] later immunized ducks against *P. lophurae* by incorporating the antigen in the aqueous phase of a water-in-oil emulsion containing killed mycobacteria.

We have been examining the adjuvant effect of a large number of miscellaneous substances on diphtheria toxoid and of some of these on *Trypanosoma congolense* antigen. A selected group of the adjuvants most effective with *T. congolense* antigen have also been tested with *T. cruzi* antigen.

The antigen was prepared by separating trypanosomes from the blood of heavily infected mice. Mouse red blood cells were agglutinated by the addition of mouse erythrocyte antiserum or phytohæmagglutinin. The trypanosomes trapped by the agglutinated red blood cells were collected by washing with saline. The separated trypanosomes were then washed twice with cold glucose saline and concentrated into a suitable volume. The trypanosomes were killed by rapid freeze-thawing. The killed trypanosome suspension (which constitutes the antigen) was pale pink to red in colour, depending on the numbers of red blood cells which it contained. It was stored at −20° until used.

Many substances were examined for adjuvant action, the results of which will be described in a later publication. In this communication, results with three substances are described: (1) *Quillaia* saponin, (2) a quaternary ammonium surface active agent, 'Arquad 2HT', (3) an alkylamine, *n*-hexadecylamine. Solutions or suspensions of these substances in water were prepared containing 1 mg/ml., 5 mg/ml. and 5 mg/ml., respectively. Mice were immunized against *T. congolense* by injecting subcutaneously a mixture of 0.1 ml. trypanosome antigen (equivalent to 1.0×10^6 trypanosomes) and 0.1 ml. of adjuvant solution or suspension. Two doses of vaccine were given with 14 days between doses. The mice were challenged by inoculating 1.0×10^5 trypanosomes subcutaneously, 14 days after the last immunizing dose. The *T. cruzi* vaccine consisted of a mixture of 0.1 ml. antigen (equivalent to 6.0×10^5 trypanosomes) and 0.1 ml. adjuvant. Groups of 9 or 10 mice were immunized with *T. cruzi* vaccine in the same way as with *T. congolense* vaccine and were challenged with 2.0×10^5 *T. cruzi* trypanosomes. The strains used were *T. congolense* strain *NIMR* and *T. cruzi* strain *Y*. After challenge, the tail blood of each mouse was examined microscopically for trypanosomes. Microscopical examinations were started 3–5 days after challenge and repeated thereafter 3 times per week.

The results with *T. congolense* (Table 1) and *T. cruzi* (Table 2) show that whereas the antigen or adjuvants given separately had no protective effect, the mixture of antigen and adjuvant provided a considerable degree of protection in mice. With both *T. congolense* and *T. cruzi* antigen, saponin proved to be the best adjuvant. The *T. congolense* vaccine gave a sterile immunity in 5 out of 6 mice, and the *T. cruzi* vaccine increased the survival time and decreased the parasitæmia. A sterile immunity was not achieved with *T. cruzi* vaccine.

Table 1. Protection of Mice against *Trypanosoma congolense* Challenge

Treatment	Mean pre-patent period, days (max. = 24)	Proportion of negative survivors at 10 days
None	7.1	1/7
'Arquad 2HT' alone	4.4	0/7
n-Hexadecylamine alone	4.8	0/6
Saponin alone	6.2	1/6
Antigen alone	5.0	0/7
Antigen + 'Arquad 2HT'	6.6	1/7
Antigen + *n*-hexadecylamine	16.1	4/7
Antigen + saponin	21.2	5/6

Table 2. Protection of Mice against *Trypanosoma cruzi* Challenge

Treatment	Mean survival time, days (max. = 33)	Mean parasitæmia*
Exp. 1		
None	12.4	186
Antigen alone	11.5	183
Antigen + 'Arquad 2HT'	18.4	107
Antigen + *n*-hexadecylamine	12.8	70
Antigen + saponin	19.3	72
Exp. 2		
None	15.0	128
Saponin alone	10.3	203

* Mean parasitæmia = number of trypanosomes seen in 20 microscopical fields (× 10 ocular, 4-mm objective) at height of infection per mouse.

Freund's adjuvant, with or without killed mycobacteria, was not effective with *T. congolense* antigen. No adjuvant activity was observed with potash alum.

The duration of protection conferred by *T. congolense* vaccine was tested by rechallenging the surviving mice at suitable intervals. Twelve weeks after the last immunizing dose of vaccine, 11 out of 22 mice were immune to infection with the same strain of *T. congolense*. At 19 weeks, 4 out of 6 mice were immune. The residual protection was further tested by injecting 1.0×10^6 trypanosomes of a different strain of *T. congolense* (*Buswale*). In this group of mice, 19 weeks after vaccination, 2 out of 5 animals remained immune to challenge.

Antigens, other than those derived from *T. congolense* or *T. cruzi* trypanosome cells, were examined for protective effect when mixed with adjuvant. Plasma or serum collected from mice heavily infected with trypanosomes but itself free from trypanosomes, injected with adjuvant in the same way as trypanosome antigen, protected against challenge. With *T. cruzi*, the plasma antigen (equivalent to Weitz's exo-antigen[4]) was more effective than trypanosome antigen. With the plasma antigen the average parasitæmia was 5 per 20 fields, compared with 272 in the untreated controls. 2/8 mice remained free from trypanosomes. The crithidial forms of *T. cruzi* grown *in vitro* and the culture medium therefrom, were found to have considerable protective effect.

Other flagellates zoologically related to *Trypanosoma* were examined for protective properties when prepared in the same way as the trypanosomes and mixed with adjuvant. No cross-protection was seen against *T. congolense* challenge, but some degree of cross-protection was observed against *T. cruzi* challenge. Protection against *T. cruzi* challenge was observed with *Crithidia fasciculata* and *Leptomonas collosoma* and, to a much smaller degree, with *T. melophagium* and *T. mega*. The culture medium in which *C. fasciculata* and *L. collosoma* had grown also showed a protective effect against *T. cruzi*.

The experiments have shown that the addition of adjuvants to weak antigens from trypanosomes and related protozoa can increase resistance to challenge in mice. Further details of these experiments will be published shortly elsewhere.

Pauline Johnson
R. A. Neal
Wellcome Laboratories of Tropical Medicine,
London, N.W.1
D. Gall
Wellcome Research Laboratories,
Beckenham, Kent

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Prolongation of Homograft Survival in Mice with Single Doses of *Cyclo*phosphamide

IT is customary, in prolonging the survival of homografts with a chemical agent, to give frequent doses of the agent over a prolonged period Effective agents are generally toxic, and their use is attended by considerable mortality, in both laboratory animals and man The immune response is not uniformly susceptible to inhibition by these agents throughout its course[1], nor is it uniformly active It is likely, therefore, that dose-schedules not closely adapted to this changing sensitivity and activity of the immune response may, on one hand, subject the recipient to unnecessary toxicity and, on the other, fail to attack the immune response sufficiently during its most vulnerable period It is therefore of some importance to establish the principles on which optimum dose-schedules may be based

The complexity of this problem necessitates a stepwise approach Initially we have investigated whether a single dose of a drug can significantly prolong the survival of a homograft and whether there is an optimum time for administering this dose

Tail or abdominal skin from ($C57Bl \times A2G$) F_1 hybrid male mice was grafted on to suprapannicular beds on the chest wall of male *A2G* recipients The animals weighed 14–22 g at the start of the experiment The drug chosen was *cyclo*phosphamide, since we found it to have the highest therapeutic index of several effective agents when used to suppress the antibody response to a bacterial vaccine[2]

At various times before or after grafting, recipients were given *cyclo*phosphamide, 200 mg/kg subcutaneously, dissolved in physiological saline, 1 ml /20 g body-weight Grafts were first inspected 9 or 10 days after grafting and then every 1–2 days thereafter The end-point adopted was the time of complete breakdown of the graft

The results are shown in Fig 1, from which it appears that a single injection of *cyclo*phosphamide is maximally effective in prolonging the survival of homografts in the mouse if given at any time from shortly after grafting to about the 4th day afterwards Administration before grafting, or on the sixth day, has relatively little effect The results are analysed statistically in Table 1

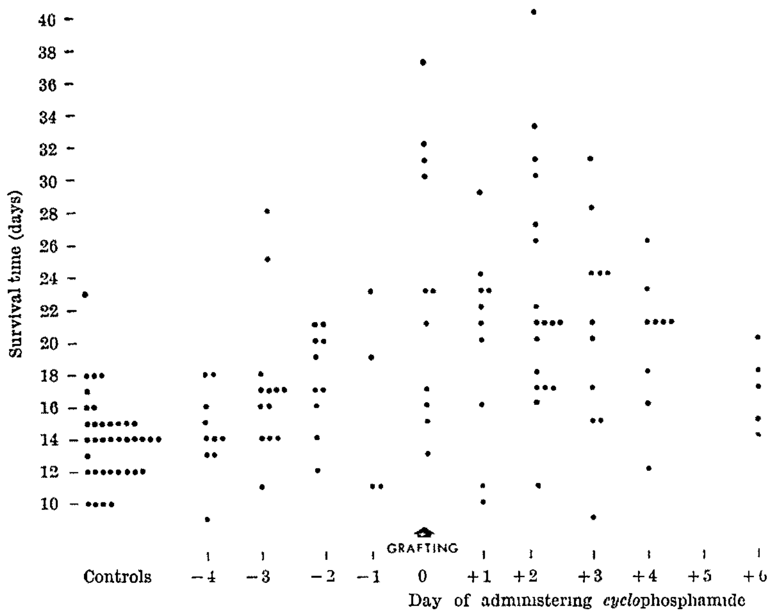

Fig 1 Effect of single doses of *cyclo*phosphamide on survival of homografts Points indicate animals in which grafts were completely rejected at the time shown in the ordinate Recipients were grafted on day 0 The *cylco*phosphamide injection on day 0 was given 1–2 h after grafting

Table 1 EFFECT OF SINGLE DOSES OF *Cyclo*PHOSPHAMIDE ON SURVIVAL OF HOMOGRAFTS

Group	Controls	–4	–3	–2	–1	0	+1	+2	+3	+4	+6
Mean survival time (days)	14 1	14 4	17 2	17 7	16	23 5	19 9	22 7	20 7	19 9	16 8
No	37	10	13	10	4	11	10	18	11	9	5
Variance	6 66	6 7	20 9	9 34	36	64 1	35 2	51 7	47 8	16 6	5 7
P*	—	> 0 5	0 02–0 05	0 002–0 01	> 0 5	0 001–0 002	0 01–0 02	< 0 001	0 01–0 02	0 002–0 01	0 05–0 1

* The significance of each difference from controls was determined by a modified *t*-test, taking into account the differing variances

We have also found that a single injection of 200 mg/kg *cyclo*phosphamide given 2 days after grafting to *A2G* recipients bearing grafts of *C57Bl* skin prolonged the mean survival time from the normal of 12 0±2 2 days to 17 0 ±2 3 days This difference is highly significant ($P = < 0\ 001$) A single injection, therefore, given at the appropriate time, significantly prolongs homograft survival even in the presence of strong *H2* incompatibility In further experiments we hope to find the optimum times of injection of two or more doses of drug

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M C BERENBAUM
Surgical Unit,
St Mary's Hospital Medical School, London, W 2
I N BROWN
Glaxo Research Ltd , Greenford, Middlesex

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PATHOLOGY

Action of Tumour Growth and Tissue Products on the Zonal Distribution of Liver Glycogen in Rats and Mice

IN 1947, Young *et al* [1] reported a decreased deposition of glycogen in the livers of tumour-bearing mice given a glucose load after a fasting period Goranson *et al* [2] were unable to demonstrate any real difference in the levels of liver glycogen in fasted controls and tumour-bearing rats and showed that the phosphorylase activity in the liver was about the same in both groups On the other hand, the blood sugar-levels of tumour-bearing animals according to Goranson *et al* [3] is reduced in alloxan-induced diabetes As pointed out by Boyd *et al* [4] the carbohydrate metabolism in human beings may be abnormal in malignant disease These authors demonstrated a decrease in glucose tolerance in cancer patients which was reversed to normal by treatment with œstrogens

It has been suggested that the disturbance of the carbohydrate metabolism in tumour-bearing individuals, including the low liver glycogen deposition, is due to the shunt of available glucose to the tumour on account of its high glycolytic rate[5,6]

We studied the zonal distribution pattern of the glycogen in the livers of rats and mice bearing tumours or injected with tumour and normal tissue supernatant and now report our results

Male Wistar rats, weighing 150–200 g, were implanted subcutaneously with the

Yoshida sarcoma and killed 2, 3, 5, 7, 9, 12 and 15 days after the implantation. Rats bearing the Yoshida sarcoma in ascitic form were killed 2, 3, 4 and 7 days after the intraperitoneal passage.

Male Swiss mice bearing the Ehrlich ascites tumour were killed 2, 3, 5, 7, 9, 12 and 18 days after the intraperitoneal passage.

The tissue supernatant was prepared in the following way: fragments of the Yoshida sarcoma or of the normal tissues to be tested were homogenized with 20 ml. of chilled saline solution in an electrical blender for 3 minutes. The resulting homogenate was centrifuged at 20,000 r.p.m. (Spinco *L*-rotor 30) for 20 min at 10°. The supernatant was removed and injected into the subcutaneous tissue of the back of each animal in the dose of 3 ml. for the rats and 1·5 ml. for the mice. The normal organs tested were liver and kidneys.

Another group of animals was injected with cell-free ascitic fluid from either Yoshida or Ehrlich tumour in the dose of 2 ml. for the rats and 1·5 ml. for the mice. The injections were always homologous. A control group of rats and mice was similarly injected with saline. The injected animals were killed at 2, 6, 18 and 24 h after the injection.

The distribution of liver glycogen in tumour-bearing animals, either in solid form or as ascites, is conspicuously different from the normal. When fully established the alteration consists in disappearance of glycogen from the periportal area and decrease in the cells of the centro-lobular zone. With rats bearing the solid Yoshida sarcoma the change is seen as early as 2 days after the transplantation. During the whole period of observation (15 days) no other change is superimposed and the liver picture is about the same as that found at 2 days. With the Yoshida ascites tumour the alteration was not detected until 4 days after the intraperitoneal transferring and was consistently more intense than that observed in the animals with the solid form of the growth. A noticeable individual variation regarding the amount of glycogen mobilization was present in the animals bearing the solid form and was less evident with the ascites tumour. Very few animals showed a nearly complete depletion of glycogen throughout the whole liver.

The Ehrlich ascites tumour-bearing mice killed from 5 days up to 18 days after the intraperitoneal passage showed remarkably constant and uniform depletion of glycogen from the periportal area and decrease in the centro-lobular zone. The individual variation was negligible in this group.

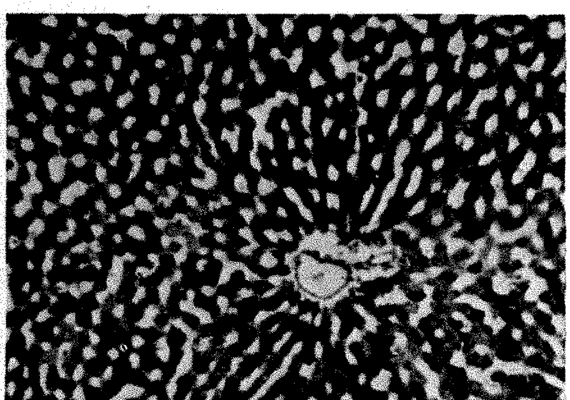

Fig. 1. Normal liver showing the even distribution of glycogen. Periodic acid-Schiff method. (× 150)

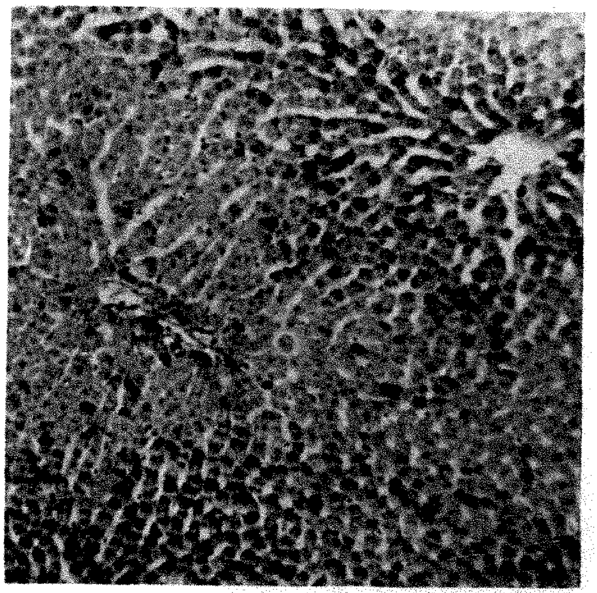

Fig. 2. Changed liver. Depletion of glycogen in the periportal area. Diminution in the centro-lobular area. Periodic acid-Schiff method. (× 150)

Rats and mice injected with homologous cell-free ascitic fluid, tumour supernatant and normal tissue supernatant also exhibited periportal glycogen depletion and centro-lobular diminution. The effect was not observed at 2 h, was irregularly seen at 6 h and was fully established at 18 h after the injection. The effect seems to be a short-lived one since at 24 h after the injection the normal glycogen distribution was again present in the treated animals.

Fig. 1 is from rat liver and shows the usual uniform distribution of glycogen.

Fig. 2 exhibits the described periportal depletion and centro-lobular diminution of glycogen observed in our experimental groups. The histological picture is reproduced in all experimental animals, either rats or mice. The animals injected with saline never showed any alteration in their liver glycogen distribution.

The observation that the glycogen depletion in the livers of rodents treated with tumour supernatant is in every way similar to that found in tumour-bearing animals invalidates the suggestion that this phenomenon is only concerned with the demands of a growing tumour for the available body glucose. On the other hand, the finding that the administration of either tumour or normal organ products can induce the same pattern of glycogen mobilization indicates that no specific tumour agent in the manner of a postulated toxo-hormone[7,8] is responsible for the observed alteration. Rather, it seems that this effect is part of a non-specific stress reaction against cell products overload, as has already been pointed out by Hilf *et al.*[9]. Perhaps the terminal cachexia of the animals bearing these tumours may be explained on terms of a chronic stress situation of which the liver glycogen change is a significant component.

J. P. Guimarães
M. C. Santos Motta

Instituto Nacional do Cancer,
Praça Cruz Vermelha 23, E. Guanabara, Brazil.

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BIOLOGY

Growth-rate of Mice in Static Magnetic Fields

EXPERIMENTS of the influence of a static magnetic field on the growth of mice were started in 1948 at the University of Budapest. I placed one half of a litter of six mice, for a period of four weeks, in the vertical field of an electromagnet and the other half of the litter in an identical dummy magnet. The aluminium cages were 38 mm in height and 75 mm in diameter, with hard rubber bottom and surrounded by an open tray, where food and water were provided. A forced air blow prevented the mice from staying for long periods outside their cage. They never attempted to escape, hereby proving the adequacy of their housing conditions.

The mice were conditioned for four days to the confinement before the magnet was energized. The field strength at the centre of the cage was 5,900 œ with an average gradient of 100 œ/cm. As can be seen from the weight curve, shown in Fig. 1, the field retarded the growth of the mice. We may note a sharp minimum on the third day, which seems to be the result of a shock due to the field, from which they shortly recover. If we disregard this minimum, the growth-rate does not start to decrease during the first few days; thereafter, however, the weight of the mice in the magnet significantly lags behind that of the litter-mate controls. The one male mouse started to lose weight on the eleventh day in the field and died shortly thereafter. This 'lethal' effect of the magnetic field on males has been observed several times since then[1] and is a phenomenon worth further investigation. The females did not exhibit any adverse symptoms. Removed after four weeks' residence in the field they mated, and gave birth to healthy litters 20 days later. (The normal gestation period of mice is 20 days.)

It is well known that there are many factors which can influence the growth-rate of mice. It is conceivable that the magnetic field acts via one or another of these factors; but it cannot be excluded that a magnetic field slows down mitotic activities in general. In the latter case, we would expect that the retardation-rate would increase with the mitotic activity of the tissue or organ in question, as is the rule with other external inhibiting agents. For this reason I investigated in 1948 and since then the effect of the magnetic field on the development of embryos in the uterus of mice and on the growth of transplanted and spontaneous tumours in mice. The observations support the foregoing assumption[1].

The effect of the magnetic field on the growth-rate of young mice is an effect which is far less sensitive to environmental conditions than most other effects, if comfortable housing conditions are provided and the position of the animals does not change relative to the magnetic vectors.

From about 20 physical phenomena, which occur in organisms placed in static magnetic fields, all with the exception of two (joule heat of induced currents and the heat generated in paramagnetic substances as a consequence of the alignment of dipoles against thermal agitation) are vector phenomena. The cumulative physical effects resulting from these phenomena, responsible for the triggering of biomagnetic effects, are, therefore, reversible effects. A frequent change in the position of the specimen relative to the field vector, or relative to the gradient vector, will lead to an attenuation, eventual annulment of the cumulative physical effect and, with it, to the disappearance of the biological effect itself. It is, however, not necessary to restrain mice in order to avoid a change in the position of the animal relative to the field. It is enough to place them in vertical fields, since mice seldom take supine positions.

During the past years further experiments have been performed with ten Alnico permanent magnets of 275 lb. each. The cages were 3¾ in. in diameter and 1½ in. high, with plastic bottoms, food and water supply, and connected to inclined exercise galleries. Two types of fields were used: in one the field strength was 4,200 œ with an average gradient of 80 œ/cm (called in the following 'homogeneous'); in the other the field strength was on average 3,600 œ and the gradient at the centre of gravity of the mouse 650 œ/cm (called in the following 'inhomogeneous'). The dummy magnets were simulated by 2-in. thick iron pole pieces, to avoid effects caused by the biological effect of cosmic radiation[2]. The dummy cages were located 2 ft. from the magnet cages, but shielded against the stray field of the magnets. The cages were cleaned by the tail movement of the mice and every second day washed in an automatic washer.

For weighing purposes the two or three mice housed in the same cage were transferred to a plastic cup and weighed with the cup. However, the error in weight, due to loss in fæces and urine, is negligible, even if the weighing is done without a cup. One droplet from a full-grown mouse weighs only 65 mg, and well-treated mice, used to the person handling them, never urinate during the weighing process. A considerable error can, however, be introduced

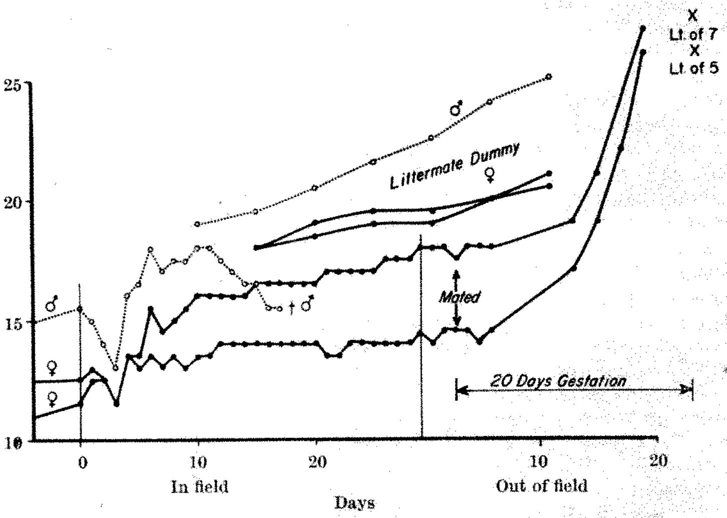

Fig. 1. Growth of five-week-old albino mice in a vertical field of 5,900 œ

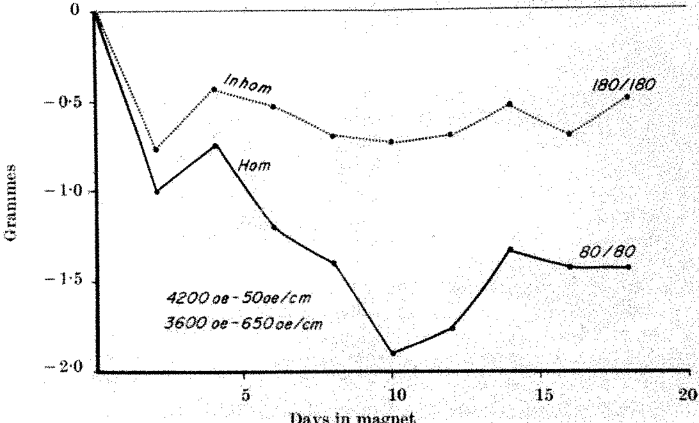

Fig. 2. Growth retardation of Swiss female mice in a homogeneous (full line) and in an inhomogeneous (dashed line) magnetic field

Table 1. SERIES: *S*; SWISS 30-DAY-OLD FEMALE MICE

Weight of three mice per cage

In 4,200 œ field				In dummy magnets			
W_0	W_{10}	W_{12}	ΔW_{11}	W_0	W_{10}	W_{12}	ΔW_{11}
38·5	58·5	64·0	22·8	42·0	64·0	67·0	23·5
41·5	47·5	56·0	10·2	42·0	71·0	75·0	31·0
45·0	65·0	66·5	20·8	43·0	61·0	64·0	19·5
45·0	69·5	74·5	27·0	45·0	70·5	72·0	26·2
47·0	64·5	68·0	19·2	45·0	71·0	72·0	26·5
47·0	55·0	57·0	9·0	46·0	69·5	73·0	25·2
48·5	61·5	64·0	14·2	46·0	70·0	71·0	24·5
49·0	67·0	70·5	19·8	47·5	70·0	72·5	23·8
51·5	65·5	66·5	14·5	52·5	74·0	76·5	27·8
53·5	67·5	69·0	14·8	53·5	75·5	75·5	22·0
46·6			17·23	46·2			25·00

Average growth: 5·74 g   Average growth: 8·33 g
Variance: 1·88 g   Variance: 1·03 g
Growth difference between magnet and dummy: −2·59 ± 0·68 g
$t = 3{\cdot}81$; $n = 18$; *P.L.*: 1 : 1,560.

Table 2. GROWTH DIFFERENCES UP TO THE ELEVENTH DAY

Series	Strain	Age	*M*/*D*	Field	$\Delta W_M - \Delta W_D$
T	Swiss	60	30/30	homogeneous	−1·66 ± 0·50
P, *S*	Swiss	30	50/50	homogeneous	−2·21 ± 0·46
C, *D*, *E*	*RF*	60	70/90	homogeneous	−0·97 ± 0·33
K, *L*, *M*, *N*	Swiss	60	120/120	inhomogeneous	−0·48 ± 0·17
Q, *R*	Swiss	30	60/60	inhomogeneous	−0·80 ± 0·38

if weighing is carried out during or around the normal feeding time of the mice. The weight of a mouse can be 2 g higher in the morning than in the evening, and since mice usually feed in groups, some amount of difference may arise between magnet and dummy groups. I have, therefore, investigated the weight variation of mice over a 24-h period, as well as recorded with electric devices their feeding habits. Their main feeding time lies between 11 p.m. and 5 a.m. and young mice have a second snack between 8 a.m. and 11 a.m. However, in the afternoon, between 2 p.m. and 6 p.m., the mice rest. Measuring the weight of a larger group of mice, individually, day by day around 4 p.m., revealed that the daily weight fluctuation does not exceed 0·55 g by young and 0·49 g by old mice. This fluctuation strictly follows a Gaussian distribution, implying that in a series where 30 animals are treated and 30 are used as controls the difference in the growth-rate can be established with an accuracy better than 0·20 g.

Table 1 shows the results of one of the series in which 30-day-old mice were treated in a homogeneous field. The first column contains the total weight of three mice per cage before placing them in the field; the second and third column, the weight on the tenth and twelfth days in the magnet, and the fourth column, the weight difference up to the eleventh day. Columns 5–8 contain the same results for the dummy groups. We can see that the average growth was 5·74 gm for the mice in the magnets and 8·33 gm for the mice in the dummy magnets. The difference in growth-rate during the first 11 days is therefore 2·59 ± 0·68 g; the effect is significant on a probability level of 1 : 1,560.

We see, furthermore, that the individual variance of the magnet group is almost twice the variance in the dummy group, indicating that the magnetic field affects individual mice to a different degree. For example, the three mice in the fourth row seem not to have been affected, while the effect of the field was twice the average in the group of the sixth row.

Twelve similar series were conducted with a total of 680 female mice. The results are summarized in Table 2 according to strain, age and field used. The growth differences up to the eleventh day of residence in the field are tabulated together with their standard errors. We can see that the total difference in weight of young mice is larger than that of older mice. The relative decrease of the growth-rate is, however, greater for the older. Young mice do not stop growing in fields of such magnitude, whereas older mice sometimes may lose weight.

Fig. 2 shows the daily change in weight of the pooled results of nine series, in which Swiss female mice were treated in homogeneous and inhomogeneous fields respectively. We can see that the effect of the homogeneous field is far greater than that of the inhomogeneous field. In both cases the minimum occurs around the tenth day of residence. We may again note the minimum around the second day, followed by a recovery, as already noted on Fig. 1. This phenomenon is now under further investigation.

I wish to express my thanks to my wife, for her help in conducting these experiments and for many discussions. This work was supported by the Biomagnetic Research Foundation.

JENO M. BARNOTHY

Biomagnetic Research Foundation,
Evanston, Illinois.

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Phytoplankton Production in the South-eastern Pacific

THE distribution of phytoplankton concentration and its photosynthetic rate in the surface waters of the south-eastern Pacific are shown in Figs. 1 and 2. Measurements were made during the following cruises: *Costa Rica Dome*, November–December 1959; *Step-I*, September–December 1960; *Swansong*, October–December 1961; and the cruise of the *Esmeralda* (Chilean Navy), July–August 1962.

The concentration of chlorophyll *a* (refs. 1, 2) was used as an index of standing stock of surface phytoplankton; the production of the phytoplankton was measured by the carbon-14 method[3] under natural light at surface temperatures.

Greater phytoplankton concentrations and production exist in regions of divergence, vertical mixing, shallow

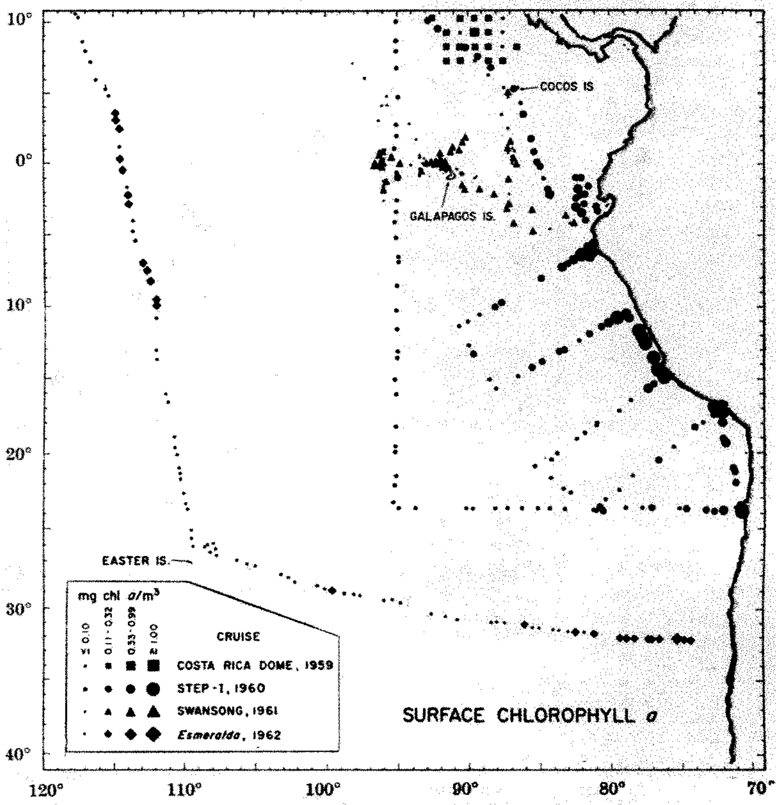

Fig. 1. Distribution of chlorophyll *a* at the surface in the south-eastern Pacific

mixed layer, or where enriched surface water is driven by wind. High production occurs along the Peruvian coast owing to replenishment of plant nutrients by strong upwelling. Because the Peruvian anchovy feeds directly on this rich source of phytoplankton it is able to exist in such numbers that guano birds and fishermen took 10 × 10^6 tons in 1962.

Production is relatively high between the Galapagos Islands and the coast, apparently the result of enriched surface water from coastal upwelling off northern Peru being driven to the north-west by the prevailing south-easterly winds. Off central and southern Peru these prevailing winds increase in force seaward. The seaward increase of the resulting Ekman transport causes a divergence reflected in moderate offshore production.

Off central Chile the prevailing southerly winds in August decrease in force westward so no divergence can be expected as off Peru, but Ekman transport may carry enriched surface water westward from the coastal upwelling regions. The divergence between the Peru Oceanic Current[4] and the Peru Counter Current[4] may also contribute to this wide zone of moderate production.

At the Costa Rican Thermal Dome[5], centred at 9° N., 89° W., upwelling and a shallow mixed layer enable production to be considerably greater than in surrounding waters.

Equatorial upwelling and vertical mixing between the South Equatorial Current and the sub-surface Cromwell Current explain the high production found near the equator. The shallow mixed layer along the north boundary of the Counter Current and along the equator, and the divergence[4] between the South Equatorial Current and southern edge of the South Equatorial Counter Current[6], all may contribute to the increased production of the region between 10° N. and 10° S. This productive strip in the eastern tropical Pacific is exploited by the Japanese long-line fishermen, who obtain best catches of tunas and billfishes there, and concentrate their effort within these limits.

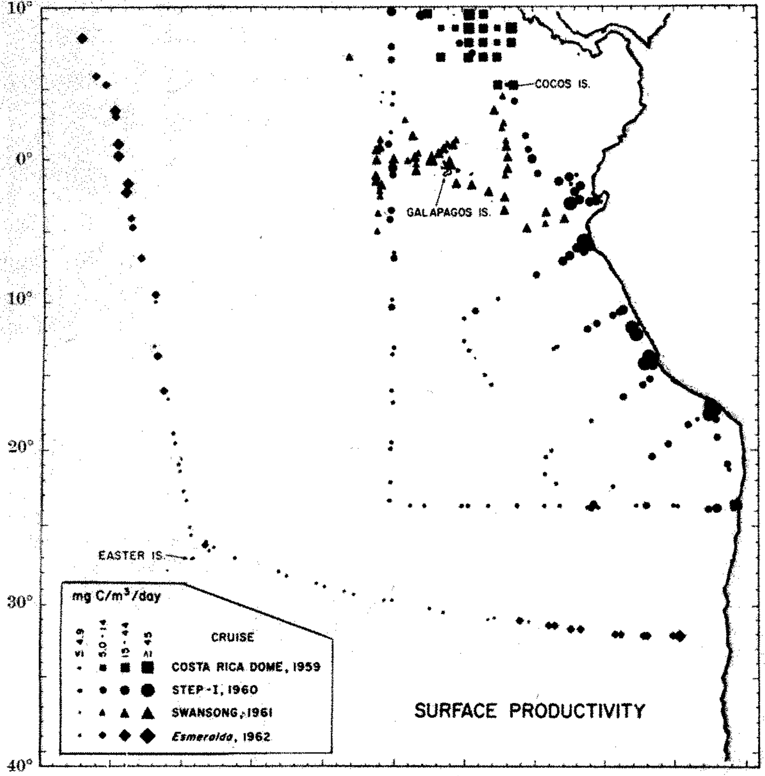

Fig. 2. Distribution of the rate of carbon fixation by photosynthesis at the surface in the south-eastern Pacific

ERIC D. FORSBERGH
JAMES JOSEPH

Inter-American Tropical Tuna Commission,
Scripps Institution of Oceanography,
La Jolla, California.

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Toxic Properties of the Saliva of *Cassis*

THE large gastropods of the genus *Cassis* feed on various achinoids[1]. Despite its toxic sharp spines, even the black sea urchin, *Diadema antillarum*, is the prey of the helmet conch, *Cassis tuberosa*. The feeding habits described by Schroeder[2] show that *C. tuberosa* actively pursues the urchin and appears to overwhelm it physically, despite the phalanx of spines that *Diadema* brings to bear on any point of attack. Even for the tough-footed *Cassis*, this is a *tour de force*, since the microneedle points of the spines readily pierce the sole of the foot. One wonders whether the helmet is quite as reckless as first appears, or whether he is aided by tricks other than a headlong charge. Schroeder also reports " . . . its long proboscis may be seen greedily feeling among the spines for the urchin's body". *Cassis* also everts his proboscis when he is broken from his shell, and then usually squirts a jet of clear fluid in different directions as he whips his proboscis about. This saliva is toxic to numerous marine organisms and conceivably could be used offensively as well as defensively. The work recorded here was undertaken to show the manner in which the poison acts on *Diadema*.

Small urchins (3–4 cm body diameter) were immersed in saliva (lot *B*) diluted 1/1,000, 2/1,000, and 4/1,000 (total volume 250 ml., excluding the volume of the urchin). There was immediate pigment release and violent waving of the spines. Movement was co-ordinated, however, and the urchins repeatedly climbed the side of the finger bowl. At the end of 20 min all three had lost the response to shading. The different doses showed a graded response to touch: when spines were lightly touched, the urchin in 1/1,000 responded by converging spines towards the point of contact; 2/1,000 yielded only general unco-ordinated spine movement, and the urchin in 4/1,000 responded only when the body was prodded. Continued prodding induced spine-waving at successively farther distances from the point of stimulus. The response to increased light (as against shadow) was also blocked in the two highest doses, in one test after exposure for 30 min. When placed in direct sun, all but the urchin at 4/1,000 began to wave their spines, and those in the control and bottom dose moved about the dish. Recovery was detectable at 1 h in the two lowest doses and was complete at 4·5 h. A second urchin placed in the previously used 2/1,000 dilution showed no suppression of reactions or of spontaneous movement.

Isolated body wall of *Diadema* placed in diluted *Cassis* saliva showed the same sequence of sensory suppression in relation to time and dose as did the intact urchin. Response to shading was lost first, then the spine convergence response to a lightly touched spine, and finally all response to even strong prodding. High concentrations evoked pigment release initially, and finally suppressed all spontaneous movement. Fragments left in the weak solutions eventually recovered.

Saliva lot *B* can be taken as an example. At 4/1,000 there was an immediate release of pigment and increased waving of spines. In three minutes the response to shading was lost in concentrations of 4/1,000, 2/1,000, 1/1,000, and 0·5/1,000. The response to prodding disappeared in 6 min at 4/1,000, in 38 min at 2/1,000, and was not lost at 1/1,000. In another series, saliva (lot *A*) decreased the response of fragments to shadow and touch in dilutions as great as 4/10,000. There was a questionable depression at 3/10,000. By means of small doses it was not possible to block the light response alone. The effect of the toxin at any effective dilution progressed and then declined, so that a level state at any one stage of inhibition was not attainable.

The spontaneous recovery of fragments under supposedly continuous exposure was subjected to further analysis. Such fragments transferred to fresh solutions were again affected. Untreated pieces placed in the old solution were inhibited little, or not at all. Dilutions in sea-water left at room temperature retained their activity, however, if not in contact with *Diadema*.

Cassis tuberosa has large paired salivary glands comprising a large hyaline posterior portion from which a duct passes through the smaller brown opaque anterior lobe. An aqueous brei of these glands inactivates *Diadema*, so the gland is indicated as the source of the toxin. The other two species of *Cassis* on Bimini, *C. madagascarensis* and *C. flammea*, also have large paired bilobed salivary glands with toxicity comparable with the glands of *C. tuberosa*.

The helmet conch is supplied with a poisonous saliva capable of incapacitating the surface organs of the black urchin and thereby decreasing his mobility and the effectiveness of his spines. It would now appear that the play of the proboscis among the spines of the urchin, as described by Schroeder[2], serves to deliver the poison to the body surface, where it is most effective.

The saliva blocks response to touch and to all the different responses to light: spine convergence on a shaded area, inhibition of spine movement under steady illumination, and increased movement under increased light[3].

The active principle of the saliva would appear to be a neurotoxin, since it first inactivates the sensory receptors or their afferent nerves. Receptors cannot be distinguished morphologically from nerves, but the physiological evidence points to a sequence of events in the receptor-effector arc[4]. The systems responding separately to mechanical and actinic stimulation[3] are separately affected by the toxin. It can block photoreception temporarily without interfering with the tactile sense, or with transmission of the impulse throughout the body. Below a damaging level, the effect is reversible, even when it has proceeded to the extent of eliminating all response and all spontaneous movement. Immobilized pieces placed in fresh sea-water regain activity in a few minutes. The urchin, or even a piece of the body wall, can inactivate the poison. Ascribing this inactivation to bacterial decomposition would appear unjustified, since the saliva is antibiotic, and solutions allowed to stand at room temperature maintain full toxicity for 24 h. Loss of activity is accompanied by a drift towards neutrality (the saliva is strongly acid), but saliva initially neutralized retains its paralysing properties.

Ivor Cornman

Hazleton Laboratories, Inc.,
Falls Church, Virginia, and
Lerner Marine Laboratory,
Bimini, Bahamas.

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Long-distance Migration of Atlantic Salmon

In recent years reports have appeared[1–4] of Atlantic salmon bearing English or Scottish tags being caught on the west coast of Greenland, and Atlantic salmon tagged in Canada[5] have also been caught in the same area.

Two further records are now to hand, both in respect of salmon tagged as smolts at this Ministry's research installation on the River Axe, south Devon.

The first, a male, was tagged on April 20, 1960, and was recaptured off Sukkertoppen (65° 20′ N., 53° 00′ W.) on October 26, 1961, length 73 cm and weight 4 kg. The second, a female, was tagged on April 11, 1961, and was recaptured near Kangerdluarssorujak (60° 40′ N., 45° 45′ W.) in the Julianehaab District of west Greenland on November 19, 1962. It was then 94 cm long and weighed 4·7 kg.

The minimum distance travelled by the first fish (the longer journey) was approximately 2,300 miles from the point of tagging to the point of recapture.

Nielsen[2] states that the duration of the freshwater life, before migration as smolts, of the west coast of Greenland salmon catch indicates that these salmon are, for the most part, of more southern, non-Greenlandic origin. As increased numbers of smolts were tagged in English and Welsh rivers last year further recaptures off Greenland of British-tagged fish are anticipated this autumn.

We thank the Ministry for Greenland, Charlottenlund, Denmark, for sending details of the recaptures.

I. R. H. Allan
M. J. Bulleid

Salmon and Freshwater Fisheries Laboratory,
Ministry of Agriculture, Fisheries and Food,
Whitehall Place, London, S.W.1.

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Hexagonal Patterns in Cell Membranes

Dourmashkin, Dougherty and Harris[1] demonstrated a hexagonal pattern on some viral and mammalian membranes treated with saponin. They took the view that the pattern was revealed by the removal of certain lipid components and suggested that their observation supported the mosaic structure proposed for cell membranes by Parpart and Ballentine[2] in which lipid cylinders, 90 Å in diameter and spaced at 150 Å centre to centre, are enclosed in a protein mesh. Muir[3] has produced the same effect in the plasma membrane of rat intestinal epithelium by *in vivo* treatment with saponin.

Kavanau[4] has proposed, on the basis of other evidence, a new theory of membrane structure and function based on the concept of a hexagonal mosaic arrangement of the lipid component in discrete bimolecular leaflets which can transform to a more open arrangement of cylindrical columns under prescribed conditions.

Bangham and Horne[5] and Glauert, Dingle and Lucy[6], however, have shown that patterns like those of Dourmashkin *et al.* can be induced in monolayers of cholesterol and in mixtures of cholesterol and lecithin by treatment with saponin. These authors suggest that their figures are formed by the insertion of saponin molecules into the film. Husson and Luzzati[7] also, using X-ray diffraction techniques, have shown that in saponin-treated red cell membranes a hexagonal lattice of 165 Å spacing can be detected, whereas only a lamellar structure is shown in cell membranes prepared by the use of hypotonic phosphate. They conclude that their results argue against an intrinsic pattern in the normal membrane.

In a recent investigation[8] of the fine structure of the marine protozoan *Gromia oviformis* we were unable to find a normal cell membrane. Instead, we found at the cell

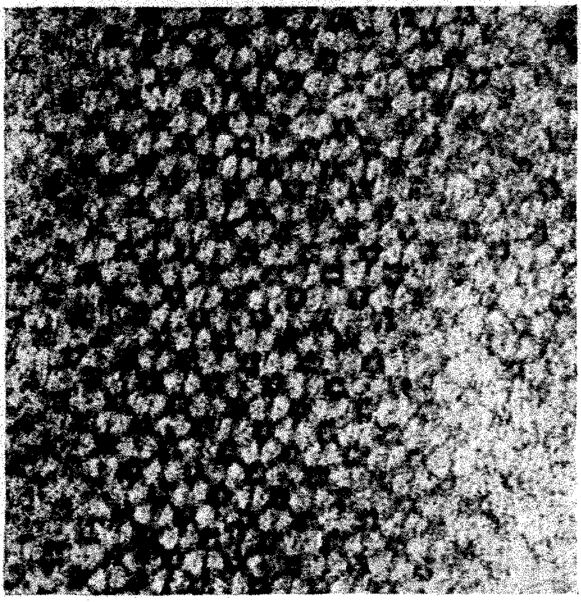

Fig. 1. Electron micrograph of tangentially sectioned 'honeycomb membrane' of *Gromia oviformis*, showing the hexagonal pattern. (× 280,000)

surface a number of layers which for convenience of reference we called 'honeycomb membranes'. These exhibited in tangential sections, Fig. 1, a hexagonal array of hollow cylinders about 100 Å in diameter, each containing a central hole of 30 Å diameter, with a centre to centre spacing of 210 Å. Linking septa connected each cylinder to its neighbours. In cross-section it was apparent that up to ten membranes were included in the complex. Kavanau has referred to this as possibly representing a case of a membrane locked in the 'open' configuration by structural modifications. We would like to emphasize one or two points which may be relevant.

Following the demonstration by Luzzati and Husson[9] of similar structures as transitional phases in lipid-water systems we have entertained the idea that the honeycomb membranes might represent a high permeability state of a normal cell membrane in response to some physical or chemical stimulus, but if this were so we are quite at a loss to say what could have induced it. Alternatively, we have considered the possibility that they might be an artefact arising during the course of fixation and embedding, but for this there appears to be no precedents, and, as far as we are aware, these processes proceeded normally. No saponin or other surfactant was used in the preparation, which was a straightforward fixation in Caulfield's solution with methacrylate embedding. Sections were stained with permanganate after mounting. Hence, it appears unlikely that the structure was produced by insertion of foreign molecules in the same way as those discussed by Bangham and Glauert.

The existence of the connecting septa between the cylinders of the *Gromia* membrane would appear to put difficulties in the way of direct transformation between the observed structure and the usual bimolecular membrane. Further, the conditions of fixing and dehydration were such as to favour Kavanau's 'closed' configuration. To this extent, then, we may say that the structure observed probably represents a stable condition, normal in the living animal. Unfortunately, no further animals were available with which to repeat the preparation; but on this occasion the structures were seen in two individuals embedded entirely and on the inner surfaces of two shells from which the cytoplasm had been dissected. This suggests that these membranes are a generally occurring feature in the species under consideration. The work of Wohlfarth-Bottermann[10] and our own unpublished observations on an allied organism, *Allogromia laticollaris*, indicate that it contains no such system, but a normal plasma membrane.

Since we were unable to demonstrate a normal plasma membrane in *Gromia*, whether the honeycomb structure is intra- or extra-cellular remains unresolved. It may be that it is more properly related to the cell wall mosaics shown by, for example, Houwink[11] in micro-organisms. Regardless of its precise position and function, however, it does represent an instance of molecular architecture apparently corresponding very closely to the membrane models proposed on one hand by Parpart and Ballentine, and on the other by Kavanau to account for the properties of such membranes in terms of their chemical composition.

W. S. BERTAUD

Dominion Physical Laboratory,
Lower Hutt, New Zealand.

R. H. HEDLEY

Department of Zoology,
British Museum (Natural History),
Cromwell Road, London, S.W.7.

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Growth of Single Cells from Higher Plants on Synthetic Media

THAT isolated single cells of higher plants can develop into cell groups and tissues was first demonstrated by the use of 'nurse' cultures[1]. Recently single cells of higher plants have been more easily grown by a 'plating' method; although the step from the 'nurse' culture to the 'plating' technique has required the addition of complex components, especially of coconut milk, to the basic synthetic media[2]. Media supplemented either by coconut milk or by yeast extract have been used in all published successful experiments in which extensive cell divisions of isolated cells have been observed. Because we know almost nothing about the active principle or principles of coconut milk and since at least with certain tissues the appearance of polyploid cells is furthered by coconut milk[3], experiments were undertaken with the view of growing single cells on chemically defined, synthetic media.

The first experiments involved a relatively fast-growing strain of *Haplopappus* cultures, which has been grown since 1959[4] on 0·6 per cent agar media containing White's nutrient[5] and 2,4-D at a concentration of 5×10^{-9} g/ml. These cultures are soft and can be spread into single cells and small cell groups by means of a spatula. They were spread in this way on the surface of the agar medium in a sterile Petri dish. It was soon apparent that under these conditions cell division occurred only in the small cell groups—up to 5 and 10 cells and more—but not in the single cells. The results were better if the cultures were grown for 2 or 3 months in test-tubes on the same medium, but with the phosphate-level (sodium dihydrogen phosphate) raised from $1{\cdot}7 \times 10^{-5}$ to $8{\cdot}5 \times 10^{-5}$ g/ml., then transferred to Petri dishes with the same high phosphate medium to which was added gibberellic acid (10^{-6} g/ml.) and spread after a growth period of 10 days in the dishes. With this method divisions were observed in about 15 per cent of the single cells. This proved that isolated single cells are able to grow on synthetic media and, in

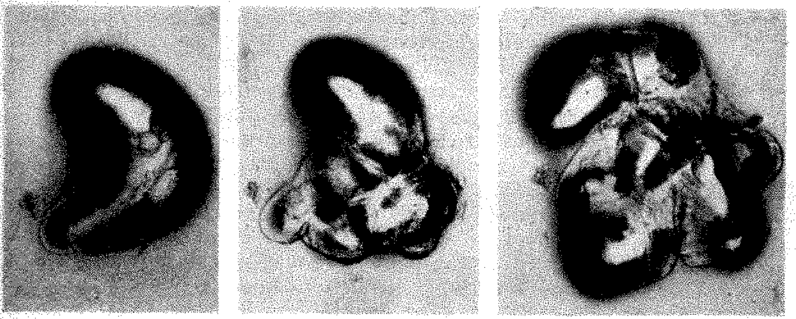

Fig. 1. Cell division in an isolated single cell of crown gall tissue of *Vitis vinifera* (× c. 125). (*a*) Single cell 8 days after isolation; (*b*) the same cell after 12 days; (*c*) 18 days after isolation

two out of 58 dishes, the formation of cell clusters (up to 50 cells) from such cells did occur. However, in most cases the *Haplopappus* cells turned brown and died after only one or a few divisions.

I therefore looked for a tissue which would spread as well as the *Haplopappus* cultures but in which this dying out of cells would not occur. A tissue answering this requirement is the crown gall tissue of *Vitis vinifera*[6], which grows excellently on a 0·7 per cent agar medium containing the macroelements according to Heller[7], Berthelot's solution of microelements[8], 5 per cent glucose and, in addition, vitamin B_1 (10^{-6} g/ml.). When using this medium, the upper parts of cultures (0·6 g) were transferred after a growth period of 8–10 weeks in test-tubes to Petri dishes and spread immediately; division of single cells could be observed (see Fig. 1 *a–c*) with an average plating efficiency of 40 per cent—in extreme cases even 70 per cent of the isolated cells divided. Some of these cells have now developed into tissue colonies of the normal size.

Although the division of the isolated *Vitis* cells occurs very regularly there are interesting variations in their behaviour. Usually division of the isolated cells starts during the first 12 days after spreading. However, others remain quiescent for between 30 and 50 days. Most of these quiescent cells were in regions of the agar free from other cells and cell groups and their division was found to occur as such regions of the agar became invaded with growing cells. It is therefore concluded that the initiation of division in such cells is dependent on them coming under the sphere of influence of actively growing cells.

I thank Prof. R. J. Gautheret of the Faculté de Science de Paris for providing me with the crown gall cultures of *Vitis vinifera*.

J. Reinert

Pflanzenphysiologisches Institut,
Freie Universität, Berlin.

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VIROLOGY

Variation of Interferon Yield with Multiplicity of Infection

CHIKUNGUNYA virus is one of the best inducers of interferon in chick cells, and many of the conditions which affect the yield of interferon have been elucidated[1,2]. This communication reports an investigation of the relationship between the amount of Chikungunya virus inoculated into chick cell cultures and the subsequent yield of interferon. Dilutions of Chikungunya virus were prepared and placed in bottle cultures of chick cells which were then placed at 37° for 24 h. Supernatant fluids were collected, heated at 65° for 30 min to inactivate the virus, and assayed for interferon using the procedure of Lindenmann and Gifford[3]. The PDD_{50}, defined as the amount of interferon preparation required to depress the plaque count to 50 per cent of the control value[3], was determined and plotted against the logarithm of the ratio of plaque-forming units/cell (Fig. 1). Less interferon was found to be produced by the cultures which received the greatest amount of virus than by those which were infected with approximately 0·1 plaque-forming units/cell. Even with 0·01 plaque-forming units/cell a considerable amount of interferon was produced. Three experiments have all given similar results. In other experiments fresh medium was placed on interferon producing cultures 24 h after infection and re-incubated for an additional 24 h. The amount of interferon found was usually less than 15 per cent of the first 24-h yield, indicating that most of the interferon was produced in the first 24 h after infection.

Similar titrations by Burke and Isaacs[4] using ultraviolet-inactivated influenza virus and chick chorioallantoic membranes showed no such zone of inhibition at high virus doses. These findings suggest that infection of cells with active plaque-forming virus inhibited the production of interferon. A comparison of the amount of interferon produced by stock preparations of Chikungunya virus with partially inactivated Chikungunya virus preparations was made and the results are given in Table 1. Considerably more interferon was produced by the partially inactivated preparation of virus than by the stock virus even though the same dilution of the virus preparation was utilized in both cases. It has already been shown that completely inactivated Chikungunya virus would induce the production of interferon in chick cells[5]. These results give evidence that interferon production by preparations of Chikungunya virus was due to 'inactive' virus in the stock preparations. It would be anticipated that conditions which tend to inactivate this virus would enhance interferon production. This would fit with the observation that the yield of interferon in an 11 h single cycle of growth of Chikungunya virus was higher at 42° than at 35° (ref. 2), since greater inactivation of the virus should occur at the higher temperature.

De Maeyer and De Somer[6] have demonstrated that production of interferon in rat tumour cells infected with Sindbis virus was inhibited at high multiplicities. Those results, as well as these reported here, may be related to

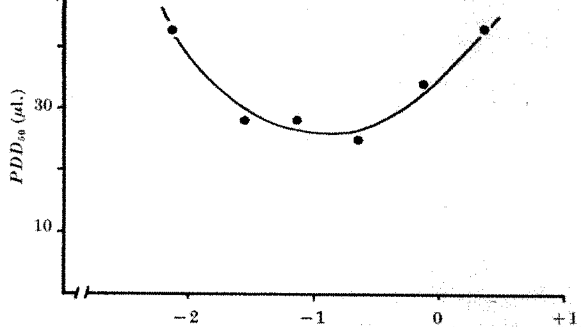

Fig. 1. Comparison of the resultant PDD_{50} of interferon with the logarithm of the input plaque-forming units/cell

Table 1. COMPARISON OF THE YIELD OF INTERFERON BY STOCK AND PARTIALLY INACTIVATED PREPARATIONS OF CHIKUNGUNYA VIRUS

Virus preparation	Plaque-forming units/cell	Total PDD_{50} units interferon produced
Stock virus	0·2	100
Partially inactivated*	0·01	186

* Stock preparation of Chikungunya virus was incubated at 35° for 4 h. Both preparations of virus were used at the same dilution.

the 'inverse interference' phenomenon described by Lindenmann[7].

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G. E. GIFFORD *

National Institute for Medical Research,
London, N.W.7.

* Present address: Department of Microbiology, University of Florida College of Medicine, Gainesville.

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Responses of Germ-free Mice to Friend Virus

CONSIDERABLE interest has developed in the germ-free animal as an assay system for potential oncogenic viruses. Theoretically it should provide a more uniform test system than the conventional animal with its unpredictable microflora which may influence unpredictably the response to the agent being studied. For this reason we undertook a comparative study of Friend virus infection in germ-free and conventional mice of the same strain. This communication concerns the results of this investigation.

The disease produced by Friend virus was first characterized by Friend[1] and later studied by a number of other workers[2-7]. Basically it consists of a neoplastic proliferation of reticulum cells with an associated erythroblastosis, polycythæmia and leucocytosis. The disease is invariably fatal in susceptible mice.

Flexible 'Trexler' plastic isolators were used for maintaining the germ-free environment. Their operation has been described by Trexler[8]. Germ-free mice (Hauschka-Mirand/*ICR* Swiss) were obtained from the Charles River Breeding Laboratories of Boston, Massachusetts.

The conventionalized mice referred to in this experiment were germ-free mice which had been purposely contaminated by placing fæces of open-animal-room mice in the drinking water. The conventionalized mice were maintained in isolators. The open-animal-room animals were mice of the same strain which were raised under ordinary laboratory conditions and were housed in open cages. These were also obtained from Charles River Breeding Laboratories.

Throughout this experiment, the germ-free and 'conventionalized' animals ate the same sterilized diet and were maintained in similar cages. Both groups were maintained in isolators and exposed to similar handling as a control of environmental factors other than fæcal microflora. The open-animal-room animals were housed in open cages but in the same room in which the isolators were located. They received the same diet as the other two groups. Weights and count determinations were made on a minimum of 10 animals per group.

Samples of diet, bedding, stools and water were regularly cultured as a check on the germ-free environment of the isolators. Routine microbiological testing was limited to a search for bacteria, fungus, parasites, and pleuropneumonia-like organisms. At the end of the experiment, the blood, cæcal contents, lung, liver and spleen from the germ-free mice were cultured as a final check on the maintenance of a germ-free environment.

Seven-week-old mice were inoculated intraperitoneally with 0·2 ml. of a cell-free filtrate prepared from the spleen of a Friend virus-infected mouse. Bacteriological studies of the filtrate were negative.

The investigations of uptake of iron-59 utilized the procedures previously reported[9]. The iron-59 solutions were checked for bacteriological sterility prior to administration.

Results showing the response of germ-free, conventionalized and open-animal-room mice to Friend virus are presented in Tables 1, 2 and 3. At 7 days no appreciable response was apparent in any group. However, at 14 and 21 days all infected groups showed some evidence of

Table 1. RESPONSE OF FRIEND VIRUS IN GERM-FREE, CONVENTIONALIZED, OPEN-ANIMAL-ROOM HAUSCHKA-MIRAND/*ICR* SWISS MICE INFECTED WITH VIRUS FOR SEVEN DAYS

Experimental groups	Weight (g)		% Hæmatocrit	WBC per mm³ ($\times 10^3$)	RBC per mm³ ($\times 10^6$)
	Spleen	Liver			
I (*a*) Germ-free without virus	0·11	1·18	32	6·9	6·97
	0·05–0·1	1·0–1·5	26–36	5·8–9·9	6·7–7·3
(*b*) Germ-free with virus	0·18	1·37	40	5·5	6·77
	0·11–0·3	1·3–1·5	35–45	4·7–7·9	6·4–7·3
II (*a*) Conventionalized without virus	0·16	1·25	42	16·1	6·70
	0·1–0·2	1·1–1·4	40–45	10·6–21·9	6·3–7·4
(*b*) Conventionalized with virus	0·22	1·37	39	8·4	6·28
	0·15–0·27	1·2–1·7	31–44	7·5–10·0	5·8–6·7
III (*a*) Open-animal-room without virus	0·10	1·77	42	8·0	7·9
	0·09–0·12	1·5–1·9	41–45	3·8–9·6	7·4–8·4
(*b*) Open-animal-room with virus	0·15	1·94	49	6·7	7·39
	0·13–0·16	1·4–2·4	46–55	5·0–8·5	6·7–8·0

Table 2. RESPONSE OF FRIEND VIRUS IN GERM-FREE, CONVENTIONALIZED, OPEN-ANIMAL-ROOM HAUSCHKA-MIRAND/*ICR* SWISS MICE INFECTED WITH VIRUS FOR FOURTEEN DAYS

Experimental groups	Weight (g)		% Hæmatocrit	WBC per mm³ ($\times 10^3$)	RBC per mm³ ($\times 10^6$)
	Spleen	Liver			
I (*a*) Germ-free without virus	0·09	1·16	47	6·5	7·38
	0·07–0·11	0·8–1·4	45–49	4·9–8·0	5·8–10·2
(*b*) Germ-free with virus	1·22	1·44	59	50·8	10·8
	0·5–1·8	1·2–1·7	44–64	7·8–77·4	9·6–14·2
II (*a*) Conventionalized without virus	0·13	1·4	44	7·2	6·12
	0·1–0·16	1·3–1·7	38–46	5·2–8·6	5·6–6·8
(*b*) Conventionalized with virus	0·49	1·52	48	6·0	6·58
	0·2–1·0	1·1–1·9	43–53	4·9–7·1	5·2–8·7
III (*a*) Open-animal-room without virus	0·15	1·8	51	8·1	8·23
	0·13–0·18	1·5–2·4	49–52	4·1–11·8	7·3–9·5
(*b*) Open-animal-room with virus	0·34	1·93	45	7·6	7·5
	0·13–0·55	1·5–2·6	42–47	5·3–12·2	6·2–12·0

Table 3. RESPONSE OF FRIEND VIRUS IN GERM-FREE, CONVENTIONALIZED, OPEN-ANIMAL-ROOM HAUSCHKA-MIRAND/*ICR* SWISS MICE INFECTED WITH VIRUS FOR TWENTY-ONE DAYS

Experimental groups	Weight (g)		% Hæmatocrit	WBC per mm³ (× 10³)	RBC per mm³ (× 10⁶)
	Spleen	Liver			
I (*a*) Germ-free without virus	0·14 0·12–0·15	1·33 1·1–1·6	44 42–45	7·9 5·7–10·9	8·29 7·7–8·7
(*b*) Germ-free with virus	2·14 1·75·2·52	1·44 1·8–1·7	71 65–75	127·5 47·8–321·0	11·7 10·9–12·9
II (*a*) Conventionalized without virus	0·16 0·12–0·19	1·69 1·4–1·8	45 30–54	14·3 10·3–17·6	6·97 4·7–8·7
(*b*) Conventionalized with virus	1·26 0·41–2·22	2·19 1·8–2·7	54 30–70	37·5 15·4–67·1	6·33 4·0–7·9
III (*a*) Open-animal-room without virus	0·14 0·11–0·20	1·95 1·5–2·6	45 41–48	10·9 9·3–13·9	8·07 6·8–8·7
(*b*) Open-animal-room with virus	1·09 0·30–2·24	2·25 1·5–2·8	52 36–66	36·1 9·6–89·9	8·35 5·9–9·9

disease but the response was considerably greater in the germ-free group as measured by splenic weight, hæmatocrit, and peripheral blood erythrocyte and leucocyte levels. The rise in erythrocyte level was accompanied by the appearance of erythroblasts in the peripheral blood and an increase in blood volume. Histological examination of the livers and spleens of all animals confirmed the greater severity of the disease in the germ-free group. However, the general pattern of the disease was the same as that described previously[3–7]. Perhaps it is of some interest to point out that the liver weights of germ-free mice were generally smaller than those of the conventionalized and open-animal-room mice.

In a previous investigation we found that the 24-h uptake of iron-59 by the spleen was an excellent parameter for following early manifestation of this disease because of its marked sensitivity as an index of erythropoiesis[9]. The results in Table 4 clearly show that the greatest increase in 24-h splenic uptake of iron-59 occurred in the germ-free group.

Table 4. ERYTHROPOIETIC ACTIVITY AS JUDGED BY 24-H IRON-59 UPTAKE OF SPLEENS FROM HAUSCHKA-MIRAND/*ICR* SWISS MICE INFECTED WITH FRIEND VIRUS FOR 14 DAYS

Groups	Average 24 h ^{59}Fe uptake (%)
I (*a*) Germ-free without virus	2·1 1·6–2·4
(*b*) Germ-free with virus	23·6 18·4–26·4
II (*a*) Conventionalized without virus	3·9 1·1–4·8
(*b*) Conventionalized with virus	18·9 6·6–36·6
III (*a*) Open-animal-room without virus	3·5 1·6–5·4
(*b*) Open-animal-room with virus	8·98 3·0–15·6

Additional data at 28 and 35 days post-infection also confirm the greater severity of the disease in the germ-free group.

These findings indicate that Hauschka-Mirand *ICR* germ-free mice are more sensitive to Friend virus infection than are conventional mice of the same strain. The enhanced susceptibility was reflected by a decreased latent period for disease production. There was no change in the basic pathological pattern of the disease. Similar studies are now under way with other murine leukæmia viruses. If the germ-free mouse proves to be generally more susceptible to leukæmogenic viruses than the conventional mouse, this could provide a valuable tool for the assay of potential leukæmogenic agents in mice.

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EDWIN A. MIRAND
JAMES T. GRACE, JUN.
Roswell Park Memorial Institute,
New York State Department of Health,
and State University of New York at Buffalo,
Buffalo 3, New York.

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Effect of Phosphate and Borate on the Infectivity of some Viruses during Purification

IN a method[1] developed in 1958 for purifying cucumber mosaic virus, infected tissue was homogenized in 0·5 M potassium phosphate buffer containing 0·1 per cent thioglycollic acid. To chelate possible metal contaminants in the phosphate, 0·001 M sodium EDTA was also added before the thioglycollic acid in subsequent tests. The filtrate was clarified by adding *n*-butanol (to an 8·5 per cent final concentration) and this was followed by low-speed centrifugation (30 min at 8,000 r.p.m. in the No. 30 rotor of a Spinco model *L* ultracentrifuge). The virus in the resultant supernatant was pelleted by ultracentrifugation (60 min at 30,000 r.p.m. in the No. 30 rotor), the virus pellets resuspended in 0·05 M phosphate buffer (*p*H 7·5) and the suspension finally clarified by low-speed centrifugation.

The same method, or with minor modifications, has been used recently for the purification of other viruses including cauliflower mosaic virus[2], potato virus *F* (ref. 3), alfalfa mosaic virus[4], celery yellow vein virus, tomato black ring virus and beet ring spot virus[5] and pelargonium leaf curl virus[6]. These viruses, except for potato virus *F* (ref. 7) and alfalfa mosaic virus[8], are spherical in shape like cucumber mosaic virus.

Attempts were made by me in 1960 to purify lettuce mosaic virus using the same procedure. These attempts were unsuccessful, the final preparations being non-infectious as indicated by the lack of local lesions on the leaves of *Chenopodium amaranticolor*.

By this time, Shepherd and Pound[9] had purified turnip mosaic virus using the cucumber mosaic virus method, but they reported that their final preparations were highly infectious only if resuspended in 0·02 M borate buffer.

Subsequent tests by me of this modification of the phosphate method failed to give infectious preparations of lettuce mosaic virus. It was then decided to substitute 0·5 M borate buffer (boric acid adjusted with sodium hydroxide to *p*H 7·5) for phosphate throughout the whole procedure and, when this was done, highly infectious preparations were made. The method has been briefly described[10] and has since been used repeatedly with the same result.

In addition to infectivity assessments, precipitin tube tests were made of the final preparations of lettuce mosaic virus purified in borate and phosphate against antiserum obtained by injection of rabbits with borate purified infectious virus. The borate preparations gave a strong precipitin reaction but the phosphate preparations gave little or no reaction.

As the phosphate method was known to be unsuitable for the preparation of the rod-shaped lettuce mosaic virus it was decided to compare the phosphate and borate methods for the purification of several viruses. Those chosen were the rod-shaped viruses causing lettuce mosaic, turnip mosaic, and potato mild mosaic (potato virus *X*), and the spherical viruses causing cucumber mosaic and arabis mosaic. The method was as follows. Two lots of tissue of similar infectivity were obtained by cutting infected leaves longitudinally. These were then homogenized (1·0 g of leaf per 1·5 ml. of buffer) in phosphate or borate buffer (0·5 M, *p*H 7·5) each containing 0·001 M sodium EDTA and 0·1 per cent thioglycollic acid. The subsequent purification procedure was the same as that described for lettuce mosaic virus except that the ultracentrifuge virus pellets were each resuspended in 0·5 ml. distilled water. The relative infectivity of the two types of final preparation at various dilutions was then determined by local lesion assay (or systemic infection in the case of arabis mosaic virus) after inoculation to *C. amaranticolor*. The results obtained with the five viruses are given in Table 1. All have been confirmed in several repeat experiments.

Table 1. INFECTIVITY ON *C. amaranticolor* OF FIVE VIRUSES AFTER PURIFICATION FROM PHOSPHATE AND BORATE HOMOGENATES

Virus and propagation host	Dilution of preparation	Mean No. of local lesions per half-leaf	
		Phosphate preparation	Borate preparation
Lettuce mosaic virus (lettuce)	undiluted	1	157
	1/10	0	57
	1/20	0	6
Turnip mosaic virus (turnip)	undiluted	2	286
	1/10	1	157
	1/20	0	24
Potato virus *X* (tobacco)	10^{-3}	6	48
	10^{-4}	3	10
	10^{-5}	0·1	7
Cucumber mosaic virus (tobacco)	1/10	250	142
	1/100	45	11
	1/500	42	1
Arabis mosaic virus (petunia)	10^{-4}	10/10*	10/10
	10^{-5}	2/10	10/10
	10^{-6}	0/10	1/10
	10^{-7}	0/10	0/10

* Denominator is the number of *C. amaranticolor* plants inoculated and the numerator is the number of plants afterwards with systemic symptoms.

As shown, the preparations of the rod-shaped viruses were of high infectivity when prepared in borate, but of very low infectivity when prepared in phosphate. By contrast, much smaller differences were obtained with the two spherical viruses, the infectivity of arabis mosaic virus being greater from the borate preparation and that of cucumber mosaic virus being greater from the phosphate preparation.

The foregoing infectivity tests were made only on the final preparations. To determine when, during the purification process in phosphate, the loss of infectivity of the preparations of the rod-shaped viruses occurred, samples were withdrawn at various stages and tested on the leaves of *C. amaranticolor*. The three viruses reacted similarly and it was found that although the original filtrates from the leaf homogenates in phosphate or borate buffer (containing sodium EDTA and thioglycollic acid) were equally infectious, those in phosphate had lost infectivity after clarification with butanol and centrifugation, whereas those in borate had not.

Possible reasons were considered for the loss of infectivity of the three rod-shaped viruses when butanol was added to the phosphate preparations. It was thought most likely that the virus particles had become aggregated and either were lost by removal in the early centrifugation or, if retained in the supernatant, were inactivated because they were aggregated. Alternatively, it was considered that they might have been disrupted by the butanol treatment. Electron microscope examination of the final preparations of the three viruses prepared both in phosphate and in borate, however, showed that neither explanation was correct. Both types of preparation contained equivalent numbers of largely unaggregated virus particles of normal size and shape. It thus appears that, following the phosphate clarification procedure, there was no change in the gross appearance of the rod-shaped virus particles although their infectivity was destroyed. Lettuce mosaic virus prepared in phosphate was also serologically inactive against antiserum prepared from infectious (borate-prepared) virus. The reasons for these effects are being further examined.

I thank H. L. Nixon, Rothamsted Experimental Station, Herts., for making the electron microscope examinations.

J. A. TOMLINSON

National Vegetable Research Station,
Wellesbourne, Warwick.

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MISCELLANEOUS

Microscopic Examination of Cell Monolayers grown in Plastic Bottles

PLASTIC culture bottles composed of a non-toxic polystyrene have been shown to be superior to polyvinyl or 'Pyrex' glass bottles for estimating the radiation sensitivity of cell monolayers cultured *in vitro*[1].

During the course of investigations involving the irradiation of monkey kidney cells grown on the surface of polystyrene bottles, it was observed that exposure of the bottles to toluol caused the dissolution of the plastic, leaving the intact monolayers floating free. This suggested a method for processing and examining the free monolayers.

The cell sheet adhering to the surface of the bottle is suitably fixed. The choice of fixative is important, as some (for example, Zenker's and Helly's) cause brittleness of the cell sheet with consequent fragmentation. Of the fixatives tried, the best results were obtained with Bouin's or Susa, although methanol or ethanol fixation for 24 h at 4° C appeared to be satisfactory. Alcohol-formalin may be used provided that prolonged washing is carried out prior to staining.

After fixation, a section of the plastic, the size of a microscope slide, is cut from the bottle by means of a hot knife and processed (washing, staining, dehydration, etc.) in ordinary staining dishes. The stained sections are immersed in toluol in individual coplin jars. Within a few minutes the plastic starts to soften, and with gentle shaking the monolayer is dislodged. As it floats free it may be teased on to a glass slide and a cover-slip applied for microscopic examination.

F. J. STANFIELD
M. E. LYMAN

Defence Research Kingston Laboratory,
Kingston, Ontario.

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[1] Hood, S. L., and Norris, G., *Rad. Res.*, **14**, 705 (1961).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 7

CIBA FOUNDATION (at the Royal Institute of British Architects, 66 Portland Place, London, W 1), at 5 p m —Prof V Ramalingaswami "Perspectives in Protein Malnutrition" (15th (Annual) Ciba Foundation Lecture)

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Prof C A Mace "Explanation in Psychology"

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S W 1), at 5 30 p m —Informal Discussion on "The Uses of Steel Vis-à-Vis Concrete" introduced by Mr J F Pain and Prof A L L Baker

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 30 p m —Mr F W Hyde "Radio Astronomy"

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Grease or Oil?"

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W C 1), at 6 15 p m —Mr Robert Maxwell "The Economics of Publishing Scientific Material"

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the EWELL COUNTY TECHNICAL COLLEGE CHEMICAL SOCIETY, at the County Technical College, Reigate Road, Ewell, Surrey), at 7 p m —Dr J W Haworth "Anaesthetics"

Tuesday, October 8

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N W 1), at 5 p m —Scientific Papers

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W 1), at 5 30 p m —Mr J D Harris "Silica and Silicates", 7 p m —Dr W F Watson "Cyclisation of Natural Rubber"

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Annual Meeting Annual Report for 1962–63 and induction of new Chairman 6 30 p m —Mr S H Grylls "The History of a Dimension" (Chairman's Address)

PLASTICS INSTITUTE (at the Wellcome Building, Euston Road, London, N.W.1), at 6 30 p m —Mr K C Waldron "Plastics in the Car Industry".

ROYAL SOCIETY OF MEDICINE, PSYCHIATRY SECTION (at 1 Wimpole Street London, W 1), at 8 p m —Dr J S I Skottowe "Our Craft" (Presidential Address)

Wednesday, October 9

BRITISH INTERPLANETARY SOCIETY (in the Lecture Theatre of the Royal Aeronautical Society, 4 Hamilton Place, London, W 1), at 9 a m —Symposium on "Advanced Propulsion"

COLOUR GROUP (GREAT BRITAIN) (in Lecture Theatre No 3 Physics Department, Imperial College, Prince Consort Road, London, S W 7), at 3 p m —Reports on the Conference of the Commission Internationale de l'Eclairage (C I E) held in Vienna, June 1963

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W 1), at 5 p m —Dr G Fielder "Strike-Slip Faulting in the Vaporum Region of the Moon", Mr S L Jain, Dr P L Robinson and Mr T K R Chowdhury "A New Vertebrate Fauna from the Triassic of the Deccan, India", Prof A Desio, Dr E Martina and Dr G Pasquarè "The Geology of Badakhshan (North-East Afghanistan)"

BRITISH INSTITUTION OF RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Dr J C Barton "Novel Types of Magnetic Recording Heads"

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS AND STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S W 1) at 6 p m —Mr H J Bernhard "Flange Theory and the Revised Standard BS 10 1962—Flanges and Bolting for Pipes, Valves and Fittings"

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with the OILS AND FATS GROUP, at 14 Belgrave Square, London, S W 1), at 6 15 p m —Dr D A Walker "Fats in Relation to Ice Cream"

SOCIETY FOR ANALYTICAL CHEMISTRY (in the Main Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S W 7), at 6 30 p m —Meeting on "Thin Layer Chromatography"

SOCIETY OF INSTRUMENT TECHNOLOGY, MEASUREMENT TECHNOLOGY SECTION (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr P Hutchins "Measurement Techniques for the Control of Efficiency and Safety of Nuclear Chemical Plant"

Thursday, October 10

INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY, ACOUSTICS GROUP (in the Physics Department, Imperial College of Science and Technology, London, S W 7), at 3 p m —Meeting on "Some Aspects of the Wilson Committee Report on Noise"

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Informal Discussion on "What is Hydrology? How is it Applied?" introduced by Mr P O Wolf

CHEMICAL SOCIETY (in the Anatomy Theatre, King's College, Strand, London, W C 2), at 6 p m —Prof H E Gunning E W T Steacie Memorial Lecture

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "A Basis for Comparison of the Operating Economy of One Nuclear Power System with Another, and with Conventional Generating Systems"

OIL AND COLOUR CHEMISTS' ASSOCIATION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W 1), at 7 p m —Mr J R Berry "Instrumental Prediction of Paint Application Properties"

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W C 2), at 7 p m —Dr G W Wilson "Optical Masers, How They Work and What They Do"

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W 1), at 8 p m —Sir Charles Harington, K B E , F R S "The Debt of Science to Medicine" (Nuffield Lecture)

Friday, October 11

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, N W 1), at 4 p m —Prof John Francis (University of Queensland) "The Cattle Industry in Tropical and Sub-Tropical Australia" *

Saturday, October 12

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 2 30 p m —Mr H T Norris "Mauritanian Society, Its Poetry and Music" *

Monday, October 14

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Patents and British Industry Today" opened by Mr P Ford

INSTITUTION OF MECHANICAL ENGINEERS, MANIPULATIVE AND MECHANICAL HANDLING MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Modern Advances in the Design and Use of Automatic Vending Machines"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S W 7), at 6 p m —Mr Eric E Shipton, C B E "Patagonian Adventure" (colour films with commentary)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the SURFACE ACTIVITY GROUP, at 14 Belgrave Square, London, S W 1), at 6 p m —Dr J M Corkill "The Structure of Thin Detergent Films"

PLASTICS INSTITUTE, ENGINEERING SUB-GROUP (at "The Ring" Blackfriars Road, London, S E 1), at 6 30 p m —Mr P J Gough "Toolmaking by Spark and Electro-chemical Erosion"

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

ASSISTANT EXPERIMENTAL OFFICER (with a degree in science, horticulture or agriculture, or equivalent) for physiological studies on vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 12)

RESEARCH FELLOW (with an M Sc or Ph D degree and/or research experience in one of the following fields neutron physics, fluctuations in electronic devices, vacuum physics and electron optics, wave optics or solid state physics including X-ray crystallography) IN THE DEPARTMENT OF PHYSICS —The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham, 4, quoting Ref 9/63/6 (October 14)

SENIOR LECTURER (with a special interest in physical-organic chemistry, preferably experience in teaching, and capable in initiating independent research) IN THE DEPARTMENT OF CHEMISTRY—Clerk to the Governing Body, Chelsea College of Science and Technology, Manresa Road, London, S W 3 (October 14)

ASSISTANT LECTURER (preferably honours graduate in zoology or physiology with suitable postgraduate experience) IN ANATOMY AND HISTOLOGY IN THE DEPARTMENT OF AGRICULTURE, Sutton Bonington, near Loughborough—The Registrar, The University, Nottingham (October 15)

READER IN BIOPHYSICS—Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, Chelsea, London, S W 3 (October 21)

STUDENT IN THE DEPARTMENT OF CHEMISTRY for research on the preparation and properties of nickel cobalt films—The Registrar, The University, Hull (October 21)

PROFESSOR OF PHILOSOPHY, PROFESSOR OF SOCIOLOGY, PROFESSOR OF PHYSICS, PROFESSOR OF CHEMISTRY, and a PROFESSOR OF MATHEMATICS—The Registrar, University of Kent, Westgate House, Canterbury, Kent (October 26)

FELLOW IN THE DEPARTMENT OF DEMOGRAPHY, Research School of Social Sciences, Institute of Advanced Studies, Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, October 28)

CHAIR OF PURE MATHEMATICS at the University of Canterbury, New Zealand—Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, University of Canterbury, P O Box 1471, Christchurch, New Zealand (October 31)

CHAIR OF SOCIOLOGY—The Registrar (Room 22, O R B), The University, Reading (October 31)

LECTURER OR ASSISTANT LECTURER IN MATHEMATICS at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (October 31)

PLANT PHYSIOLOGIST, Principal Scientific Officer grade or Senior Principal Scientific Officer grade (active research worker with wide experience of research in plant physiology, particularly as applied to practical problems in the production of economic crops)—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (October 31)

SENIOR LECTURER IN THE DEPARTMENT OF PSYCHOLOGY, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Brance Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, October 31)

TEMPORARY RESEARCH ASSISTANT (graduate in botany, biochemistry or chemistry) IN THE DEPARTMENT OF BOTANY, to assist in aspects of amino-acid metabolism in plants being conducted by Dr L Fowden—The Secretary, University College, Gower Street, London, W C 1 (October 31)

LECTURERS (2) IN EDUCATION AT THE UNIVERSITY OF OTAGO, Dunedin, New Zealand—The Secretary, The Association of Commonwealth Universities (Brance Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, University of Otago (London and New Zealand, November 2)

Chair of Zoology—The Secretary, Queen's University, Belfast, Northern Ireland (November 30)

Research Assistant at the Radcliffe Observatory, Pretoria—The Secretary to the Radcliffe Trustees, 7 Lincoln's Inn Fields, London, W C 2 (December 1)

Animal Nutritionist (with a degree in agriculture with postgraduate training or experience in biochemistry, animal physiology or animal nutrition, or an honours science degree with postgraduate training or experience in animal husbandry, and preferably experience of nutrition work in the tropics) in the East African Agriculture and Forestry Research Organization, for investigation of metabolism of tropical and temperate type cattle with a tropical environment—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S W 1, quoting Ref T/RC 264/364/02

Biochemist, Basic grade (university graduate in chemistry or biochemistry, or A R I C) in the Chemical Pathology Department, for duties which will include assays of protein bound iodine and other specialized estimations, with training in all aspects of clinical biochemistry—The Clerk to the Governors, St Bartholomew's Hospital, London, E C 1, quoting Ref No AC/321

Biologist (with postgraduate experience in microbiology) to join a group carrying out research on problems concerned with the genetics of bacteria and bacterial viruses—The Director, Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, Ducane Road, London, W 12

Lecturer (man or woman, with good academic qualifications and preferably some teaching experience) in Mathematics—The Principal, Avery Hill Training College, Bexley Road, Eltham, London, S E 9

Lecturer (with at least a good honours degree in chemistry, some teaching and research or industrial experience, and an active interest in research) in Inorganic Chemistry, to teach to Grad R I C , B Sc General and H N C standards—The Principal, South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex

Non-Medical Biochemist (basic grade)—Dr M Lubran, Pathology Department, West Middlesex Hospital, Isleworth, Middlesex

Research Assistant (qualified for registration for Ph D degree and with a special interest in organic chemistry) in the Department of Chemistry—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W 8

Senior Technician (Grade II) (able to supervise and organize technicians and technical services) for the teaching section of the Department of Geology—The Assistant Bursar, The University, Reading

Technician or Senior Technician (female) for research work in mammalian physiology—The Administrator, University Laboratory of Physiology, Oxford

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

General Register Office Census 1961 England and Wales County Report—Monmouthshire Pp xxiii+96 (London H M Stationery Office, 1963) 20*s* net [307

The British Drug Houses, Ltd The Determination of Fluidity of Cotton, Rayons and Cellulose Acetate using "Shirley" Cuprammonium Hydroxide Solution Pp 8 (Poole The British Drug Houses, Ltd , 1963) [18

Foundations of Scientific Policy By Dr Stephen Toulmin Pp 6 (London Dr Stephen Toulmin, The Nuffield Foundation Unit for the History of Ideas, 1963) [18

Government of Northern Ireland Ministry of Agriculture Leaflet No 135 Careers in Agriculture Pp 8 (Belfast Ministry of Agriculture, 1963) [78

Bulletin of the British Museum (Natural History) Botany, Vol 3, No 2 The Diatom Genus *Capartogramma* and the Identity of *Schizostauron* By R Ross Pp 47–92+plates 1–2 (London British Museum (Natural History), 1963) 14*s* [78

Department of Scientific and Industrial Research The Work of the Torry Research Station and Humber Laboratory for Research on the Problems of Handling and Preservation of Fish and Fishery by-Products—a Brief Description Pp 24 (London Department of Scientific and Industrial Research, 1963) [78

Morganite Research and Development Limited The Northfields Laboratories—Descriptive Brochure Pp 11 (London Morganite Research and Development Limited, 1963) [78

Department of Scientific and Industrial Research Building Research Station Building Research—Quarterly List of Publications, List No 25, April 1963 Pp 7 (London Department of Scientific and Industrial Research, 1963) [78

General Register Office Census 1961 England and Wales County Report—Denbighshire Pp xxiii+55 13*s* 6*d* net County Report—Anglesey Pp xii+46 11*s* 6*d* net County Report—Carmarthenshire Pp xxiii+56 13*s* 6*d* net (London H M Stationery Office, 1963) [78

Gateways Guide to Courses of Further Education in Middlesex, 1963–1964 Part 1 Vocational Courses Pp 55+xi Part 2 General Interest and Recreational Courses Pp 50+v (London Middlesex County Council Education Committee, 1963) [128

The Edinburgh School of Agriculture The Edinburgh and East of Scotland College of Agriculture Calendar for 1963–1964 Pp 66 (Edinburgh The Edinburgh School of Agriculture and The Edinburgh and East of Scotland College of Agriculture, 1963) [128

General Register Office The Registrar General's Quarterly Return for England and Wales Births, Deaths and Marriages, Infectious Diseases, Weather, Population Estimates, Quarter ended 31st March, 1963 (No 457, 1st Quarter 1963) Pp 32 (London H M Stationery Office, 1963) 2*s* 6*d* net [128

Other Countries

United States Department of the Interior Geological Survey Bulletin 1131 Geology and Mineral Deposits of the Turtle Lake Quadrangle, Washington By George E Becraft and Paul L Weis Pp v+73+plates 1–6 Bulletin 1134 Ordovician Graptolites of the Basin Ranges in California, Nevada, Utah, and Idaho By Reuben J Ross, Jr and William B N Berry Pp v+177+plates 1–14 (Washington, D C Government Printing Office, 1963) [307

Ministère de l'Agriculture de la Province de Québec Le Conseil des Recherches Agricoles Recherches Agronomiques—Sommaire des Résultats, 1961–1962 Pp 20 (Extrait du Rappoty du Ministère de l'Agriculture de la Province de Québec pour l'Annèe finissant le 31 Mars 1962) (Québec Ministère de l'Agriculture de la Province de Québec, 1963) [307

United States Department of Commerce Weather Bureau Technical Paper No 48 Characteristics of the Hurricane Storm Surge By D Lee Harris Pp v+139 (Washington, D C Government Printing Office, 1963) 70 cents [307

Canada Department of Mines and Technical Surveys Geological Survey of Canada Miscellaneous Report No 7 The National Parks in Ontario a Story of Islands and Shorelines Pp iv+70 50 cents Memoir No 313 Devonian Stratigraphy of Northeastern Alberta and Northwestern Saskatchewan By A W Norris Pp xi+168 2 50 dollars Paper 63–18 Rocks and Minerals for the Collector Sudbury to Winnipeg By Ann P Sabina Pp iii+69 75 cents (Ottawa Queen's Printer, 1963) [307

National Academy of Sciences—National Research Council, Washington Publication 1078 Damage to Livestock from Radioactive Fallout in Event of Nuclear War (A Report by the Subcommittee on Livestock Damage of the Advisory Committee on Civil Defense, National Academy of Sciences—National Research Council) Pp vi+93 (Washington, D C National Academy of Sciences—National Research Council, 1963) 2 dollars [307

Bulletin of the American Museum of Natural History Vol 125, Article 2 Types of the Tachinidae (Diptera) in the American Museum of Natural History By Paul Henri Arnaud, Jr Pp 101–138 (New York American Museum of Natural History, 1963) 1 dollar [307

Records of the Australian Museum Vol 25, No 10 (19th June, 1962) The History and Significance of the Fossil *Casuarius lydekkeri* By Alden H Miller Pp 235–238 Vol 25, No 11 (19th June, 1962) Two New Scleractinian Corals from Australia By John W Wells Pp 239–242+plates 16–18 (Sydney The Australian Museum, 1962) [307

United States Department of Agriculture Agriculture Handbook No 240 Controlling Insects and Diseases of the Pecan By Max R Osburn, William C Pierce, A M Phillips, John R Cole and George L Barnes Pp 52 (Washington, D C Government Printing Office, 1963) 25 cents [307

Chicago Natural History Museum Fieldiana Botany Vol 29, No 10 Tropical American Plants, V By Louis O Williams Pp 545–586 (Chicago Chicago Natural History Museum, 1963) 75 cents [307

Transactions of the American Philosophical Society New Series, Vol 53, Part 4 The Western Journals of Dr George Hunter, 1796–1805 Edited by John Francis McDermott Pp 133 (Philadelphia The American Philosophical Society, 1963) 3 dollars [307

Canada Department of Mines and Technical Surveys Geological Survey of Canada Geological Maps—Preliminary Series Map 30–1963 Yukon Territory and Northwest Territories Map 1039A Alberta and Northeastern British Columbia showing Oil and Gas Fields (information to January 1, 1963) Ninth edition (Ottawa Director, Geological Survey of Canada, 1963) [307

Bulletin of the Florida State Museum, Biological Sciences Vol 7, No 4 Catalogue of Fossil Birds By Pierce Brodkorb Pp 179–293 (Gainesville, Fla Florida State Museum, 1963) 1 40 dollars [18

World Health Organization Information Circular on Insecticide Resistance, No 35, September 1962 Pp 16 (Geneva World Health Organization, Division of Environmental Health, 1962) [18

Republic of Cyprus Ministry of Commerce and Industry Geological Survey Department Bulletin No 1 The Mineral Resources and Mining Industry of Cyprus By T B S Gorman and L M Bear Pp xv+208 (Nicosia Geological Survey Department, 1963) 20*s* [18

United States Department of the Interior Geological Survey Bulletin 1063–E Geology of the Edgemont NE Quadrangle, Fall River and Custer Counties, South Dakota By Garland B Gott and Robert W Schnabel Pp v+127–190+plates 12–16 Bulletin 1121-J Geology of the Duck Creek Pass Quadrangle, Montana By Willis H Nelson Pp iv+56+plate 1 Bulletin 1130 Geology of the Mount Pinchot Quadrangle, Southern Sierra Nevada, California By James G Moore Pp vii+152+plates 1 and 2 Bulletin 1141–D General Geology of the Jackson Mountains, Humboldt County, Nevada By Ronald Willden Pp iv+65+plates 1–3 (Washington, D C Government Printing Office, 1963) [18

New Zealand Marine Department Fisheries Technical Report No 8 Biological and Economic Aspects of the Elephant Fish *Callorhynchus milii* Bory in Pegasus Bay and the Canterbury Bight Pp ii+54 (Wellington Government Printer, 1963) [18

Canada Department of Mines and Technical Surveys Geological Survey of Canada Miscellaneous Report No 6 Jasper National Park Behind the Mountains and Glaciers By David M Baird Pp vi+184 1 50 dollars Index to Publications of the Geological Survey of Canada (Supplement for 1961, 1962) Pp vii+71 35 cents Paper 62–25 Geology of the Barite, Gypsum, Manganese, and Lead-Zinc-Copper-Silver Deposits of the Walton-Cheverie Area, Nova Scotia By R W Boyle Pp i+36+map 75 cents Paper 62–33 Dahadinni and Wrigley Map-Areas, District of Mackenzie, Northwest Territories By R J W Douglas and D K Norris Pp ii+34+2 maps 75 cents (Ottawa Queen's Printer, 1963) [18

The Organization for Economic Co-operation and Development Pp 254 (Paris The Organization for Economic Co-operation and Development, 1963) [18

Records of the Dominion Museum, Wellington Vol 4, No 14 (29 March, 1963) The Southern Black-Backed Gull (*Larus dominicanus*) Lichtenstein—Measurements, Plumage Colour, and Moult Cycle By F C Kinsky Pp 149–219 Vol 4, No 15 (24 May, 1963) The Littoral Marine Mollusca of the Snares Islands By R K Dell Pp 221–229 Vol 4, No 16 (24 May, 1963) A Moa-Bone from the Sea-Floor in Cook Strait By C A Fleming Pp 231–233 (Wellington Dominion Museum, 1963) [18

European Organization for Nuclear Research, CERN Annual Report, 1962 Pp 165 (Geneva European Organization for Nuclear Research, CERN, 1963) [18

Chicago Natural History Museum Fieldiana Geology Memoirs Vol 4 The Paleoecological History of Two Pennsylvania Black Shales By Rainer Zangerl and Eugene S Richardson, Jr With contributions by Bertram G Woodland, Robert L Miller, Richard C Neavel, and Harry A Tourtelot Pp xii+353 (55 plates) (Chicago Chicago Natural History Museum, 1963) 10 dollars [68

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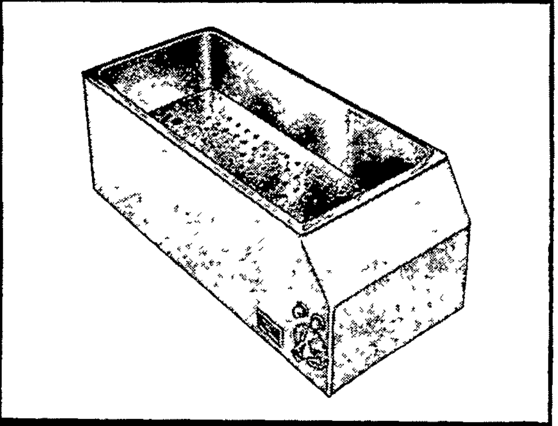

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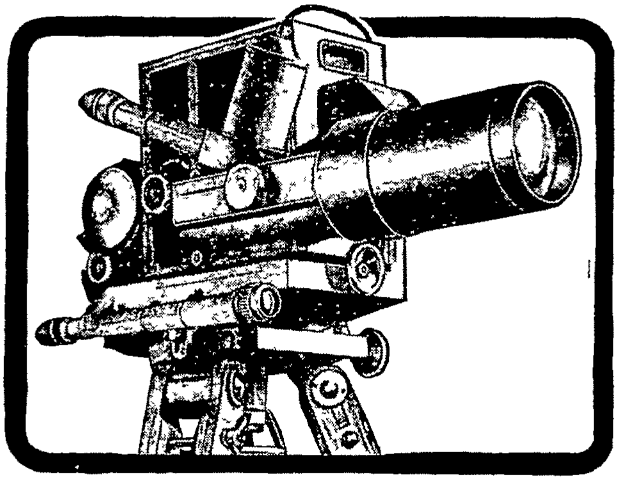

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- 3 pressure keys operate electric controls in compact switchboard
- Can be operated by remote control
- The camera's laterally unreversed, upright pictures can be copied without reprinting

PENTAZET

16mm and 35mm, the scientist's high-speed camera
For more information about the Pentazet, write to:
The Rank Organisation
RANK STUDIO EQUIPMENT
Woodger Rd • Shepherds Bush • London W12 • Tel• SHEpherds Bush 2050

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ADVERTISEMENTS SHOULD BE ADDRESSED TO T G Scott and Son, Limited, 1 Clement's Inn, Strand, London, W C 2 Telephone HOLborn 4743 Telegrams Textualist, Estrand, London

APPOINTMENTS VACANT

SOUTH AUSTRALIAN INSTITUTE OF TECHNOLOGY

NORTH TERRACE, ADELAIDE,
SOUTH AUSTRALIA

HEAD OF DEPARTMENT OF PHYSICS

Applications are invited for the above position The salary is that of a University Reader in S A , £A3,000 by £A100 to £A3,300

Applicants should hold at least a Master's degree in Physics and should preferably have had some industrial and teaching experience

This Department, until now a part of the Mathematics and Physics Department, provides a degree course in Industrial Physics in the Faculty of Technology of the University of Adelaide It also gives service courses in Physics at various levels for other degree courses in Technology and Applied Science The Department is also responsible for a variety of subjects for Diploma and Certificate students, including Radiography, Radiotherapy and Optometry Postgraduate short courses on special topics are being developed

Subject to the general direction of the Head of the School of Applied Physics, the appointee will be required to supervise and develop the work of his Department

At the completion of six years of service, the appointee may apply for paid leave of absence for up to one year to obtain further experience abroad Other assistance may be granted at the discretion of the Council

The appointee will be eligible to join the S A Government Superannuation Fund He will be assisted in meeting reasonable travelling and removal expenses

Applications, giving personal, academic and professional details, as well as an approximate date for commencement of duty, should reach the Agent-General and Trade Commissioner for South Australia (from whom further information may be obtained) at 50 Strand, London, W C 2, not later than November 15, 1963

ROWETT RESEARCH INSTITUTE

BUCKSBURN ABERDEEN
(ANIMAL NUTRITION AND ALLIED SCIENCES)

Applications are invited for the post of Scientific Officer/Senior Scientific Officer in the Physiology Department A good honours degree in physiology or biochemistry is required An additional veterinary qualification would be an advantage, as would research experience in the field of animal metabolism A suitably qualified candidate could work for a higher degree Salary range £791 to £1,309 or £1,438 to £1,772, placing according to age, qualifications and experience F S S U superannuation Further particulars available

Apply, with fullest particulars, naming three referees, to the Secretary of the Institute, by November 1, 1963

UNIVERSITY OF ALBERTA

FACULTY OF AGRICULTURE
VACANCY
ASSISTANT PROFESSOR OF ANIMAL PHYSIOLOGY
DEPARTMENT OF ANIMAL SCIENCE

Ph D or equivalent in animal physiology, minor in biochemistry preferred Duties include fundamental and applied research with farm and laboratory animals with emphasis on environmental physiology , teaching , limited extension work Starting salary $7,500 to $9,000, depending on qualifications , to begin between January 1 and July 1, 1964

Apply, giving names and addresses of three references, to Head, Department of Animal Science, University of Alberta, Edmonton, Alberta Closing date November 30, 1963

TEA RESEARCH INSTITUTE OF CEYLON

DIRECTOR

Applications are invited for the post of Director in succession to Dr D L Gunn, C B E High academic qualifications and extensive experience of research and of the administration of research are required

The post may be filled either by direct appointment or by secondment from a research institution or university for a period of at least three, but preferably more, years

The Institute is situated at an altitude of 4,500 feet above sea level in a climate in the past found satisfactory by expatriates, including their young families, during lengthy careers with the Institute It is financed by a cess on exports of tea and has a gross annual income of about £200,000 It has ten research divisions headed by specialist officers in addition to advisory divisions The work of the Institute is of great importance to the tea industry in Ceylon and to the economy of the Island The working scientific language is English

The terms are salary scale £4,250 by £125 to £5,250 free of tax , entertainment allowance of £400 per annum , a free furnished house , free use of an official car with driver for both official and private purposes , provision exists for grants for education of children in Europe , overseas leave of six weeks annually on full pay with passages, covering also wife and up to four children , there is a district medical scheme to which the Institute is a subscriber, half of approved medical expenses of the Director and his family are paid by the Institute , contributory provident fund arrangements of 15 per cent of salary from each side, which may be used to continue existing arrangements, such as F S S U , or to make new arrangements The duration of the contract is subject to negotiation It is desired to fill the post as soon as possible

For citizens of Ceylon, India, and Pakistan, the salary scale is Rs 2,000 by Rs 75 to Rs 2,300 per mensem, plus Dearness Allowance of Rs 181 60 per mensem for a married man or Rs 139 10 for a single man Education allowance and overseas leave cannot be offered and the entertainment allowance is Rs 250 per mensem, but the other terms are the same

Formal applications in full detail, together with any specific enquiries from suitably qualified persons, should be addressed both to (a) The Chairman of the Tea Research Institute of Ceylon, "Lynwood," 50 Edinburgh Crescent, Colombo 7, Ceylon, and also (b) Dr F R Tubbs, C B E , Director, East Malling Research Station, East Malling, Maidstone, Kent, England, to arrive before November 11, 1963

Members of the Institute's Scientific Advisory Committee in the United Kingdom, i e , Mr F C Bawden, F R S , Rothamsted Experimental Station, Harpenden, Herts , Professor T A Bennet-Clark, F R S , Department of Botany, University of East Anglia, Earlham Hall, Norwich, Norfolk, Nor 05G, and Professor G E Blackman, F R S , Department of Agriculture, University of Oxford, England, may also be consulted on general aspects of the Institute and its work

SCS

Scientific Civil Service

PRINCIPAL RESEARCH FELLOWSHIPS IN GOVERNMENT ESTABLISHMENTS

To enable scientists of high calibre to undertake short-term research in the Scientific Civil Service, awards of PRINCIPAL RESEARCH FELLOWSHIPS are announced

TENURE Period of 1 year to 3 years (with possibility of extension to 5 years)
VALUE Fixed annual stipend between £1,890 to £2,640 Superannuation under F S S U

QUALIFICATIONS A candidate must produce evidence (including published work) of a considerable period of fruitful research and be accepted as a leading worker in his/her field

EXAMPLES OF POSTS

1 Royal Aircraft Establishment, Farnborough, Hants—Work in the field of ASTRONAUTICAL ENGINEERING
2 Royal Botanic Gardens, Kew—TAXONOMIST for monographic work in difficult plant groups
3 Post Office Research Station, Dollis Hill, London—Advanced developments in TELECOMMUNICATION SCIENCE

Research Fellows will be expected to propose their own fields of research

The award of SENIOR or JUNIOR RESEARCH FELLOWSHIPS will continue as heretofore

Senior Fellowship between £1,420 to £1,755 annually

Junior Fellowship between £975 to £1,310 annually

A high standard is set and candidates must hold 1st or 2nd Class Honours Degree and have completed 3 years (Senior) or 2 years (Junior) of postgraduate research

INQUIRIES to Civil Service Commission, 23 Savile Row, London, W 1, quoting S/570

AIR MINISTRY

SCIENTIFIC ADVISER

Scientific Officers/Senior Scientific Officers required in Whitehall and R A F Command H Q in the Home Counties

The SCIENTIFIC ADVISER controls OPERATIONAL RESEARCH into a wide variety of problems affecting the efficient functioning of the R A F to-day and its development to meet future demands Previous experience in operational research not essential, but flexibility and originality of mind, wide range of interests and leadership are necessary

APPLICATION FORM and booklet from Ministry of Aviation, Est 2/3 A 3, Room 516, The Adelphi John Adam Street, W C 2, quoting HC/2D/0909

D.S.I R.

GEOLOGICAL SURVEY AND MUSEUM, London

At least 2 posts, based in London, concerned with GEOPHYSICAL SURVEYS in the field Male candidates preferred The posts are graded Geologist (Scientific Officer) or Senior Geologist (Senior Scientific Officer) Good promotion prospects Non-contributory pension
At least 1st-year university qualifications in Geology and in Physics or Mathematics required Experience in Applied Geophysics an advantage

APPLICATION FORMS from Civil Service Commission, 23 Savile Row, London, W 1, quoting S/5756/63 Closing date October 18, 1963

WARREN SPRING LABORATORY, Stevenage, Herts

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER in Chemical Engineering Division for work covering wide field in chemical engineering and applied chemistry, including automatic and computer control of plant, gas-solid and gas-liquid suspensions, technology of powders and slurries, applied catalysis and surface chemistry Interest in plant as well as laboratory work required

APPLICATION FORMS from Director at above address Closing date October 15, 1963

DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND

MARINE LABORATORY, Aberdeen

EXPERIMENTAL OFFICER/ASSISTANT EXPERIMENTAL OFFICER for STATISTICS SECTION, to assist with design analysis of experiments, analysis of statistics of commercial fisheries, production of statistical tabulations Male candidates must be prepared for periods of duty at sea Degree, including statistics with mathematics or economics, or professional qualifications in statistics plus appropriate experience essential

PARTICULARS from Establishment Officer, Room 166, St Andrew's House, Edinburgh, 1 Closing date October 15, 1963

MINISTRY OF AVIATION

EXPLOSIVES RESEARCH AND DEVELOPMENT ESTABLISHMENT, Waltham Abbey, Essex

MECHANICAL ENGINEER (graded SENIOR SCIENTIFIC OFFICER), with wide interest in physical properties of metals and non-metals, to join team studying various practical physical and mechanical engineering problems calling for novelty in approach and experimental flair Research experience in mechanical engineering or the physics of solids essential
WRITE for booklet and application form to Dr G H S Young at above address, quoting HC/2D/0923

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

SALARIES S O £791–£1309
(Increments and special £°5 award for approved post-grad experience)
S S O £1438–£1772
(Starting salary may be above minimum)
Salaries are supplemented in the London Area

QUALIFICATIONS
S O 1st or 2nd Class Hons Degree or Dip Tech in appropriate subject Age under 29
S S O As above plus at least 3 years postgraduate research Age 26 to 31

ASST. EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER

SALARIES A E O £490 (age 18) – £691 (age 22) – £858 (age 26) – £1053
E O £1164–£1431
Salaries are supplemented in the London Area

QUALIFICATIONS
Degree, Dip Tech, H N C or equivalent in appropriate subject Under age 22, minimum qualification is 2 G C E 'A' levels in Scientific subjects A E O age 18–27 E O 26–30

Full details of careers in the SCIENTIFIC CIVIL SERVICE are available on request from Civil Service Commission, 23 Savile Row, W 1.

ORGANIC CHEMIST

Commencing salary up to £2,000 per annum with F S.S U superannuation

The Animal Health Trust require an Organic Chemist to assist the Head of the Forensic Laboratory in the direction of research and routine screening of body fluids for pharmacologically active agents

Applicants should have a good honours degree in chemistry or biochemistry with postgraduate experience in toxicological analysis and/or detoxication and show evidence of ability to direct and co-ordinate work of junior staff

Application forms from Head of Forensic Laboratory, Equine Research Station, Newmarket, West Suffolk

Closing date for receipt of applications November 18, 1963

CANADA

FISHERIES RESEARCH BOARD
PHYSIOLOGIST, ENDOCRINOLOGIST or BIOCHEMIST
$7,300 to $10,500

DUTIES. To conduct research on hormones in marine life in such a manner as to complement a continuing biochemical research programme on adrenalcortical and sex hormones in fish

QUALIFICATIONS. A Ph D degree Demonstrated research ability essential Post-doctoral or other experience in the metabolism, mode of action, or isolation and identification of steroid hormones desirable

RESEARCH ASSOCIATE
$5,200 to $8,500

DUTIES· Under the general guidance of senior scientists to assist in research related to studies on tissue culture and fish diseases

QUALIFICATIONS· Graduation from a university of recognized standing with a Bachelor's degree or better Demonstrated research ability
Each of the successful candidates will be appointed to a salary commensurate with qualifications and experience
Submit complete résumé to Director, Fisheries Research Board of Canada P O Box 429, Halifax, Nova Scotia, Canada

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BRISTOL COLLEGE OF SCIENCE AND TECHNOLOGY
ASHLEY DOWN, BRISTOL, 7
Department of Chemistry and Biology

Applications are invited from Organic or Biochemists for appointment as

TECHNICAL ASSISTANT

to assist in research in synthetic and metabolic studies

Experience with radioactive tracers would be an advantage but not essential

Candidates should hold a good H N C or be graduates

Salary £650 per annum Superannuable post

Applications should be addressed to the Secretary and Registrar Please quote reference CST 63/70

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY

Research Assistant required for work on the Cross-linking of High Polymers in solution The applicant should have an honours degree in chemistry or physical chemistry (or a good G R I C), with preferably some Research experience The successful applicant will be engaged on research sponsored by the Department of Scientific and Industrial Research Salary on appointment £825 per annum rising to £850 per annum in the second year Superannuation under F S S U

Applications, giving full details, should be addressed to Professor R H Peters, Department of Polymer and Fibre Science, the Manchester College of Science and Technology, Sackville Street, Manchester, 1, as soon as possible

Return to Research in Britain

Are you a British scientist in Canada thinking of returning to work in Britain? You may find what you are looking for, without having to go home first, in the Civil Service, the United Kingdom Atomic Energy Authority, or the Central Electricity Generating Board.

Research Fellowships and Permanent Appointments

There are openings in all branches of science. The order in which they are most numerous is (i) physics, mathematics, engineering, and metallurgy; (ii) chemistry and biochemistry; (iii) biology. The research ranges from the most fundamental to the most applied.

A selection board of scientists will be coming to Ottawa on 7th January, 1964

If you would like an appointment with them, would you write by 11th November, 1963,
to: Mr. P. C. Davidge,
Senior UKAEA Representative in Canada,
c/o Atomic Energy of Canada Ltd., Chalk River, Ontario.

An announcement to British scientists in the U.S.A. will be made in November

**Issued jointly by the Civil Service Commission,
the U.K. Atomic Energy Authority, and the Central Electricity Generating Board.**

CENTRAL ELECTRICITY GENERATING BOARD

require

SCIENTIFIC ASSISTANTS

Young men and women with an interest in experimental work are invited to apply for posts as SCIENTIFIC ASSISTANTS in the Materials Division at the Central Electricity Research Laboratories, Leatherhead, Surrey

Vacancies exist for Scientific Assistants to work under the supervision of graduate Scientists on investigations into the behaviour of special steels and ceramics at high temperatures using mechanical tests, optical and electron microscopy and X-ray techniques

Applicants should have obtained at least five G C E "O" level passes and preferably, at least two "A" level passes in science subjects

Scientific Assistants are encouraged to study for higher qualifications by part-time day release

Salaries according to age (minimum starting salary £350 per annum) with prospects of obtaining £1,080 per annum

Applications, stating age, qualifications, experience, present position and salary to N Berryman, Personnel Officer (Headquarters), 24/30, Holborn, London, E C 1, by October 14, 1963 QUOTE REF N/322

LOWER INDUS PROJECT

The team engaged on this large scale investigation and development project which covers an area of about 15 million acres is to be increased by TEN AGRICULTURAL SPECIALISTS preferably men with overseas experience in irrigated Agriculture

The new posts will be filled by men with experience in one or more of the following crop trials, reclamation of saline land trials, agricultural surveys, crop sampling surveys, economic surveys, studies into the water requirements of crop plants Project planning and feasibility assessment will be required of at least two of the appointed Specialists

All Appointments are initially for two year contracts Salaries and Allowances are negotiable and will vary with the particular specialization Free furnished accommodation will be provided in each case

Essential details only in the first instance, to The Personnel Manager, Hunting Technical Services Limited, 6 Elstree Way, Borehamwood, Hertfordshire

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PILKINGTON BROTHERS LIMITED
Glass Manufacturers, require a

PHYSICIST

age between 25 to 30 years, at their Research Laboratories to carry out basic research work covering theoretical and experimental investigations of heat transfer and model studies of viscous and non-viscous flow in glass-making processes

The work involves design and analysis of experiments at small-scale laboratory and pilot plant level

A good honours degree in Physics is essential with some experience of university or industrial research

The salary is in a range which can rise to £2,000 per annum

Applications should be made, quoting RES/J 3, to L P Laidlaw
GROUP STAFF DEPARTMENT,
Pilkington Brothers Limited,
Grove Street, St Helens, Lancashire

APPLICATIONS ARE INVITED FOR A Post-doctoral Fellowship in haemoprotein chemistry Sound background in physical organic chemistry and/or modern analytical techniques are essential —Apply to Dr G R Schonbaum, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada

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Instrumentation Division Manchester

AEI

SCIENTIFIC APPARATUS SALES

AEI have a number of opportunities in the Analytical Instruments Section of their Scientific Apparatus Sales Department The Department is a rapidly expanding one, and makes a wide range of scientific equipment including mass spectrometers, X-ray spectrometers, electron microscopes, and particle accelerators

CHEMISTS An Honours graduate is required with strong interest in organic chemistry and keenness to prove himself in a new and expanding field The work involved is the introduction of mass spectrometry methods to organic chemistry groups throughout the world

TECHNICAL SALES STAFF Several Honours graduates in chemistry, physics, or other scientific discipline are required for commercial work The duties cover liaison between prospective users and AEI Application Laboratories

The preferred age limits for both appointments would be 24 to 30 Freedom to travel overseas would be essential and a foreign language would be an advantage

Please write, giving brief details of age, qualifications, experience, and present salary, and stating which type of post interests you, to

STAFF MANAGER (E25) ASSOCIATED ELECTRICAL INDUSTRIES LTD · TRAFFORD PARK · MANCHESTER, 17

BEA

RADIOLOGICAL PROTECTION

We wish to recruit two men for interesting new posts concerned with radiation protection measures for our aircraft and equipment at London Airport

SUPERVISOR

To investigate and report on radioactive contamination found and make recommendations on the protection measures necessary Applicants should have a B Sc or other appropriate professional qualification and the ability to organize and administer a monitoring and protection programme Salary range £1113 to £1308 per annum

ASSISTANT

Preferably aged 21 to 25, to record levels of contamination and generally assist the Supervisor in his duties Although a scientific background is desirable, we will consider those of high intelligence who have a keen interest in this work and the capacity for development under training Salary range £735 to £894 per annum

Apply in writing to Personnel Officer, Head Office (NA), British European Airways, Bealine House, Ruislip, Middlesex

COMMONWEALTH OF AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF PLANT INDUSTRY

FELLOWSHIP IN MOLECULAR BIOLOGY

Applications are invited for appointment to a Research Fellowship in Molecular Biology in the Division of Plant Industry, C S I R O , Canberra

The Division of Plant Industry has recently established a Unit of Molecular Biology This Unit at present consists of five scientists and is now seeking to appoint another Fellow The work presently in progress includes the study of replication and expression in RNA viruses , the evolution of enzymes and the mapping of active sites, molecular mechanisms of adaptation, of regulatory processes, and of differentiation

The Division of Plant Industry, situated in Canberra, comprises some 130 scientists conducting pure and applied research in Genetics, Plant Breeding, Biochemistry, Physiology, Microbiology, Inorganic Nutrition, Ecology, Agricultural Physics, Agronomy, and Plant Introduction

The Division is well equipped with instruments for the characterization of macromolecules, including preparative and analytical ultra-centrifuges, isotopic equipment, recording spectrophotometers, and electron microscope, and electronic computer facilities

Applicants should possess a Ph D degree in an appropriate field, or have had post-graduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability

Dependent upon qualifications and experience, appointment will be made at a salary of not less than £A1 979 per annum up to £A3,015 per annum Appointment of an applicant having outstanding qualities of leadership in research and eminence in the broad field of study would be considered up to a maximum of £A3,873 per annum Salary rates for women are £A188 per annum less than the corresponding rate for men

The Fellowship is initially for three years with the possibility of extension if mutually desired

Consideration may be given to the continuation of F S S U or, alternatively, if a secondment is arranged with the present employer, the Organization may be able to arrange to reimburse the employer's share of other superannuation schemes

Return fares paid for the appointee and his dependent family Further particulars supplied on application to
Mr P F Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2,
to whom applications (quoting Appointment No 130/614) should be addressed by November 2, 1963

AUSTRALIA

If you are thinking of taking up an appointment in Australia you are cordially invited to write to us for helpful information and advice on the various aspects of Australian conditions and affairs
"Australia—A Guide for Newcomers" is one of a number of publications designed especially to help those contemplating residence in Australia, and a copy will be freely sent on request

Please write, call or telephone

THE NATIONAL BANK OF AUSTRALASIA LIMITED
Information Department
Chief London Office 6-8 Tokenhouse Yard, EC2 Telephone MONarch 8070

UNIVERSITY OF MANCHESTER

Applications are invited for the posts of Lecturer and Assistant Lecturer in the Department of Physics in the Faculty of Science The research interests of applicants should lie in the High Energy Physics field, in which the Department has an active programme Duties to commence as soon as possible Salary scales Lecturer, £1,250 to £2,150 per annum, initial salary according to qualifications and experience , Assistant Lecturer, £1,000 to £1,150 per annum Membership of the F S S U and Children s Allowance Scheme

Applications should be sent not later than November 15, 1963, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained Applications by airmail letter in the first instance will be received from overseas candidates, who should state the names and addresses of at least three persons to whom reference can be made

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UNIVERSITY OF EDINBURGH

DEPARTMENT OF MEDICAL PHYSICS

LECTURESHIP

Applications are invited for the post of Lecturer in the above Department Salary scale Lower £1,250 by £110 to £1,690 , Higher £1 805 by £115 to £2,150 by £110 to £2 700 per annum, with placement according to qualifications and experience, and with superannuation benefit and family allowance where applicable The successful applicant will include among his duties those of University Radiation Protection Officer and will be expected to undertake and supervise research in this and related fields The successful applicant will be expected to take up duty in October, 1963, or as soon as possible thereafter

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than October 12, 1963

CHARLES H STEWART,
Secretary to the University

SOUTHERN RHODESIA

MINISTRY OF AGRICULTURE

Applications invited from holders of Agricultural degrees (pass or honours) with majors in appropriate subjects Post-graduate experience a recommendation

1 POULTRY OFFICER—Matopos Research Station
Design and execution suitable research programme, including the conducting of egg-laying tests and poultry advisory work

2 AGRICULTURAL CHEMISTS—Chemistry Branch
One post Analytical Laboratory, Salisbury, concerned with analysis by chemical and spectrographic methods, agricultural products and materials Duties analytical and advisory
The other post Soil Survey Section , soil surveys for Land Settlement, Irrigation and other schemes Staff usually spend winter months in the field and for the remainder of the year are occupied in the Salisbury offices and laboratories on analysis of the samples collected and compilation of maps and reports

3 SEED RESEARCH OFFICER, SALISBURY
Applicants should have an honours degree in either Plant Pathology or Microbiology Duties include investigation of seed-borne diseases, detection and control, and various aspects of seed physiology

4 ANIMAL HUSBANDRY OFFICER—Matopos Research Station
Applicants should possess Degree with Animal Husbandry major subject A diploma in sheep and wool a recommendation Duties include physiological and nutritional studies on exotic and indigenous beef breeds and assisting in all matters pertaining to beef production in the lower rainfall areas of Southern Rhodesia

Salaries are in the Professional Officer scale £900 by £100 to £1,200 by £75 to £1,350 by £60 to £1,770 by £65 to £1,900 per annum

Entry point depends on qualifications and experience

Pension Benefits—Good leave conditions—Low Income Tax—Good sporting facilities—Attractive climate—*Fares Paid*

Applications to and further details from Secretary (R), Rhodesia House, 429 Strand, London, W C 2

Closing date October 16, 1963

GOVERNMENT OF UGANDA

VACANCY FOR A BIOCHEMIST

The Department of Technical Co-operation invites applications for the post of Biochemist, Uganda Duties To work with other members of the research staff in establishing modern biochemical techniques for research into adaptation and diseases of domestic livestock in Uganda The programme is concerned principally with the study of serum proteins in relation to immunity to tropical animal diseases and the estimation of drug levels in the animal body Qualifications Candidates must possess a first- or second-class honours degree in chemistry, or veterinary qualifications with additional specialization in biochemistry Experience of the application of modern bio-chemical techniques to the study of animal diseases desirable but not essential Terms Appointments on contract for one tour of 21 to 27 months in the salary scale £1,266 to £2,448 a year Gratuity (taxable) of 25 per cent of emoluments on satisfactory completion of contract Initial salary according to experience Government quarters usually available at a low rental Free passages for officer, wife and dependent children Education allowances Generous leave

Applicants should normally be nationals of the United Kingdom and Great Britain Apply to the Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S W 1, stating full name and giving brief particulars of qualifications and experience quoting RC 217/153/04/C1

UNIVERSITY OF LONDON

CHAIR OF MEDICAL MICROBIOLOGY AT ST THOMAS'S HOSPITAL MEDICAL SCHOOL

The Senate invite applications for the Chair of Medical Microbiology tenable at St Thomas's Hospital Medical School (salary in the range £3,645 to £4,445 a year)

Applications (ten copies), must be received not later than November 20, 1963, by the Academic Registrar, University of London, Senate House, W C 1, from whom further particulars may be obtained

ROYAL PERTH HOSPITAL

WESTERN AUSTRALIA

HOSPITAL MICROBIOLOGIST

Applications for the above post are invited from medical graduates with suitable experience in Microbiology The Hospital Department of Microbiology is under the control of the Director of Hospital and University Services in Microbiology

The successful candidate will be required to assist the Director (Professor N F Stanley) in the administration of the routine laboratory and clinical services of the Hospital Department of Microbiology, to act in the absence of the Director and to carry out special investigations from time to time

Royal Perth Hospital is the main teaching hospital of the Medical School of the University of Western Australia The Hospital Department of Microbiology is well equipped, adequately staffed for the performance of all modern microbiological techniques, functions in close association with the University Department of Microbiology in the adjacent School of Medicine and the Virus Research and Diagnostic Laboratory of the Department of Public Health

SALARY Within the range of £A3,800 to £A4,350 per annum, commencing rate according to qualifications and experience Salary will commence from the date the appointee takes up duty

CONDITIONS OF SERVICE Include annual leave, long service leave (three months on full pay after each seven years service) and sick leave Study leave may be granted as a privilege for some special purpose approved by the Board Contribution to the State Superannuation Scheme is required, or alternatively, the Hospital would consider subsidizing the appointed officer's existing commitments under an approved scheme or endowment policy

TRANSPORT EXPENSES A travel grant equivalent to minimum First Class sea passage will be provided for successful applicant and family (under 16 years of age), together with an allowance of £S200 for the cost of removal of personal effects, etc (excluding furniture and motor vehicle), subject to a bond to remain in the Hospital's service for a minimum period of three years

A memorandum of Information including further details of the Department of Microbiology is available upon request

Further information concerning Royal Perth Hospital and the State of Western Australia may be obtained from the Agent General for Western Australia, Savoy House, 115 Strand, London, W C 2

Applications in writing should include date of birth, qualifications, experience, present and previous appointments, a list of publications, a recent photograph and the names and addresses of three referees

CLOSING DATE November 30, 1963

Joseph Griffith,
ADMINISTRATOR

AUSTRALIAN NATIONAL UNIVERSITY

SENIOR LECTURER IN PHYSICS OR THEORETICAL PHYSICS

Applications are invited for appointment to the new post of Senior Lecturer in Physics or Theoretical Physics in the School of General Studies of the University The appointee will be required to take up duty on January 1, 1964, or as soon thereafter as possible Applicants should hold the degree of Doctor of Philosophy in Physics or equivalent qualification Some teaching experience is desirable but not essential Research interests in the Departments of Physics and Theoretical Physics are in the fields of atomic collision processes, atomic diffusion in metals, the design and use of shock tubes, and theoretical physics The salary range for a senior lecturer is at present £A2,450 by £A110 to £A3,000 per annum Commencing salary will be [illegible] accordance with qualifications and experience [illegible] are at present under review [illegible] formation and the forms which [illegible] an application may be ob- [illegible] cretary Association of Com- [illegible] es (Branch Office) Marl- [illegible] Mall, London, S W 1 Ap- [illegible] ndon and Australia on [illegible]

[illegible] COUNCIL REQUIRE [illegible] preferably not under [illegible] technical aspects of [illegible] and equipment, for [illegible] Colindale Avenue, [illegible] ale rising to £1,281 [illegible] provision —Written [illegible] fessional referees), [illegible] ting ref E10/29

GHANA ACADEMY OF SCIENCES

Applications are invited for the following vacancies in the Cocoa Research Institute, Tafo

Secretary Duties Applicant to be responsible to the Director for the general administration of the Institute, knowledge of accounting will be an advantage Qualifications Applicants, who must be between the age of 20 to 45, should hold a degree of the A C I S or the A C C S Salary in range £1,050 to £1,900 per annum

Entomologist Duties Responsible for research work on insect pests of cocoa Qualifications Good honours degree or higher degree in zoology with postgraduate experience in entomology Salary To be determined according to qualification and experience is in the range £1 050 to £2,300 per annum (for Ghanaians) and £1,260 to £2,750 per annum (for expatriates)

Spectrographer Duties To include running and maintenance of a spectrographic laboratory concerned with mineral analyses of plants and soils Qualifications A good honours or higher degree in physics or chemistry with postgraduate experience in the running and maintenance of a spectrographic laboratory concerned with mineral analyses of plants and soils Experience in handling and measuring radioisotopes would be an additional advantage Salary To be determined according to qualifications and experience is in the range £1,050 to £2,300 per annum (for Ghanaians) and £1,260 to £2,750 per annum (for expatriates)

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For further information and application forms, please write, stating the post in which you are interested to the Director of Recruitment, Ghana High Commission, 248 Tottenham Court Road, London, W 1

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Further particulars may be obtained from the Secretary, Royal College of Advanced Technology, Salford 5, Lancs, to whom applications should be submitted by October 31, 1963, quoting reference ME/55

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Potential applicants for the Chair, for which applications were invited by advertisement last month, are notified that the

CLOSING DATE FOR APPLICATIONS HAS BEEN POSTPONED

until October 30, 1963

Full particulars may be obtained from the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1, or from the Registrar of the University

Applications (in duplicate), should reach the Registrar of the University not later than October 30, 1963

UNIVERSITY OF MELBOURNE

DEPARTMENT OF STATISTICS

Applications are invited for a position of Senior Lecturer in Statistics The salary range is £A2,450 to £A3 000 per annum Initial salary will be determined according to qualifications and experience Superannuation similar to F S S U in Great Britain will be provided

Conditions of appointment may be obtained from the Secretary Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 Applications close in Australia and London on November 30, 1963

COMMONWEALTH AGRICULTURAL BUREAUX

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(Faculty of Technology in the University of Manchester)

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(SECOND ADVERTISEMENT)

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Applications (three copies), with curriculum vitae and the names of two referees, should be sent to Dr P H Tuft, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, not later than October 31, 1963

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Application forms and further details may be obtained from the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 Applications close on November 30, 1963, with H McCredie, Secretary, Rothmans University Endowment Fund

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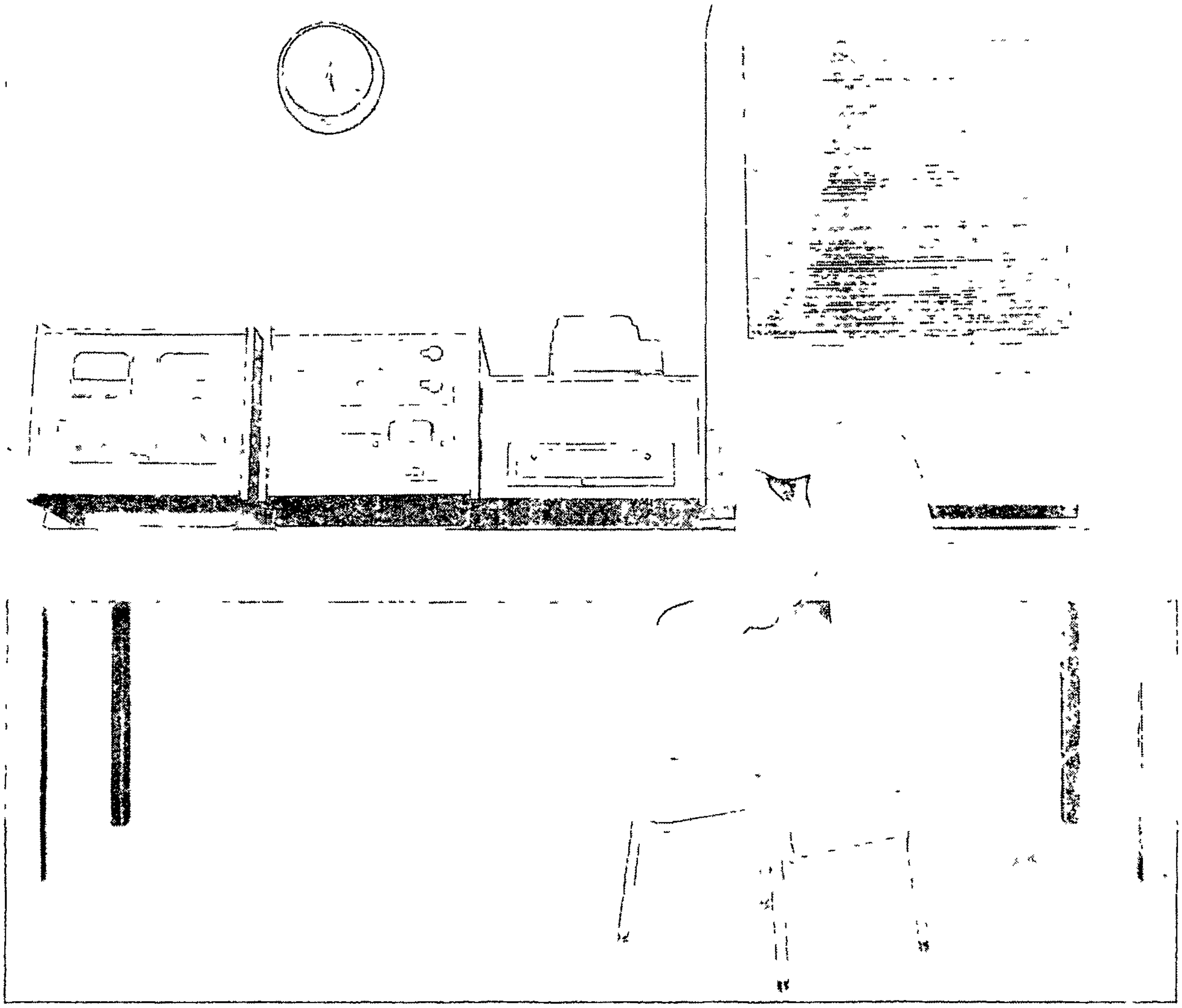

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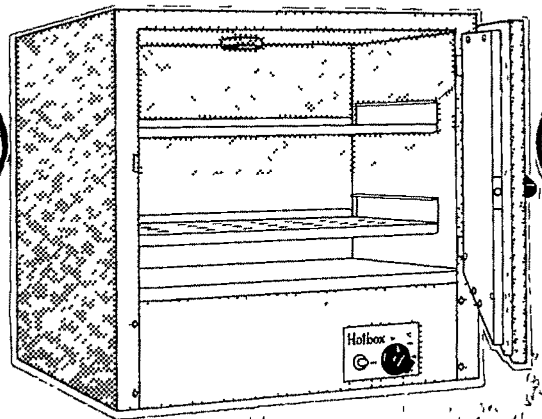

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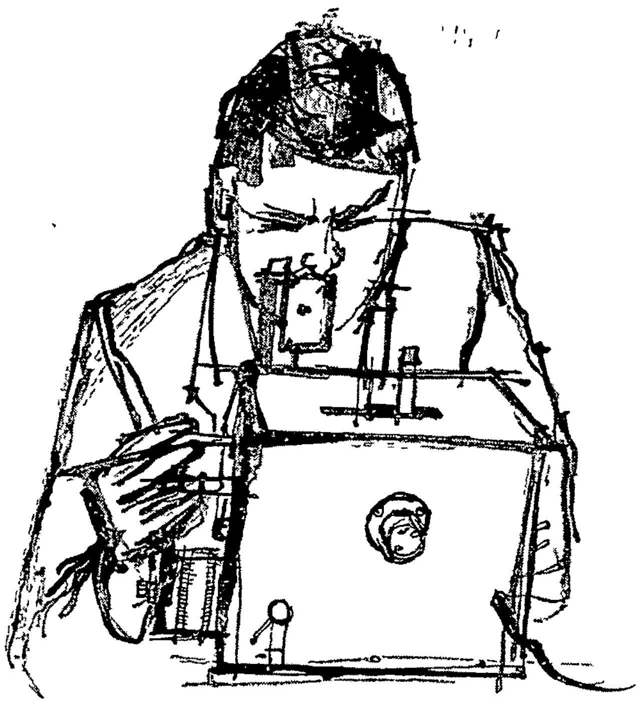

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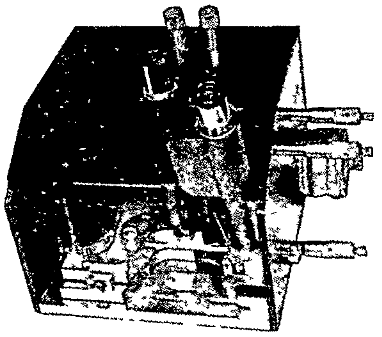

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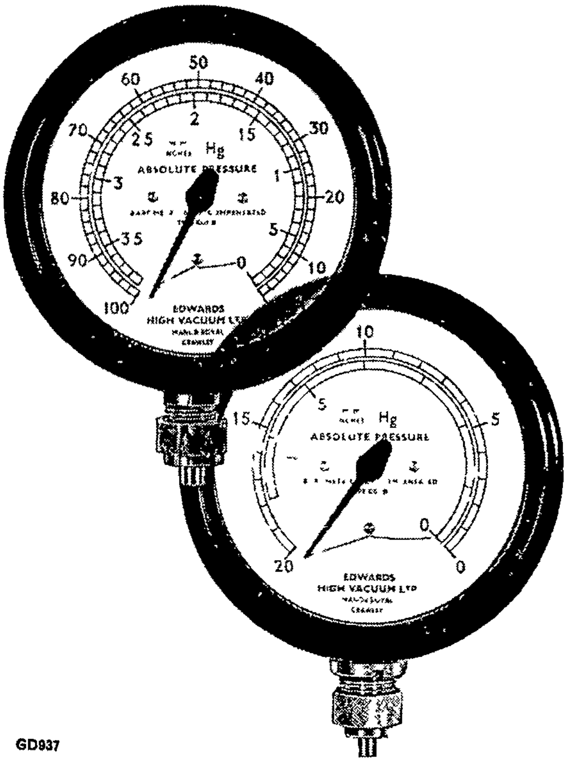
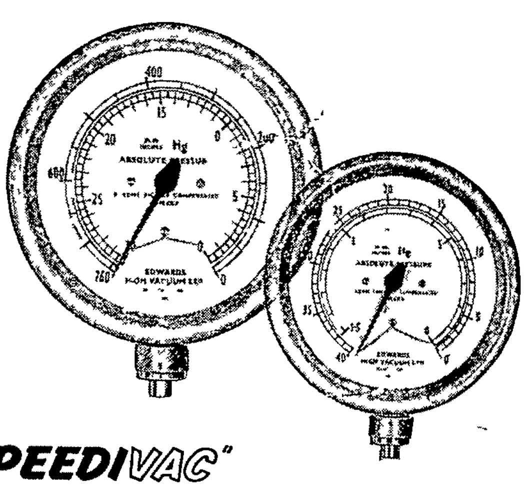

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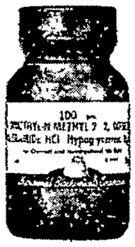

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(¹) J E Rall J Roche R Michel O Michel S Varonne Biochem Biophys Acta 62 622 (1962) (²) A L Lehninger B L Ray M Schneider J Biophys Biochem Cytol 5 97 (1959) (³) A L Lehninger B. L Ray Biochem Biophys Acta 26 643 (1957) (⁴) D F Tapley J Biol Chem 222 325 (1956) (⁵) A. L Lehninger, ibid 234 2187 (1959)

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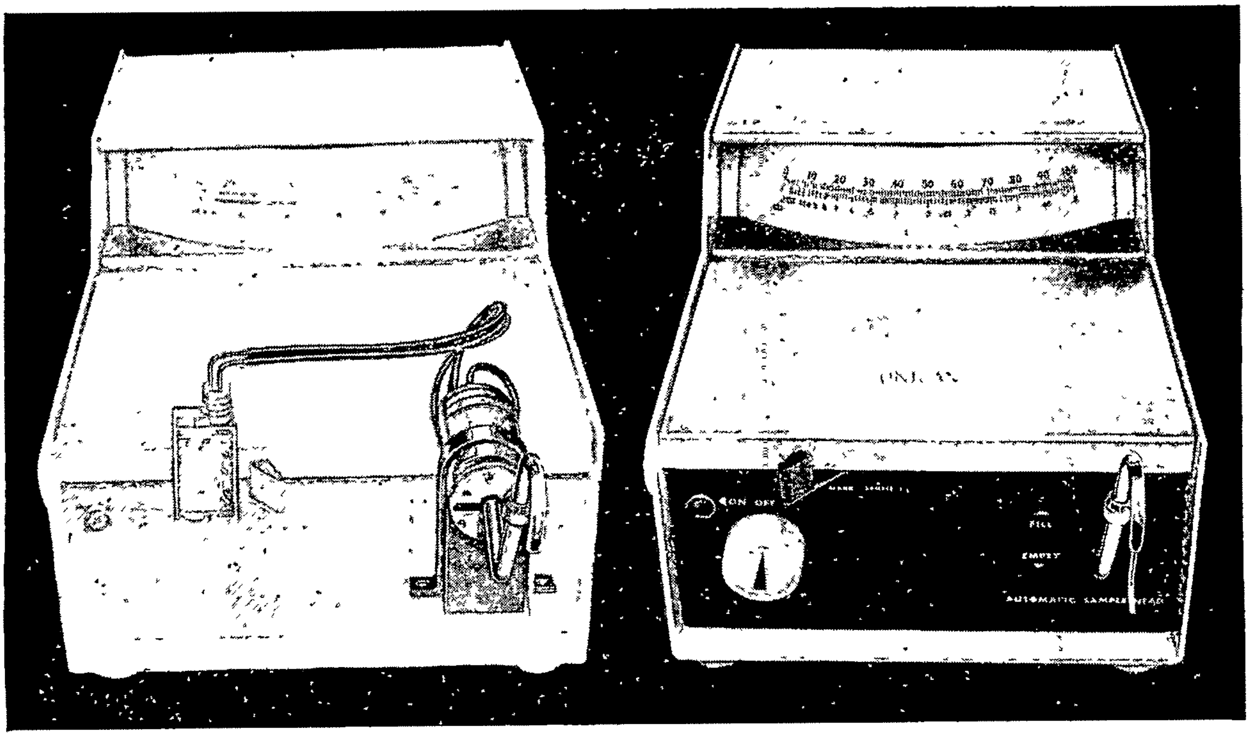

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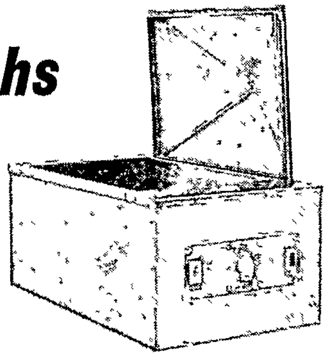

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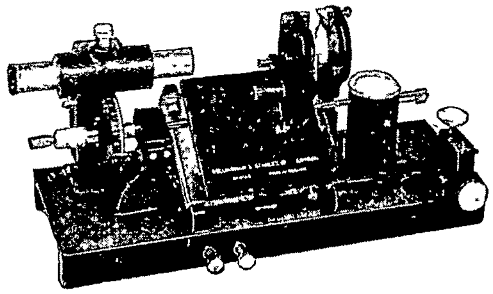

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A MODEL FOR TECHNICAL ASSISTANCE

MR J G HARRAR'S review, as president of the Rockefeller Foundation, in the annual report of the Foundation, 1962*, is of special interest both for its survey of the philosophy that has guided the Foundation during the fifty years of activity which were completed in the spring of 1963 and its attempt to assess the function of the Foundation in the years ahead This survey of its activities during the past half-century directs attention to the Foundation's efforts in advancing human knowledge and its application year by year to those ills and problems which limit the freedom and satisfactions of mankind Mr Harrar shows clearly how the Foundation's early efforts to deal with problems of public health on an international scale led first to programmes for developing schools of public health, to training a growing body of physicians, nurses and technicians to practise in this field, and then to a more general entry into the field of medical education

Even at this stage of his retrospect, it is apparent that the Rockefeller Foundation has set a pattern on which, a generation or more later, the soundest efforts in the field of technical assistance have been modelled This is no less true of its work in other fields, such as the national sciences or the social sciences, both of which the Foundation created Divisions in 1928 In this context the programme for the agricultural sciences which arose in 1943 out of the Foundation's increasing concern with human nutrition and food production gains special interest The story of the correction in the past twenty years of the deficient position of Mexico with respect to its basic foods is not only fascinating in itself, but also of profound significance in relation to the problem of feeding the world's expanding population to-day It corroborates all that Prof. Martin Jones said at Aberdeen on August 30, in his address to Section M (Agriculture) of the British Association, on the need to help and educate the peoples in every country to develop their own resources for the production of their basic needs for food

In 1929 the Rockefeller Foundation also established a Division of Humanities, the programme for which included the study of history as important for background and perspective in contemporary thinking and in interpreting change and continuities in the world to-day it was one of the first to introduce reforms in serving the needs of library development From all these activities the Foundation has drawn a rich body of experience as a basis for judgments on the lines of future policy Much of this experience is highly significant for others who are concerned with technical co-operation in the developing nations, and it is notable that the careful and intensive examination of this experience by the Foundation which has marked the year 1962 has led the Foundation to decide to place increasing emphasis on the ecological approach to human problems

This, in Mr Harrar's judgment, is the fundamental decision taken during the year, and it is in line with a marked emphasis on the same ecological approach which characterized discussions at the recent meeting of the British Association at Aberdeen The note was struck by Sir Eric Ashby in respect of higher education in his presidential address It was implicit in that on land use in the Scottish uplands in joint sessions between the Sections for Agriculture, Geography, Botany and Forestry, as well as in others arranged by Section N (Sociology) and on planning jointly between Section F (Economics) and Section X (General) Moreover, in Section D (Zoology), Dr F Fraser Darling's presidential address on the unity of ecology was followed by a joint discussion of the subject with Section K (Botany)

The concept that a given society, whatever its stage of development, stands in intimate relation with all aspects of its environment and that a change in one necessarily affects the others now commands wide acceptance outside the political world Even there the Beeching proposals and the reaction to them have forced the politician to recognize the value of an ecological approach, which can sometimes assist advance simultaneously on several fronts and avoid some of the internal torques and tensions induced by rapid development in one sector and slow advance in others Without such an approach, there may be little hope of meeting successfully the immeasurably greater challenges of to-day and the future While it could scarcely be said that in discussing British national parks in his presidential address to Section E (Geography) at the Aberdeen meeting, Prof H C Darby was concerned with ecology, the experience with national parks in Britain is, in fact, as emphatic a demonstration of the need for an ecological approach to some problems as is the experience and functioning of the Nature reserves themselves

The process of consolidation which the Rockefeller Foundation began in 1962 does not substantially change its pattern of operation, though it brings together in a more precisely focused effort activities which were hitherto separate Nor does it affect the abiding conclusion, drawn from the Foundation's experience and which Mr Harrar reiterates in this review, that the best investment a private philanthropy can make is in the development of the talented and promising individual within an area of opportunity Essentially all the progress in which the Foundation can claim to have had some role has derived from the energy, ability and dedication of those who have been assisted through contributions to their training or professional activity Now through this policy there has been created throughout much of the world a corps of competent individuals who can begin to take increasing responsibility for building up local projects and institutions

Mr Harrar does not suggest that help from the Rockefeller Foundation has been other than modest in proportion to the total needed, but the objective in each instance has been to provide support at critical moments and in such a manner as to assume maximum leverage towards progress Hope for the future, he believes depends on success in achieving educational and professional opportunities for an ever-increasing number of men and women with the intellectual capacity and motivation to contribute to social progress To this end the Foundation expects to continue its programme of fellowships, scholarships and study awards, to assist the intellectual development and learning of individuals, under which more than 16,000 scientists and scholars have already benefited At the same time, the pattern of the training awards will be integrated with other types of work in an over-all ecological approach

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* The Rockefeller Foundation President's Review, 1962 Pp v+87 (New York The Rockefeller Foundation, 1962) See also p 121 of this issue of *Nature*

To-day Mr Harrar believes the training process has been carried to a point at which it is readily possible to identify opportunities for concentration A half-century of effort in many areas by the Foundation and other agencies has now trained individuals over a wide spectrum of needed skills to staff national institutions and programmes As emerging indigenous institutions accept increasing responsibility for training their nationals, outside aid can be concentrated on selected institutions rather than on individuals, to help build them into positions of excellence and permanence in which they can satisfy ever larger proportions of the national educational needs and aims Ultimately, some of these institutions can be expected to extend their influence beyond national boundaries to become regional centres

Mr Harrar does not discuss the political presuppositions of stability and order implicit in such a policy, but, in the climate of to-day, this policy is clearly wise In accordance with it, the Foundation decided in 1962 to centre its concern for institutional development primarily on selected universities in the less-developed countries By thus narrowing its objectives and utilizing an ecological approach for its attainment, the Rockefeller Foundation believes it can promote a better relation between the universities and their environments In many less-developed countries there is, for example, a serious lack of balance between the ability of the institutions to serve society and the demands made on them to produce the trained and competent specialists required in a host of fields.

This lack of balance may be due to various causes Sometimes it is simply one of size the universities have neither the student capacity nor the staff to handle the training of the necessary personnel Elsewhere it may be due to the tardiness of the universities, often among the most conservative institutions in the social structure, to adapt the courses they offer to the manifold kinds of skills and competence demanded by complex societies Yet again, relatively sudden influxes of large numbers of students have so diluted the authority of the university and academic quality that the very purpose and function of the university have been weakened

The Rockefeller Foundation fully recognizes that these different situations require handling in different ways, nor does it contemplate any attempt to transplant the American system Quite apart from the increasing extent to which the Governments of the developing countries now recognize the importance of bringing their university systems into consonance with present-day requirements, the form which any satisfactory system takes must develop from within the universities themselves and from the societies which support them What the Rockefeller Foundation seeks to do is rather to assist those centres of higher education which are already emerging as models which others can emulate, and, by thus building on established points of strength, help to raise the level and tone of the institution as a whole

This new policy is as firmly in line with the basic objectives of the Foundation as the philosophy behind it is in harmony with that which inspired the concentration on investment in the development of the talented and promising individual All nations need the strength which comes from stable institutions of higher learning to serve the intellectual and other needs of the population, and where such institutions do not provide a growing body of alumni contributing uniquely to national economic and social stability, democratic institutions are unlikely to function Further, without in any way repudiating the principle of the international interchange of scientists and scholars, which is always rewarding, the Foundation is now dedicated to the principle that a gradual, steady shift to education at home is the solution towards which the developing countries should progress

Besides this establishment of a limited partnership with several universities aimed at overall development of the university pattern and process, the Rockefeller Foundation had, even earlier than 1962, fostered the establishment of an international research institute working to increase production of a vitally needed food in a highly important part of the world The International Rice Research Institute is part of this programme of institutional development and is expected also to contribute to the development of a university and ultimately to become part of it Support for this programme will be given both directly through the reinforcement of faculty, provision of equipment and library materials and, indirectly, through training awards for junior faculty and the support of visiting specialists In so far as it is able, the Foundation will seek to promote a balanced university development, aiding the whole university and not selected departments or schools separately

It is obvious that the re-orientation of the work of the Rockefeller Foundation is of profound significance for those concerned with technical assistance generally, and this latest report is one that will doubtless be closely studied by the Secretary for Technical Co-operation, Mr Robert Carr Moreover, not only does the experience of the Foundation bear closely on the problems that Britain is now encountering in expanding her educational assistance overseas but also it is relevant in the field of research It should not be overlooked that this record of half-a-century's effort deals with the closure of projects and transfer of staff to new areas. It is not simply a record of new ventures, and Mr Carr might well ponder the implications of this record for Britain's own overseas research effort for which his Department is now responsible, though so far silent

What stands out above all from this record is the imagination and insight with which the private institution has used its opportunities and the high sense of responsibility with which it has formulated and executed its programmes While a private foundation cannot command the resources which are at the disposal of a Government, it is sometimes free from the limitations which are inevitable in Government effort and especially in dealing with other countries Whatever the difference in the extent of resources and in the manner in which programmes are executed, the objectives of Government policy and of Foundation policy can well be identical The improvement of the communication of knowledge and its application, the infusing of cultural and moral values much more pervasively through the intricate fabric of contemporary society, the encouragement of creative activity, whether by assisting the talented and promising individual, or by helping the development of new patterns and institutions which will sustain such work—these are no monopoly of the Rockefeller Foundation or any other private institution The latest review by the president of the Foundation is especially a challenge to Britain to match the new opportunities which confront her in this changing world with vision and resource and generosity not inferior to those which in former generations were brought to the service of Empire and Commonwealth

CHEMISTRY AND THE DEVELOPMENT OF MEDICINE

Chemistry in the Service of Medicine

Edited by F N L. Poynter Pp 207 (London Pitman Medical Publishing Co , Ltd , 1963) 25*s*.

THIS book, *Chemistry in the Service of Medicine*, consists of twelve papers, which were read at the second British Congress on the History of Medicine and Pharmacy, organized by the Faculty of the History of Medicine and Pharmacy of the Worshipful Society of Apothecaries, and held in London during September 1961 These papers are authoritative historical reviews and complement each other, hence a clear picture is thereby obtained of a rapidly developing situation in which both chemistry and medicine stimulate each other and profit by each other's advances Many of the distinguished contributors have made significant advances in their respective fields, such now being the subject of historical review It is amply demonstrated in this publication that history is not simply a record of things of the past but intimately connected with the present which it helps to shape

The objectives of the Congress are carefully explained by Dr W S C Copeman, chairman of the Faculty, in a short preface The introduction is by Sir Alexander (now Lord) Todd, who traces the growth of the relationship of science, in particular chemistry, in the development of medicine He points out that medicine did not really begin to develop to any great extent until there had been a rapid expansion in our knowledge of the basic sciences He speculates as to the future developments in the inter-relationships of medicine and the basic sciences

The first paper, by Dr A G Debus, is entitled "Paracelsian Doctrine in English Medicine", and the second, by Dr F W Gibbs, "Boerhaave and the Place of Chemistry in Medicine". Both these papers deal with the early history of medicine The third paper, by Prof D McKie, is entitled "Chemistry in the Service of Medicine 1660–1800" In this, the growth of chemistry during the period is described It is shown that during this period chemistry invaded the territory of physiology and entered the service of medicine but as an independent subject The fourth paper is by Prof F G Young and is entitled "The Rise of Biochemistry between 1800 and 1900" The development of biochemistry during this period is described, but it is pointed out that although there were many distinguished British chemists, they took little or no interest in the application of their chemical knowledge to the solution of biological problems, which was in markedc ontrast to many of their illustrious Continental colleagues This state of affairs is discussed in relation to the educational system then existing in Britain Reference is also made to the lack of encouragement given to the late Sir Frederick Gowland Hopkins when he expressed his intention to apply chemistry to the study of biological processes

The fifth paper, by Prof W D M Paton, is entitled "The Early Days of Pharmacology" The distinction between *materia medica*, therapeutics and pharmacology is explained and the development of the subject from the beginning of the nineteenth century is reviewed, but Prof Paton estimates that the subject has shown its greatest degree of expansion during and following the period 1930–40 The inter-relationships existing between chemistry and pharmacology with regard to their respective advances are discussed In the sixth paper, Dr W D Foster traces the development of chemical pathology from its beginnings in the seventeenth century until its establishment as an essential part of modern medicine about the year 1925 Dr Foster quotes an authority, who, in 1924, stated that the use of chemical pathology in clinical medicine was limited to the investigation of glycosuria, renal disease and disturbances associated with acid-base balance The seventh paper is by Sir Charles Dodds and is entitled "Chemistry and Endocrinology" The historical development of endocrinology in relation to chemistry is described Considering the multitude of chemical compounds produced by such an organ as the adrenal gland, Sir Charles Dodds suggests that chemistry owes a considerable debt to medicine and in particular to endocrinology The eighth paper, entitled "The Contribution of Chemistry to Cancer Research", is by Prof A Haddow Prof Haddow considers that the main contribution of chemistry to cancer research is along fundamental lines Chemical constitution and carcinogenesis are discussed.

The ninth paper is by Dr T F Macrae and is entitled "Chemistry and Nutrition" The development of our knowledge concerning the availability of food, food additives, vitamins, proteins, carbohydrates and fats is discussed The tenth paper is entitled "Chemistry and Genetics" and is by Prof D G Catcheside, who discusses the development of the subject Prof Catcheside points out that the history of the advances in the chemistry of genetics has been largely influenced by the rapprochement between genetics and biochemistry Recent advances owe much to the newer techniques of chromatography, electrophoresis and the use of isotopes The eleventh paper, entitled "From Antiseptics to Antibiotics", is by Dr W A R Thomson, who concludes his paper by suggesting that a careful co-ordination of the two complementary methods of attack on micro-organisms—antibiotics and immuno-therapy—is necessary The twelfth and final paper is entitled "Origin and Rise of Synthetic Drugs" and is by Dr F L Rose Drugs used in the treatment of malaria and sleeping sickness and the sulphonamide drugs are some of the subjects discussed Reference is also made to the use of organic substances capable of producing a colorimetric reaction in the study of the fine detail of cell structure

Most of the papers are followed by a list of references of great historical significance S L TOMPSETT

HIGH-ENERGY PHYSICS

Proceedings of the 1962 International Conference on High-Energy Physics

At CERN, Geneva, 4th–11th July 1962 Edited by J Prentki (Sponsored by the International Union of Pure and Applied Physics) Pp xxiv + 950 + 2 plates (Geneva CERN—European Organization for Nuclear Research 1962) 60 Swiss francs

THE first seven pioneering conferences on "High-energy Physics" were held annually during 1951–57 at the University of Rochester Since 1958, however, the conferences have been sponsored by the Commission on High-energy Physics of the International Union of Pure and Applied Physics and the successive annual conferences have been held at CERN, Kiev and Rochester The conferences are now to be held biennially, and the 1962 conference was held during July 4–11 at CERN. The *Proceedings* have now been published and consist of a large volume of 950 pages, recording the substance of 208 papers presented to the conference together with the discussions on them, which were organized in twenty parallel, one special and thirteen plenary sessions There are 810 illustrations and drawings, including two colour plates The papers recorded represent only a selection of the total number presented to the conference, and in order to speed up the process of publication the editing and checking were reduced to a minimum It is with considerable pride that the Scientific Information Service of CERN, which was responsible for the publication, was able to announce the publication of the *Proceedings* in record time on October 25, just fifteen weeks after the conclusion of the conference

The *Proceedings* follow the general plan of the conference, which was divided effectively into two parts. The first three days, July 4–6, were for the specialists and consisted of parallel sessions devoted to pion and nucleon physics, strange particle physics, cosmic-ray and high-energy physics, and the theory of elementary particles Following the week-end, which was free of scheduled lectures, there were three days of plenary sessions at which the results of the previous sessions were intended to be summarized and put into perspective for the conference as a whole by 'rapporteurs', one for each subject division Naturally, with so much information reported and discussed, the 'rapporteurs' had no easy task and their lectures to the plenary sessions as given in the *Proceedings* show that many quite openly admitted their inability to summarize or survey the work presented at the conference They dealt generally with one or other aspects of the particular topic assigned to them Nevertheless, with the whole of the *Proceedings* available to the reader there should be no difficulty in finding out the progress reported in any special field

Prof V F Weisskopf, director-general of CERN, gave the concluding address and in his brief remarks surveyed in a masterly and clear fashion the progress achieved both experimentally and theoretically in high-energy physics during the years between 1960 and 1962 Many new facts had emerged Results obtained from scattering experiments of nucleons by nucleons or pions by nucleons show that the nucleon at very high energies gets more diffuse and larger so far as scattering with strongly interacting particles is concerned It implies the absence of a core The various groups of isobars, in particular the baryons and their excited states now seem to fit in well with the simple quantum numbers, but it is still unknown what this means There are indications, too, that the list of the excited states is limited, unlike the excited states of the hydrogen atom The discovery of the existence of two neutrinos has opened up a new field of research, neutrino physics, and a special session of the conference was devoted to the neutrino experiment, which was described by M Schwartz Members of Columbia University, New York, and of the Brookhaven National Laboratory, conducted the experiment in which it demonstrated that the neutrons emitted in π–μ decay differs from that emitted in nuclear β decay The increasing amount of evidence that the muon is equal to the electron in all its properties except that of mass, and other details about weak interactions, such as whether the $\Delta Q = \Delta S$ rule holds, or whether the isotopic spin changes by more than $\frac{1}{2}$, leave, however, weak interaction theory still much where it was in 1960, in spite of more than 300 papers on the subject during the two years between the conferences This is the conclusion arrived at by L B Okun of the Institute of Theoretical and Experimental Physics, Moscow, who acted as 'rapporteur' for the sessions on weak interactions (theoretical) His report is followed by a bibliography for the period January 1961–May 1962 in which are listed some 320 papers.

Prof Weisskopf concludes with the remark that we do not understand the meaning of what we observe "We are trying, not without success, to introduce analogies and ways of speaking It could be that all we have found follows from field theory The theoretical situation is problematical but full of interesting ideas The experimental situation is very much better than anyone would have guessed two years ago The world within the nucleon is much richer and much more interesting than we ever expected "

The *Proceedings* lists 357 scientists in addition to those from CERN as having attended the conference and altogether some 760 people participated in the conference either as authors of papers or in the discussions At the first conference in 1951 there were only about 50 participants It is difficult to forecast what will be the number at the next conference in 1964 at the Joint Institute for Nuclear Research, in Dubno, and whether the progress to be reported will be significant and worthy of the very large effort now devoted to high-energy physics. The next *Proceedings* will doubtless have to be published in two volumes S WEINTROUB

TRANSISTOR COMPENDIUM

Der Transistor

Physikalische und technische Grundlagen Von Dr H Salow, Prof Dr H Beneking, Dr H Kromer und Dr W v Munch (Technische Physik in Einzeldarstellungen, Band 15) Pp viii+426 (Berlin, Gottingen, Heidelberg Springer-Verlag, Munchen J F Bergmann, 1963) Dm 82

THIS book is in effect a collection of four monographs, each of which was written by one of the authors, as follows (1) "Physical Background of Transistors" (Kromer, pp. 73); (2) "Technological Background of Transistors" (Salow, pp 137), (3) "General Circuit Theory of Transistors" (Beneking, pp 139); (4) "Physical and Technical Properties of Transistors" (von Munch, pp 64)

Part 1 starts immediately with the electron energy band model, then passes rapidly through carrier statistics and current transport phenomena to the theory of the plane *p-n* junction diode and *p-n-p* transistor In both cases, as is to be expected, the author deals with both the 'diffusion' and 'drift' type of device, although the treatment is necessarily condensed Finally, some of the ways in which the behaviour of practical transistors depart from the simple low-injection-level, one-dimensional theory are discussed

In the second part, there is a relatively detailed account of the methods of growing cystals of germanium and silicon and of the various etchants which are used for surface treatment there are some beautiful photographs of etch pits This is followed by an account of the various processes by which transistors of different geometrical construction and impurity profile are made, the reasons for the different geometries being given It is arguable, however, whether or not too much emphasis has been given to this part Although a considerable amount of detail has been presented, it is obviously not enough to enable an uninitiated reader either to grow a crystal or to make a device, it would therefore seem better to err on the side of giving too little rather than too much In Part 3 the author deals with the usual topics of bias stabilization, small-signal parameters, and equivalent circuits (with a bias to the hybrid-π) and then proceeds to examine amplifiers, with particular reference to stability, and oscillators Finally, switching properties are considered

The last part is of limited scope, dealing in the main with circuits for the measurement of the h-parameters in the low-frequency and high-frequency bands, and the effect of temperature on static characteristics There is a brief reference to the measurement of noise at low frequencies The section is concluded, out of character, with a series of short descriptions of 'other junction devices'

In general it can be said that each author has contributed something useful to the whole, but that the work lacks cohesion and the emphasis is not evenly distributed One would like to have read more of avalanche breakdown and noise, which are very cursorily dealt with, and less of crystal growing Part 1 could have been expanded considerably with advantage None the less, the book is not without value as it stands Although it cannot be regarded as a useful introductory book to the serious student of transistors, it will no doubt be a very useful adjunct to such a book—for readers of German F J HYDE

TELEMETRY

Telemetry

By R E Young (Temple Press Monographs on Rockets and Missiles) Pp vii+78 (London Temple Press Books, Ltd, 1963) 17s 6d

THE cover note claims that this book sets out to give a comprehensive treatment of the subject of telemetering in missiles which will be suitable both for students and those engaged in the testing and manufacture of missiles I am afraid that both groups will be disappointed

In particular, I think that the student would, for his own good, be well advised not to read this book because of the vague and often incorrect statements it contains It consists of five chapters which deal with a variety of subjects, including some very elementary standard proofs concerning transmission lines, aerials and receivers

Unfortunately this elementary mathematics has suffered in some instances from printer's errors or incorrect extracting from the original reference Mistakes like this can only confuse the student so that when the author adds in parenthesis "see any standard text-book on transmission lines" this is undoubtedly a sound piece of advice

Modulation methods, a most important subject in radio telemetering, are dismissed in very general terms in only three pages, no mention being made of wideband improvement and the significance of modulation index in frequency modulation systems The emphasis on high-speed oscillography and the treatment of data handling in general are not in line with modern practice nor are the arguments the author uses in discussing digital telemetry particularly valid

Many of the facts and figures quoted as typical of present practice are wrong For example, when describing the use of a piezo-electric material in vibration transducers the author states that barium titanate is suitable in temperature environments up to 250° C without any deterioration of its properties This temperature is, of course, above the Curie point of the materials

It is impossible to detail in a short review many of the inaccuracies regarding limiters, discriminators, commutation and the generation of telemetering waveforms Perhaps the greatest single criticism is the fact that after a number of vague references throughout the book the reader is left with no clear and concise picture of typical British telemetering systems Since this is probably the first book to be published in Britain on this subject it would have been a good opportunity to record the contributions made to the art by scientists and engineers in the United Kingdom and Commonwealth

Regrettably it pales to insignificance when compared with American publications on a similar subject

M A PERRY

CARCINOMA OF THE UTERINE CERVIX

Het Experimentele Carcinoma Colli Uteri

(English Summary—Résumé Français) Door Dr Michel Thiery Pp 542 (Brussels Arscia Uitgaven N V, Presses Académiques Européennes S C, 1963) n p

IN this research monograph, the experimental induction of carcinoma of the uterine cervix is described in considerable detail The anatomical and methodological information will be very valuable to experimentalists in the field, and some of the observations on the progression of cellular atypia and carcinoma *in situ* to frank invasive carcinoma will provide ammunition for those who now press for mass screening of the human female population by cervical smears or other methods

The first three chapters are devoted to descriptions of the experimental methods used and of the anatomy of the test animals The favoured technique involved the repeated painting of the uterine portio with a suspension of 3,4-benzpyrene in acetone under direct vision Mice of several different strains and hamsters proved more or less responsive to this method of tumour induction, rats and guinea pigs were resistant Mice were used in most of the investigations

Chapters 4 and 5 are concerned with the histopathological features of the lesions observed in treated animals hyperkeratosis, epithelial dysplasia, carcinoma *in situ*, and squamous cell carcinoma The close parallelism between these induced lesions and 'spontaneous' lesions of the human cervix is stressed Particular attention is directed to the histopathological diagnosis of early stromal invasion, the features of the subepithelial connective tissue layer which takes the place of the basement membrane in the mouse, and to the squamo-columnar junction Cyclical variations in the vaginal epithelium continued during treatment though their timing was frequently disturbed The majority of tumours were squamous cell carcinomas, but one-fifth of them were mucoepidermoid in nature Finally, modes of local spread and metastasis are considered and the occurrence of associated pathological conditions reported

Chapter 6 is perhaps the most interesting and valuable of the whole book it is concerned with the histochemical characteristics of normal and pathological cervicovaginal epithelium Both glycogen content and the presence of periodic acid–Schiff-positive mucus showed cyclical variations The former does not aid in distinguishing different grades of preneoplasia or neoplasia, it only provides evidence of the degree of cellular differentiation of the lesion Differences in the histochemical patterns of –SH– and –SS– group reactivity were observed between normal and pathological epithelium, but differences between different lesions were not sufficiently marked or constant to be of value in differential diagnosis

The DNA content of the basal nuclei of squamous epithelium was found normally to reach a maximum during œstrus, keratinization was associated with a decrease in DNA content In the abnormal hyperkeratotic epithelium of treated animals, however, the DNA-level was as high as for œstrus epithelium, even in sprayed animals In general, there seemed to be a positive correlation between the DNA value and the position of the lesion on the progressive path between pre-neoplasia and neoplasia, and the author concluded that Feulgen-histophotometry might be a valuable diagnostic tool From a study of the levels of sixteen different enzymes, the author inferred that enzyme histochemical procedures can be successfully applied to the identification of specific cell-types and to the differentiation of various pathological lesions In particular, he was impressed by the fact that squamous cell carcinomas showed a very high 5-nucleotidase activity which made their detection in lesions at the stage of early stromal invasion a relatively simple matter

In the penultimate chapter the evidence for the precancerous nature of various lesions is reviewed, and the *tempo* of their progression to neoplasia discussed Finally, a comparison is made between the experimental tumours and those arising in the human cervix Despite anatomical and genetic differences, the author concludes that "on the whole the parallelism is truly striking For this reason the experimental tumour might eventually offer new opportunities for the study of certain aspects of cervical carcinoma in man"

The book is well written, well illustrated, well documented and well produced, its greatest limitation for most workers is that it is written in the Dutch language

F J C ROE

CHANGES OF CLIMATE

Changes of Climate

Proceedings of the Rome Symposium organized by Unesco and the World Meteorological Organization (Arid Zone Research—XX) Pp xii+488 (Paris Unesco, London H M Stationery Office, 1963) 28 francs, 40*s* , 8 dollars

THE study of climatic change is an attempt to reduce into some semblance of order changes in the Earth's climate on diverse scales in both space and time It must consider the operation and effect of a large number of geophysical mechanisms, and involves the collation of results from many different scientific disciplines It is an open secret that, perhaps because the subject is so complex, specious arguments have tended to go unchallenged and occasional dubious conclusions have found their way into text-books of climatology A forum was obviously needed where ideas could be presented and criticized the October 1961 symposium in Rome provided this The volume under review contains the text of forty-five papers presented at the symposium together with a brief summary of the ensuing discussions.

Throughout the volume a laudable concern is shown for the physical significance of results, particularly those inferred from analyses of time series There is a distinct tendency to adopt a more precise approach to the subject than hitherto Also it should be noted that the problem considered was to assess whether any significant changes in climate can be resolved from available data and to understand the mechanisms involved, it was not to attempt any prediction of future trends The papers are divided into four main sections

The first section deals with changes during the period of meteorological records Most of these papers try to show the way in which the evidence falls into a pattern in which the physical significance depends on the combined results of several different variables However, even when distinct trends can be firmly established there was difficulty in interpreting these in terms of what had happened to the atmospheric circulation Section 2 is devoted to changes during late geological and early historical records The net result here is to show how various disciplines can combine to pose questions which meteorologists, without a quantitative theory of global climate, find great difficulty in answering Occasionally in the first two sections there is a tendency to consider mean atmospheric and mean oceanic circulations as though they are complete physical entities in themselves rather than part of a statistical description of highly fluctuating flow-patterns This is particularly noticeable when cause and effect are discussed the importance of deviations from the average flow (eddies) were sometimes forgotten There are obviously many interrelated mechanisms going on that are extremely difficult to assess adequately in a qualitative way, and one is left with the feeling that a more precise treatment along the lines indicated by the papers in the next section is urgently required

Theories of changes of climate are the subjects of Section 3 Here there is a call for an attempt to produce a rough model which can represent realistic seasonal variations, later the model might be used to discriminate between likely and unlikely hypotheses A pointer to the probable line of future attack is given by two papers using a quantitative approach and extremely simple models to describe the effect of a hypothetical increase in solar constant, and to test the validity of possible mechanisms of climatic change Simple numerical techniques might well be profitably applied to some of the other theories advanced Section 4 concerns the significance of changes of climate —on vegetable cover, patterns of land use, geomorphological features, etc The importance of *variability* of rainfall to agriculture in semi-arid regions is emphasized, and the final paper stresses the practical implications of statistically insignificant changes in climate when a threshold, peculiar to the area and the crop, is crossed

The whole publication is excellently produced in a paper-covered volume, but a summary of each paper in English as well as in French (vice versa for some papers) would have improved the presentation For nearly 500 pages of valuable material the price is extremely low

G B Tucker

RUSSIAN APPROACH TO FISH ECOLOGY

The Ecology of Fishes

By G V Nikolsky Translated from the Russian by L Birkett Pp xv+352 (London Academic Press, Inc (London), Ltd , New York Academic Press, Inc , 1963) 75*s*

THIS most stimulating book is especially welcome for three reasons First, no other book covers the same ground, and it should be a very useful reference work for both academic biologists and those concerned with fishery problems Secondly, this book and English translation make the great contribution of Soviet ichthyologists and ecologists available to the many who cannot read Russian Thirdly, and in the long run perhaps most important, this book is to be welcomed for its refreshingly unifying approach to biology

Throughout the book one is aware of Prof Nikolsky's appreciation of the unity of the organism and environment, and that it is the adaptive interactions to particular elements in the environment which ensure the development and existence both of the organism and of the species It is made quite clear that ecological studies must be based on knowledge of the structure and functions of the organs, and that such studies, at all stages of the life-cycle, make the only basis for rational fishery development

The book is divided into three parts In the first, Nikolsky considers "Interrelationships between Fishes and their Abiotic and Biotic Environment" It is here that the reader is most conscious of Nikolsky's continual awareness of the interchange between the organism and all the many facets of the environment proceeding simultaneously some factors having greater effect at one stage of the life-cycle and others at another, some having greater significance at one stage of the evolution of the species and others at another, a continual flux and web, with continual interaction of the different factors This interaction so often appears to be forgotten by those designing ecological experiments

The abiotic relationships considered are the density and pressure of water and adaptive changes of the swim bladders of different fish groups, water salinity and osmoregulatory mechanisms, gases in solution and adaptations for aerial respiration, the effects of temperature, light, sound, electrical and electromagnetic forces, and the fishes' reactions to bottom deposits and water movements There is also mention of work on X-rays and radioactivity on fish, very relevant to the modern problem of dumping atomic waste From experience in high latitudes, attention is directed to the many ways in which ice affects fish

The discussion on biotic relationships begins with those which are intraspecific This includes a useful summary of work on shoaling in fishes, of great importance for the design of fishing gear When discussing interspecific relationships, Nikolsky comments on the differences in faunistic complexes at different latitudes, the more intensive predator–prey relationships in lower latitudes with corresponding increase in the development of protective adaptations (such as great fecundity, care of young, armour), and that lower-latitude fish tend to be more stenophagic The interrelationships of fishes and other organisms are then treated group by group, from bacteria, viruses and plants, and from Protozoa to Mammalia, whether these other organisms be food or enemies of the fishes.

The second part of the book, entitled "Fundamental Links in the Life Cycles of Fishes", is a series of discussions on reproduction and development, size, growth and age of fishes and ways of estimating these, population dynamics, migrations, overwintering and hibernation behaviour, and feeding relationships There is inevitably some repetition of Part 1

Part 3 is much shorter, and a discussion on the significance of fishes in the life of mankind and the biological basis of a rational fishery Here it is emphasized that fundamental knowledge of the ecology and behaviour of the fish, at all stages of the life-cycle, is the only rational basis for fishery development One point raised is that whereas in temperate waters much can be done to manipulate age classes of fish to give highest sustained yields, in tropical waters production is unlikely to be increased by this method, as the life-cycles of the fishes are so much shorter and age groups represented in the catchable population fewer

To compress the whole 'ecology of fishes' into some 300 pages is bound to lead to generalizations which are rather sweeping and thereby lose in accuracy, and some of the parts concerning fishes outside the U S S R have to be read with discretion A rather surprising omission when discussing the role of cœlenterates in the lives of fishes is that there is no mention of corals, and the whole groups of fishes which gain food and shelter from them References to work in the tropics, for example, are rather out of date One particularly puzzling point, to which no reference is given, is the "intestinal incubation of eggs in *Tachysurus barbus*" figured Could these possibly have swallowed their eggs when disturbed, as fishes practising oral incubation so often do ?

One is very grateful to the translator for making this book available in English, but the impression is given that he is not a zoologist The choice of "abiotic" for 'physicochemical factors' becomes misleading in some contexts, and "upstream" is used when 'higher up in the water' is probably meant Curious punctuation occasionally gives misleading ideas, and one could wish that the English names used for the fish were the common ones, as the names used are sometimes confusing (The translation of Nikolsky's *Special Ichthyology* seems more successful in this respect)

The 140 figures illustrating the text are helpful, though a few graphs lack information concerning the units used, or which curve is which Three figures present a visual summary of the biotic links in the sea and fresh water and "the proper and improper conduct of a fishing industry"

Despite minor drawbacks, this is a splendidly stimulating and thoughtful book, which should have a profound effect on the approach to fish ecology It is a book which should be in the possession of every ecologist interested in fish, and of all those concerned with the management of fisheries, though the price is unfortunately high

R H McConnell

DEVELOPMENTS IN PARASITOLOGY

Advances in Parasitology

Vol 1 Edited by Dr Ben Dawes Pp xvi + 347 (London Academic Press, Inc (London), Ltd , New York Academic Press, Inc , 1963) 70*s*

It is not possible, in a relatively short review, to tell prospective readers of the wealth and detail of the information contained in this well-written and well-produced book

Introduced by a short preface by its editor, Prof. B Dawes, the book begins with two chapters devoted to the parasitic Protozoa In the first of these, Clay G Huff sums up recent researches done on the species of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* parasitic in birds. This valuable article contains a detailed section on the avian species of *Plasmodium* which discusses the natural immunity and susceptibility of the avian and mosquito hosts, the effects of X-irradiation on the development of these Protozoa in their mosquito hosts, the cytology and genetics of the species discussed, the growth of their sexual phases *in vitro* and *in vivo*, the development of the gametocytes and their infectivity, the periodicity of the asexual multiplication, the effects of viruses, bacteria and yeasts and coccidia on the parasites, their cytochemistry, fine structure, immunology and pathology and their exoerythrocytic phases Shorter, but no less interesting, sections follow on recent work on *Haemoproteus* and *Leucocytozoon*

In the second essay on Protozoa, C. Horton-Smith and P Long discuss the pathology and life-cycles of the species of *Eimeria* which cause coccidiosis of the domestic fowl and the turkey, the oocyst production of these species, the DNA, RNA and other chemical substances encountered in the coccidian life-cycle and modern knowledge of the immunology of this economically important disease

The rest of the volume is devoted to parasitic nematodes and to the larval phases of monogenean trematodes. A valuable essay by W P Rogers and R I Sommerville describes in detail the significant and promising work that has been recently done on the infective stages of parasitic nematodes and their significance in parasitism In a short review it is impossible to indicate the importance of the investigations here described They include studies of the nematode life-cycle as a whole, the physiology, behaviour and survival of the all-important infective stages, the process of infection of the host by these stages through the gastro-intestinal tract or through the skin and the problems faced by the nematode when it has to pass from the world outside the host to the environment it encounters inside the host's body The mechanisms of the vital ecdyses are here involved, and the authors discuss the problems of infectiousness and their hypothesis of infectiousness considered as an adaptation to parasitism

In the next article, D Poynter discusses the lungworm of cattle, *Dictyocaulus viviparus*, which causes parasitic bronchitis, or husk, in cattle, the pathology of this economically important disease, the biology of the infective stages on the pastures from which the cattle pick them up and the remarkable recent use of X-irradiated larvæ of the parasite, to make the vaccine developed by workers at the Glasgow School of Veterinary Medicine, which is proving an effective means of protecting cattle from the disease. This vaccine marks a 'break-through' in the field of the immunology of parasitic infections the importance of which can scarcely be overestimated

There follows a useful article by John E Larsh, jun , on recent work on experimental trichinasis in hamsters, rats and mice, and the volume concludes with an article by J Llewellyn on the morphology of the larvæ of monogenean trematodes and their post-miracidial development

So rapid have been the advances in our understanding of the parasite as an organism living, like other creatures, in conflict with the risks of its mode of life and with the challenge of its environment, that everyone who has to deal with parasitic life can scarcely afford not to read this book and to learn how the application of the methods of biochemistry, biophysics and experimental biology in general have altered, in a few productive years, the whole structure of this branch of biology The promise of this kind of work is incalculable Not only the biologist but also the medical man and the veterinarian and everyone else who is concerned with the mitigation of the suffering and loss of life inflicted by the parasite will welcome this volume and will hope for successors to it which will deal with the fields of work not covered in these pages

G. Lapage

Photo-Electronic Image Devices

Proceedings of the Second Symposium held at Imperial College, London, September 5–8, 1961 Edited by J D McGee, W L Wilcock and L Mandel (Advances in Electronics and Electron Physics, Vol 16) Pp xv+654 (New York and London Academic Press, 1962) 130*s*

ADVANCES in photo-electronics have largely centred around image devices, photomultipliers and other components for converting radiation into electronic signals The spur to increase sensitivity, signal/noise ratio and spatial resolution and to improve reproducibility has kept technologists busy and has led to excursions into allied branches of applied physics, particularly electron optics and vacuum technology The discovery of new physical effects likely to have far-reaching influence is not at present a primary aim of the subject, which, however, does not lack effects requiring clarification and justification on fundamental grounds If the subject is compact, that is more than can be said of the workers in it, many of whom owe their first allegiance to particular fields of application Prof McGee, in organizing two symposia on the key-image devices, has thereby brought together scientists who might otherwise never have met

The report on the second symposium on "Photo-Electronic Image Devices", held at the Imperial College of Science and Technology during September 5–8, 1961, is very factual, it shows no diminution of activity since the 1958 symposium, stresses the attention being directed to details to improve performance and leaves no doubt as to the contributions being made by users (compare the transistor, for which no user has also been a designer or technologist) Several astronomers are now using electronic cameras following pioneering work by Lallemand, Duchesne and a few others The precautions needed to prevent the photographic plate spoiling the vacuum and hence the photocathode are central themes of two papers, a bakeable electronographic plate may still be required, however, despite its inability to match the large gain conferred by the conventional developing of a photographic plate Image intensification is a fruitful subject at the moment and has received much attention, the television camera tube, on which so much effort has already been expended, did not figure so prominently, but infra-red and ultra-violet versions were described Nuclear physicists are extending the versatility of scintillation counters, as several papers show, and further progress can be expected, based on applying techniques developed for other devices

One or two laboratories surveyed their own work over the past few years, but review papers were otherwise absent, their inclusion in any symposium is a gamble for they may turn out to be mere catalogues or to be insufficiently critical But two or three good reviews here would have improved an already impressive and readable report

J R TILLMAN

Diseases of Turfgrasses

By Prof Houston B Couch (Reinhold Books in Agricultural Sciences) Pp xiii+289 (New York Reinhold Publishing Corporation, London Chapman and Hall, Ltd, 1962) 80*s* net

THE production and maintenance of high-quality grass turf, whether for ornament or recreation, requires skilled management which involves effective control of diseases affecting turf grasses A comprehensive treatment of this specialized topic would certainly be welcomed by turf specialists and plant pathologists, it is disappointing, therefore, to find that Prof Couch's book does not fulfil the promise of its title

The main text of the book occupies 180 pages and is divided into four chapters, the first of which deals briefly and in very general terms with the nature and causes of disease in plants Chapter 2 (124 pp) is concerned with fungal diseases These are well described and the control practices (where any are available) are clearly explained The coverage on fungal diseases is certainly comprehensive and includes many diseases (for example, those affecting inflorescences, such as smuts and ergot) which might well have been omitted Indeed, their inclusion only serves to exaggerate the omissions referred to below The third chapter deals concisely with nematodes, and the main text concludes with a short chapter on the fundamentals of disease control

Two notable omissions from this book are physiological and virus diseases Their exclusion can scarcely be justified in a work which claims to be comprehensive To be told (p 5) that physiological diseases are already well covered in standard works on turf-grass management will scarcely satisfy the plant pathologist, particularly when no specific references to sources of information are given Equally the omission of virus diseases, apparently on the grounds that "information concerning them is not yet sufficient to evaluate properly their importance", is scarcely acceptable The same might be said of a great many of the fungal diseases described The omission of virus diseases is all the more serious because recent work is showing that they may, in fact, be seriously damaging in agricultural swards, presumably they may be equally important in sports turf and lawns The inclusion in this book of the symptoms, host-ranges and mode of transmission of the many grass viruses now known would have been most valuable if only in directing the attention of turf specialists to this group of diseases

More than a third of the book is devoted to five appendix tables Apart from the first (which is a short list of fungicides and nematocides), these constitute a particularly clear index of diseases and host species cross-referenced under their common and technical names Certainly in this 100-page appendix the reader will have no difficulty in locating the items of information contained in the other 180 pages

ELLIS GRIFFITHS

The Morphology of Pteridophytes

The Structure of Ferns and Allied Plants By Dr K R Sporne Pp 192 (London Hutchinson University Library, 1962) 12*s* 6*d* net

THIS condensed but clear account of the morphology of the pteridophytes is evidently aimed primarily at an undergraduate public Available at a most reasonable cost, it will surely be deservedly popular as a student text An excellent feature of this book is the attention directed to the integration of the results of modern research with classical morphology Not only are more obvious discoveries like *Stylites* described, but also recent developments in all aspects of the subject are included Rather more than half the ninety items included in the bibliography were first published since 1945

Great pains have evidently been taken over the production of the beautifully drawn figures, which are a joy to study Nevertheless, one must regret that it has been found necessary to overcrowd the illustrations on certain of the figures (for example, Figs 14–17), so as to confuse the eye, and to over-reduce certain of the illustrations (for example, Figs 12*A*, 16*E*, and 17*A*) to such a degree that their evident quality is obscured One appreciates with approval the intention to keep the price of this book as low as possible, but it is a pity that a larger page-size could not have been used

Errors appear to be commendably few There is an unfortunate misprint on the fourth line of p 166, where *Phyllitis* is intended but *Athyrium* is printed, which may seriously mislead the unwary It is not true that there are eleven species of *Equisetum* in the British flora, and *Phylloglossum drummondii* occurs also in south-western Australia, but these are small blemishes in an admirable text This book is a valuable addition to the botanical literature, and is most warmly recommended

J D LOVIS

LYON PLAYFAIR, 1818–98*

By PROF R. V. JONES, C.B, C B E

Department of Natural Philosophy, University of Aberdeen

THE Scottish family of Playfair has an extraordinary record of distinction in public service There have been Playfairs in high positions in medicine, the law, the armed Services and the Civil Service Playfair of the axiom was both an eminent mathematician and a geologist Another member of the family was Principal Playfair of St Andrews, who was in office from 1800 until 1819 He married a Miss Lyon, a relative of the Lyons of Glamis, and they had four sons The eldest of these, George, went into medicine and served, as so many of the family had done, in India, he became chief inspector-general of hospitals in Bengal He married a Miss Ross of Edinburgh and, of their four sons and three daughters, it is their second son, Lyon, born at Meerut on May 21, 1818, who is of especial interest now

The boy returned to Scotland in 1820, and in due course went to the parish school This he said was excellent Of his later education at a grammar school he was not nearly so complimentary In his autobiography he says "To my mother and her surroundings I owe all my early knowledge, precious little of which I derived from the Grammar or Classical School to which I was sent at a later period" He retained, as will be seen, this critical attitude to the then current form of education throughout his life His best holidays, he says, were spent with his grand-uncle, a minister, in the manse at the gate of Glamis Castle

At the age of fourteen he was enrolled as a bejant in the University of St Andrews, and attended the ordinary courses for the M A degree Of the subjects in the course, mathematics pleased him most, but he broke away from his curriculum whenever possible to slip into the lectures in chemistry and natural philosophy After this, he was sent to work as a clerk in the office of his Uncle James, a merchant in Glasgow His fellow clerk was named Ramsay, and when, after a short experience of mercantile life, he obtained his uncle's permission to study for medicine, he went to board at the house of Ramsay's mother The Ramsay family is worthy of mention, since one of Mrs Ramsay's grandsons was the famous chemist of University College, the discoverer of helium on the Earth (in 1894)

Instead of going straight into medicine, Playfair decided to study chemistry seriously, since it was his favourite subject, he therefore chose to go to the Andersonian College (now the Royal College of Science and Technology in Glasgow) rather than to the University itself, because this would give him an opportunity to study with Graham, who was professor at the College, and one of the most distinguished chemists of his time In Graham's classes there were two students of a similar age to Playfair with whom he formed friendships The first of these was David Livingstone The second student was James Young, who, as James Watt had been before him, was employed to repair laboratory instruments He had been engaged by Graham as an assistant, and was later to participate with Playfair in the foundation of the shale oil industry

After Playfair had been with him for two years, Graham was called to University College, London Glasgow thereby lost its attraction for Playfair, who moved over to Edinburgh to carry on with his medical studies He obviously enjoyed the social life of the University, and helped to form a dining club which developed into 'The Red Lions', a famous social function of the British Association Playfair had to stop his medical studies, since he contracted eczema, and he left for India on his father's advice Again he started in a mercantile career, this time in Calcutta, but once again his interest strayed back to chemistry, and he gained the acquaintance of the professor of chemistry in the local college The professors there advised his father to send Playfair back to England, and he therefore returned in 1838 to join Graham at University College In 1839 Graham advised him to go to study under Liebig at Giessen

Liebig at this time was writing his great book on agricultural chemistry, and he invited Playfair to undertake the English translation Liebig's views were to be announced in a general way at the 1840 meeting of the British Association in Glasgow, and since Liebig himself was unable to go, Playfair went to represent him and was promptly appointed secretary of the Chemistry Section

Industrial Chemistry

Playfair returned to Giessen and graduated as a doctor of philosophy, but the Glasgow meeting had given him the opportunity of meeting various chemical manufacturers, and in the spring of 1841 he accepted an offer from a Mr Thomson of Clitheroe to become chemical manager of his print works

Playfair knew nothing of the process of calico printing, but he quickly saw that Mr Thomson's works were likely to fail sooner or later, since Thomson insisted on expensive hand productions, which were unlikely to survive in the competition with mechanical reproduction processes. Playfair therefore thought of moving, but before he left Clitheroe, he encountered the great labour riots of 1842 in Lancashire The rioters were out to smash the machines, and Playfair went to the rescue of a neighbouring mill He met the advancing rioters, and told them that he was perfectly well aware that their force was irresistible, and that they could stop the works He suggested to them that instead of doing widespread damage, they could secure their object of stopping the works by removing the plugs from the boilers They accepted his offer of acting as a hostage, while they removed the plugs Characteristically, he afterwards recorded that from the conversations that he had with them during his detention, many of their demands were reasonable

While at Clitheroe, he gave several lectures in Manchester, and accepted the office of honorary professor of chemistry in the Royal Institution in Manchester This brought him into contact with several men of distinction who were at that time in Manchester, most notably Dalton and Joule Dalton was president of the Manchester Literary and Philosophical Society, and was re-elected annually There was always one dissentient vote in the ballot, Playfair says, this was that of Dalton himself, who used to vote for one of his friends On the last vote before Dalton's death, there was the one dissentient vote as usual—but this time Dalton had voted for the door-keeper

In October 1842 Playfair was surprised to receive a letter from Faraday, offering him the professorship of chemistry in King's University, Toronto Playfair thereupon visited Faraday in London, and indicated that he would like to accept the proposal This intention was not, however, favourably regarded by various of Playfair's friends in London, who thought that his loss would be too serious A few days later, to his great surprise, Playfair received an invitation from Sir Robert Peel, who was then Prime Minister, to visit him at Drayton Manor, his home near Tamworth Playfair thought that this letter might well be a practical joke, and spent a day or two checking its authenticity He then went to stay with Peel for a few days, and Peel explained to him that several men of science had asked him to persuade Playfair to stay in Britain He even offered Playfair a written promise that he would make it his duty to obtain suitable employment for Playfair in Britain, but the latter would not accept the document, saying that the Prime Minister's appreciation was all that he could have wished

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* Substance of a lecture given on September 3 before Section X (General) of the British Association for the Advancement of Science meeting in Aberdeen

For a time, Playfair returned to Manchester, but he was quickly drawn into an investigation on behalf of the British Association for the Advancement of Science with Bunsen, who came across from Heidelberg, into the economy of blast furnaces This investigation, which showed that with the existing design of furnaces more than 80 per cent of the fuel escaped from the furnace in the form of gases, resulted in a great improvement in efficiency The report was published in the annual volume of the British Association for 1845 In the meantime, in 1843, Peel had asked Playfair to sit on a Royal Commission into the state of large towns This was the first of the many public committees on which Playfair was to serve, he took a special interest in the large towns in Lancashire, since he was well placed to study them The findings were terrifying Owing to the effect of such features as bad sewerage and overcrowded tenements (one-seventh of the population of Liverpool lived in cellars) the expectancy of life was very short, in Manchester it was twenty-two years, in Liverpool twenty, in Ashton sixteen As a result of the findings of the Royal Commission, on which Edwin Chadwick, the veteran sanitary reformer, was prominent, the conditions in the towns were much improved

Just as this inquiry was drawing to a close in 1845, Peel offered Playfair the appointment of chemist to the Geological Survey, and Playfair became a regular guest at Peel's home, along with such men as Wheatstone and George Stephenson Playfair records how after dinner on one of these parties, George Stephenson put forward the theory that his locomotives derived their original power from the Sun through the plants that were fossilized into coal, the geologists present laughed, and Stephenson gave up the argument Peel afterwards asked Playfair what he thought of Stephenson's view, and the latter told him that it could be supported Peel told Playfair to explain the arguments to Follett, a lawyer, who was also a guest Next evening, the conversation was duly steered in the same direction again, to give Follett a chance to intervene, briefed by Playfair, he completely routed the geologists Stephenson, listening in amazement, exclaimed "Of all the powers in Nature, the greatest is the gift of the gab"

While Playfair was still in Manchester he formed a close friendship with Joule, who was then engaged on his experiments on the conservation of energy Joule and Playfair undertook several investigations together into atomic volume and specific gravity, and published several joint papers in the *Journal of the Chemical Society*, the most important result was the discovery that the increase in the volume during the process of solution of a hydrated salt was due to the water released from the salt itself Playfair persuaded Joule to become a candidate for the chair of natural philosophy of St Andrews, one of the electors, however, objected to a slight physical deformity that Joule had, and so created another instance of a university losing a great opportunity

It is one of the regrettable facts in the history of scientific publication that the Royal Society rejected Joule's first account of his conservation experiments Playfair tells how this was due, among others, to Wheatstone, who was his close personal friend Playfair and Wheatstone spent a whole Sunday afternoon walking on Barnes Common discussing Joule's experiments and their consequences, if true, to science Playfair failed, unfortunately, to convince Wheatstone, and so the Royal Society rejected the paper Playfair continued his work with Joule, after he transferred to the Geological Survey, the main premises of which were in Jermyn Street, London He himself had a temporary laboratory in Duke Street, here he discovered the nitroprussides Among his assistants were Edward Frankland and James Dewar. The Geological Survey ran the Geological Museum which developed, under the directorship of Henry de la Beche, into the School of Mines

Playfair's attempts to pursue his researches became increasingly interrupted by Government demands on his services The first of these was to report on the state of Buckingham Palace Playfair found, after some drastic experiments involving the explosion of gunpowder with some olfactory mixture in the Palace kitchen, that the carbon monoxide fumes from the charcoal fires were ascending into the royal nurseries The sewerage system was also rudimentary. Playfair's report was so frank and brutal that the Government did not dare to publish it He had also to look into the state of Eton College, and the Board of Health asked him to report on graveyards, from which experience he became an advocate of cremation, and to analyse all the water proposed for the supply of towns The Admiralty asked him to determine the best coals for steam navigation, Playfair was connected with this investigation for many years

Another field of activity for Playfair about this time was that of safety in mines Lyell and Faraday were Government commissioners, and had reported on the dangers of fire-damp This was, of course, a considerable time after the invention of the safety lamp, but there was still much carelessness in the mines, and explosions were repeatedly occurring Playfair was asked to investigate a particularly bad explosion at Jarrow, apart from investigating the explosion itself, Playfair conducted a programme of analysis of fire damp from many mines His direct connexion with safety in mines did not last very long; but years afterwards it turned out to be valuable A serious strike of miners was imminent in the Newcastle district, and, although he was in Brittany at the time, both the owners and the men decided to send for Playfair to arbitrate between them The strike was averted

The Corn Laws

The investigations that Playfair was called on to undertake took him farther and farther away from pure science As one of these tasks he was asked to report on the foul state of the Serpentine At this period, by far the most important of the investigations that he was asked to carry out was one in the autumn of 1845, when Peel sent for him and told him how worried he was about the effects of the potato disease in Ireland Peel asked Playfair to nominate two others to act with him on a commission of inquiry, and to report Playfair found that the prospect for Ireland was starvation, and he reported to Peel that this would be so unless the Corn Laws were repealed This obviously placed Peel in a very difficult position, since his own party was responsible for the Laws Peel replied to Playfair "I am indeed sorry that you are compelled to make so unfavourable a report, but the knowledge of the whole truth is one element of security" This readiness of Peel to face unpleasant facts shows why Wellington said of him "Of all the men I ever knew, he had the greatest regard for truth" It is, of course, one of the most celebrated incidents in Britain's history of party government how Peel turned on his own party, and carried the repeal of the Corn Laws, in view of the present interest of the functions of science in Government, it is interesting to realize that Peel had a man of science behind him.

The Great Exhibition of 1851

The culmination of all this public work came in Playfair's appointment to a key position in the planning of the Great Exhibition of 1851. This Exhibition had probably started as a concept of the Prince Consort and Sir Henry Cole. The Society of Arts organized a committee to carry through the arrangements, but the task was really too great. A Royal Commission was therefore formed, containing eminent men of both political parties. The detailed arrangements were in the hands of an executive committee, substantially with the membership of the body previously selected by the Society of Arts. However, the two bodies were in danger of drifting apart, and Peel asked Playfair to be a member of both bodies to ensure that they kept in step. Playfair himself had misgivings about leaving his scientific work, and objections were raised by his fellow professors on the grounds of the upset to their teaching programme. Peel again intervened, this time so strongly that neither Playfair nor his colleagues could refuse.

Playfair at once came into close contact with the Prince Consort, and this rapidly developed into friendship. One of Playfair's many contributions was to organize the classification of the various sections. His system was accepted by all the authorities concerned, including the foreign representatives, with the exception of France, since the French had devised a logical and philosophical classification for themselves. Playfair and the French representative agreed that they should test the rival classifications by seeing how easy it was to classify a simple object on the two systems. The Frenchman suggested that they should try his walking stick. Playfair turned to his class of 'Miscellaneous Objects' and soon found the walking stick under the sub-section 'Objects of Personal Use'. The French Commissioner looked for a long time in vain through his logical classification, but ultimately found it under the heading 'Machines for the Propagation of Direct Motion'. Playfair's system was thereupon adopted.

Much of the success of the Exhibition must have been due to Playfair's wisdom and energy, the Prince Consort was very impressed and offered him the post of Gentleman Usher in his household. In this position, Playfair was very frequently consulted by the Prince on all kinds of matters. The Exhibition building, originally erected in Hyde Park, was taken down and erected again in 1854 at Sydenham as the Crystal Palace. As part of its educational value, it was to be decorated with copies of great statues from all over the world, along with an ethnological collection of effigies of savages. Playfair records that most of the figures were nude, and that just before the opening there was a written representation to the Queen, signed by most of the Bishops, stating that they could not attend the State opening unless all the nudes were properly draped. The Prince Consort called for Playfair's advice, his light-hearted suggestion was that the Prince should ask the Bishops for a loan of their aprons during the ceremony.

In the winter of 1851–52 the Society of Arts organized a series of lectures on the results of the Exhibition, in which the speakers underlined the need for scientific education. In this series Playfair's lecture was outstanding. He held that the Exhibition showed that "wherever a nation used science in its manufactures that nation was in the ascendant", and suggested that "in the establishment of institutions for industrial instruction you, at the same time, create the wanting means for the advancement of science in this country". Playfair also gave two brilliant inaugural lectures at the School of Mines urging the importance of ensuring the developing harmony between the advance of pure science and the progress of industry. The second lecture, which was given in the autumn of 1852, was on "Industrial Instruction on the Continent" and included much information that he had gained earlier in the year when, at the request of the Prince Consort, he had toured the Continent. He pointed to a curious weakness in the national outlook in Britain, which even now has not been fully overcome. "In this country we have eminent 'practical' men, and eminent 'scientific' men but they are not united and generally walk in paths wholly distinct ... from this absence of connexion there is often a want of mutual esteem and a misapprehension of their relative importance to each other". In 1853 he continued this theme in a lecture to the 'People's Club' in Sheffield on "Science in its Relation to Labour".

In August 1851, before the Exhibition had ended, the Prince had roughed out a scheme for using the profits. This involved buying a large area in South Kensington, very near to the site of the Exhibition, and building on it an institution for "the furtherance of the industrial pursuits of all nations". Playfair, with whom the Prince discussed his scheme, favoured a widespread system of centres of technical education throughout the country united with a university of mines and manufactures on the Kensington site. The Prince provided a friendly brake on his enthusiasm by pointing out that Playfair had omitted any reference to religious education in his scheme, and that it might be wrecked by religious opposition on this account. This was not a fanciful objection, for religious bias was formidable: in 1845 Playfair's former master, Liebig, was even disqualified from consideration for the chair of chemistry at King's College, London, because he was a Lutheran and not an Anglican. Following the Prince's wishes, the South Kensington site was in the meantime purchased out of the £186,000 profit of the Exhibition, and, although it took many years, the ultimate formation there of the Royal College of Science is due to the foresight of the Prince, Playfair and their associates.

The Science and Art Department

One step on the way was the formation of the Science and Art Department in 1853, with Playfair at the head of its science side. It was mainly concerned with educational matters, and it founded science schools in various towns. In the course of this work, Playfair paid a visit to Edinburgh, and persuaded the Town Council to provide the land, and the University the original collections, for a museum, he then succeeded in obtaining a parliamentary vote, and thus founded the Scottish National Museum.

The Science and Art Department seems to have had some other functions besides its educational ones, since it was asked in 1852 to design a State funeral carriage to carry the body of the Duke of Wellington. Playfair records that it was made of bronze and was so heavy that it broke a sewer when crossing St James's Park; and this delayed the funeral procession, one of the spectacles of the century, for one to two hours.

At this time Playfair was living in the Putney area, and saw much of Wheatstone, who lived in Hammersmith. They spent many of their Sundays together, amusing themselves by deciphering the cipher advertisements in the personal column of *The Times*. One turned out to be from an Oxford student in a reading party at Perth, who was corresponding with a young lady in London. When he proposed an elopement, Wheatstone and Playfair decided that it was time to step in. They therefore inserted an advertisement in the same cipher reading "Dear Charlie, Write no more. Our cipher is discovered".

About the same time, Playfair frequently saw Babbage, the inventor of the calculating machine, who resented the way that he had been treated by the Government. In the course of trying to improve the public recognition of science, Playfair had suggested to the Prince Consort that it would be a happy gesture to make one or two scientists Privy Councillors, he mentioned Faraday and Babbage as two who were well fitted for this honour The Prince asked him to sound the two men, but while Babbage was delighted with the suggestion, he made it a condition that he alone should be appointed, as reparation for the Government's neglect of his inventions This unfortunate attitude caused the Prince Consort to drop the idea

The prospect of the Crimean War caused Wheatstone and Playfair to submit one of their ciphers to Lord Palmerston, it has since become one of the classical methods of enciphering Playfair produced inventions for incendiary shells and poisonous gas shells, but these were not taken up at the time In 1855 the mastership of the Mint became vacant Playfair would very much liked to have had this appointment, but he found that his late master, Graham, was anxious for it He therefore supported Graham, who was awarded the honour

Chemistry at Edinburgh

In 1858, the chair of chemistry at Edinburgh became vacant Playfair had applied for this post on an earlier occasion, when it was awarded to Gregory On the present occasion, he saw a chance to get back to research, and his attainments and distinctions were such that he would almost certainly get the chair if he applied for it But his friends in Edinburgh insisted that he should have some testimonials One of these he obtained from his pupil, Frankland, now a professor himself His letter to Frankland asks the latter to confirm various of his qualifications, including "that as President of the Chemical Society I have shown that I studied Chemistry"

Playfair had to resign his appointment as Gentleman Usher to the Prince, and he threw his full energies into reorganizing the Chemistry Department He introduced a system of tutorial classes, which rapidly became a feature of other departments throughout Scottish universities He also devised a system of merit certificates, which again extended throughout the Scottish universities He also decided, perhaps from his experience at Court, that graduation ceremonies needed to be revived to produce a feeling of pride among graduates All these reforms are strong features of the Scottish universities to-day

As always, Playfair collected good stories One of his colleagues at this time was James Simpson, who introduced chloroform into surgery Playfair says that in Scotland there used to be an antipathy to objects not mentioned in the Bible, and that on this account, for example, potatoes were only slowly adopted by the Scots This attitude gave Simpson much trouble, since not only was chloroform not mentioned in the Bible, but also it was positively in direct contradiction to the curse on woman that she shall endure sorrow in bringing forth children Simpson adroitly answered the religious objections to chloroform by saying that God, when he performed the operation of taking one of Adam's ribs out, first put him into a deep sleep such as chloroform produces

Both the Prince of Wales and Prince Alfred were sent by their parents to be tutored by Playfair in Edinburgh When the Prince Consort died prematurely in 1861, Playfair suffered a great personal loss, but such was his reputation by this time that the public demands on him inevitably continued He was asked to organize the Jury Department of the International Exhibition of 1862 His work on royal commissions still went on In 1861 or 1862 he was president on a Royal Commission into the British herring fisheries, which repealed the closed season for herring The finding of the Commission was that the closed season enabled the predators on the herring, such as cod and ling, themselves to multiply They were normally caught with herring as bait, with the result that the absence of such bait during the herring close season resulted in fewer casualties among the cod and ling Playfair estimated that the number of these fish that were as a result not caught would in fact themselves eat more herring than those that were caught by all the fishermen of the United Kingdom

In 1865 he served on the Royal Commission on cattle plague, now known as foot-and-mouth disease Playfair himself suggested the measures that have ever since been accepted, of closing markets in affected districts, stopping all transit of cattle, and slaughtering and burning any suffering from the disease These severe measures resulted in great personal unpopularity for Playfair, but his conviction that he was right carried him through

In 1867 he was again a judge at an international exhibition, this time that in Paris Britain did not do nearly so well in that exhibition as she had done in 1851 Playfair saw his chance to drive home the lessons that he had been preaching ever since 1851 He wrote a famous letter to Lord Granville, which the latter asked should be published in *The Times* The following is an extract

"I have just returned from Paris I am sorry to say that, with very few exceptions, a singular accordance of opinion prevailed that our country had shown little inventiveness and made little progress in the peaceful arts of industry since 1862 Deficient representation in some of the industries might have accounted for this judgment against us, but when we find that out of 90 classes there are scarcely a dozen in which pre-eminence is unhesitatingly awarded to us, this plea must be abandoned So far as I could gather them by conversation, the one cause upon which there was most unanimity of conviction is that France, Prussia, Austria, Belgium and Switzerland possess good systems of industrial education for the masters and managers of factories and workshops, and that England possesses none A second cause was also generally, though not so universally, admitted, that we had suffered from the want of cordiality between the employers of labour and workmen, engendered by the numerous strikes, and more particularly by that rule of many Trades' Unions, that men shall work upon an average ability, without giving free scope to the skill and ability which they may individually possess"

This letter was one of the factors that later resulted in the appointment of the Royal Commission on scientific instruction of 1871

The House of Commons

In 1867, the Reform Bill was passing through Parliament, and Playfair represented to Gladstone that it should provide for the representation of the Scottish universities Gladstone agreed, and Playfair found himself approached to be a candidate He accepted after some hesitation, and was elected as a Liberal, despite the fact that the University was predominantly Conservative So far as he could, Playfair stood aside from party politics For the first year or so he decided to learn the business of the House of Commons without making any speeches His first speech was in fact made in 1869, and was in support of the abolition of religious tests in the English universities In this matter, he held that the Scottish universities, which had long before abandoned such tests, were well in advance of those in England In 1870, his attention was switched to matters of postage, when he successfully carried through the introduction of the postcard

Education was a very topical subject at that time, and Playfair was naturally called on to speak on many occasions Irish problems were continually before Parliament, and one of the Bills was that for opening Trinity College, Dublin, to Roman Catholics Playfair, with his liberal views on religion, was a strong supporter of the Bill It was ultimately defeated, because it was likely to interfere with a larger scheme being contemplated by Gladstone When this latter Bill was introduced, it contained some curious compromises, one of which was to exclude priests from lectures on philosophy and history Playfair opposed such measures on principle, pointing out that they forced the student to be "either a bigot or an infidel" His opposition, against a Bill introduced by his own party, was so effective that he carried the day This caused the Government to resign It will be remembered that Playfair had already played a part in the upheaval in the Conservative Party over the Corn Laws, he now caused a comparable upheaval in the Liberal Party Disraeli, however, could not form a Government, and so the Liberals went back Gladstone's respect for Playfair evidently remained, because he offered Playfair the office of Postmaster-General Playfair was just beginning to enjoy administering this post when, early in 1874, Gladstone's Government came to an end, and a Conservative Government was returned at the election

Education in Science and Technology

Outside Parliament, in 1870 he addressed the Midland Institute in Birmingham on his life-long theme of the inosculation of the arts and science This address was full of his usual good sense, coupled with his flare for humorous illustration In this instance he drew a lesson from the behaviour of the natives of Tahiti, who, when they received a present of iron nails from Captain Cook, very prudently sowed them in the ground in order to raise a crop for the next season

Playfair then made an important series of speeches on educational matters. The first of these, in 1870, was on primary education In this he urged that instruction in experimental science should be more widespread "The pupil", he said, "must be brought in face of the facts through experiment and demonstration. He should pull the plant to pieces and see how it is constructed he must apply with his own hand the magnet to the needle He must see water broken up into its constituent parts and witness the violence with which its elements unite . one of the first lessons he must learn from science is not to trust on authority but to demand proof for each assertion All this is true education, for it draws out faculties of observation, and connects observed facts with the conceptions deduced from them in the course of ages, gives discipline and courage to thought, and teaches a knowledge of scientific method which will serve a lifetime. Nor can such education be begun too early" . "Those who are believers in the teaching of the great Secondary Schools of this country will deem my aspirations to improve Primary Education low and utilitarian Frankly I admit the latter Such a style of education will never realize Lord Brougham's hope that the time may come when every working man in England will read Bacon, but it may contribute to the fulfilment of Cobbett's desire, that the time might come when every man in England could eat bacon"

Also in 1870, he addressed the Philosophical Institution of Edinburgh on technical education He concluded "It is for the United Kingdom to determine whether she desires her history to be that of a country which was raised to the highest place among States by the genius of mighty men, though she lost that position by a blind reliance on the practical empiricism of her people, or whether her future history is to be that of an enlightened nation which, seeing that a general diffusion of science and art is giving to other countries advantages in industrial competition, added this intellectual power to the practical aptitude of her population"

Another of these addresses was that on teaching universities and examining boards, and was primarily directed towards saving Ireland from a system of State examinations at university-level He stood very strongly for the system of each university conducting its own examinations, with a maintenance of standards being ensured by the appointment of external examiners In this, he was surely right

A particularly important speech was that on universities in their relation to professional education, which he made to the St Andrews Graduates' Association in 1873 This contains the passage

"Our Latin and Greek education still goes on, though, for its practical uses, it is as much a survival as the two buttons behind our coats are survivals of the time when the slits and flaps of our ancestral coats had to be buttoned away Their existence survives their utility Medical men no longer write or speak Latin and yet a fourth of a professional man's life is spent in the venerable deception that it is indispensable to him . If liberal culture be, as it should be, a part of professional training, then the culture should be made to bear directly on the training, and not remain a mere survival of an education that is only known to us in history New professions are arising around, but for these our old universities make no provisions, and old professions have completely changed their aspects, yet the schools and colleges remain as of old Perhaps the most robust men of our time have been our engineers and mechanicians, for they have given more impulse to civilization than any other class of men. But I look in vain for a single representative man among our Telfords, Watts, Stephensons, Arkwrights, Wedgwoods, whose intellect was nurtured on ancient classical learning, or who could find anything in school life to aid the development of their genius . . If liberal culture be, as it should be, a part of professional training, then the culture should be made to bear directly on the training, and not remain a mere survival of an educational condition that is only known to us in history . And yet our Prime Minister, Mr Gladstone, and Home Secretary, Mr Bruce, make orations on the same day, telling us that the old classical system is that which is best fitted for mental culture, under all conditions "

Reviewing the results of all his efforts for technical education, Playfair in the late 'eighteen-eighties could point to promising progress "I now feel that this work may be left to younger men, but I hope that they will not take their hands from the plough till England has adapted its system of education to the changed condition of competition."

The 'Playfair Scheme'

The fall of the Liberals and the advent of a Conservative Government in 1874 did not reduce official demands on Playfair's time Disraeli asked him to be chairman of a Commission to reorganize the Civil Service This Commission reported in 1875, and was the origin of the 'Playfair Scheme' which divided the Civil Service into two divisions, the upper of which was to be recruited by highly competitive examination at university-level, and the lower of which was primarily clerical This made Playfair extremely unpopular with the clerical side, but his scheme has essentially survived to the present time

Deputy Speaker

In 1876 Playfair was on another Royal Commission, this time on the Scottish universities.

When the Liberals returned to power in 1880, Playfair was offered the post of Chief Whip; but he did not feel adapted for this kind of duty, and so he declined He did, however, accept the office of Chairman and Deputy Speaker of the House of Commons, and this led to the most troubled period of his career It was, in any event, a remarkable post for any man of science to fill, but in addition, it happened that this particular time was about the stormiest in Britain's entire history of Parliamentary Government The basic trouble was the unsatisfactory state of affairs in Ireland, which led the Irish members into a policy of concerted obstruction to all parliamentary procedure It was an extremely difficult time for the Speaker It was even more difficult for Playfair, who had to deputize for the Speaker, frequently long into the next day, but without quite having all that authority which the Speaker by tradition possessed, and in the face of deliberate Irish tactics directed towards exhausting whoever was in the chair There were rules for dealing with obstruction in Parliament, but these had hitherto applied only to individual members It became necessary to extend them to cases of members acting in concert, and during 1881 the Speaker himself suspended twenty-eight Irish members *en bloc* In July 1882, Playfair was in the chair when after nineteen hours' debate on a single clause of one Bill, leading men of both parties pressed him to close the discussion by suspending the obstructing members After detailed warnings, Playfair then suspended sixteen members This action inexplicably brought widespread criticism, even though he had acted strictly according to procedure Some thought that he had been too lenient, others too strict, probably everyone concerned was very much out of temper with the way things had been going, and irrationally blamed Playfair as the man in control, even though the sanctions that he had at his disposal were quite inadequate for the task Playfair was very unhappy, and resigned shortly afterwards It is perhaps possible that someone with a very strong personality might have been able to exert a greater control than Playfair The ability to control unruly audiences seems to require some indefinable personal quality Playfair, when dealing with the rioters in Lancashire, showed that he had this quality to an unusual degree, and if he was unable to control Parliament in these particular circumstances, it is most likely that most other men would have fared even worse In any event, the incident made such an impression that the circumstances were never repeated, in fact, the men who were afterwards most enthusiastic about Playfair's patience and impartiality were the very Irish members whom he had suspended

Despite his retirement as chairman, he continued to be active in Parliament In 1883 he successfully repelled a determined attack on the Act regarding vivisection, which had been carried through Parliament by him in 1876 This Act brought vivisection under control, and made it a condition that no animal should be the subject of a painful experiment without having been previously anæsthetized The opponents of the Act wished to ban vivisection altogether, but Playfair's powerful defence, on the grounds of the value of controlled vivisection to mankind, entirely saved the Act Another attack, also in 1883, was on compulsory vaccination, here the Government were so worried about the outcome that the Cabinet decided to make the matter one for an open vote Here again, Playfair's rational defence of compulsory vaccination was so much more convincing than the emotional arguments of his adversaries that he carried the vote with an overwhelming majority despite the original misgivings of the Cabinet

Playfair was elected president of the British Association for 1885, and took as the subject of his Presidential Address "Science and the State" For an appreciation of this address, see *Nature*, **200**, 7, 1963

Towards the end of Gladstone's second Government, in 1885, Playfair became aware that Gladstone intended to introduce a Bill creating a Minister for Scotland Playfair, who was proud of the record of Scottish education, was against the separation of Scottish affairs under a separate Minister His opposition was all the greater because this would involve the severance of English from Scottish education "This I highly disapprove", he said, "as I think it will be ruinous to Scotch education" His point appears not to have been so much any beneficial effect on Scottish education that might be gained by following an English example, as the fact that attempts might be made in Parliament to bring the Scottish pattern more into line with that in England if Parliament felt that the Scottish differences were being protected by a Scottish Secretary This may appear paradoxical, but Playfair presumably had much parliamentary experience behind his views

Vice-president of the Council

Playfair evidently felt very strongly on the matter, because he decided not to stand as a Liberal candidate for the Scottish universities again, since he felt that even his Liberal supporters were not united on this important educational point Instead, he stood, still as a Liberal candidate, for South Leeds, which was predominantly a working district The Liberals suffered a setback generally in the election, but Playfair himself fought a very successful campaign He appears to have addressed the working men practically as his intellectual equals, and they appreciated it Gladstone, when forming his third Government in early 1886, offered Playfair the post of vice-president of the Council, the holder of this post was effectively the Minister of Education In this office, Playfair carried through a Bill for the reform of the medical profession which gave, incidentally, direct representation to the great body of medical practitioners on the General Medical Council Gladstone's Government fell in 1886 again, on the Home Rule Bill for Ireland On this issue Playfair firmly supported Gladstone in his belief that home rule ought to be given Playfair himself was again returned for South Leeds, but the Conservatives came into office under Lord Salisbury

Celebrations

The following year was the Golden Jubilee of Queen Victoria; Playfair, as a Privy Councillor, witnessed much of the proceedings In 1887 there was also a major celebration of the Declaration of Independence in Philadelphia, the Americans were anxious to have Britain represented at their celebrations in Philadelphia, and they invited Gladstone and John Bright Neither of these men could go, and so Playfair went as the representative of the United Kingdom As we would expect, Playfair's speech was excellently fitted to the occasion He had to reply to the toast of "England, our Mother Home" The speech is too substantial to report at length He replied, he said, "With confidence, because England and the United States now know that they are the chief guardians of political liberty and constitutional Government throughout the world, and that they ought to be linked for ever more by the bonds of friendship and kinsmanship"

Later, on the same visit to the United States, he had the duty of presenting to President Cleveland a memorial in favour of a treaty of arbitration between Great Britain and the United States, signed by 233 members of the House of Commons, and originally prompted by the Peace Society of Working Men

At home, Playfair's measures were as practical as ever. In the same year, 1887, he carried through a Bill which for the first time legalized the sale of margarine as a substitute for butter

There was an election in 1892, which resulted in the return of the Liberals, Playfair again won South Leeds Gladstone, himself an octogenarian, had regretfully decided that he would have, in the face of public opinion, to shed some of the older of his colleagues, he therefore did not invite Playfair back to office, but offered him a peerage as a mark of appreciation Playfair accepted, and became Baron Playfair of St Andrews Even though he was now seventy-four, his public work continued He was appointed a Lord-in-Waiting to the Queen He had to refuse the chairmanship of several further Commissions, because his time was already full with the Aged Poor Commission, and with the work of the 1851 Commission

Tension with the United States

In 1895, Playfair's good relations with the United States were put to a severe test At that time, there was a border dispute between Venezuela and British Guiana In the disputed area which both countries claimed, the Venezuelans had granted concessions to various United States nationals As a result, the United States was drawn into the dispute, and President Cleveland, the same President to whom Playfair had only a few years before carried the Memorial of Arbitration, issued what was in effect an ultimatum to Britain He announced that he himself would set up a Commission and impose its solution on Britain, if necessary by force of arms This outburst, which appears to have been quite unwarranted, produced a severe shock Feeling both in the United States and in Britain mounted towards the stage where war might break out In these circumstances, Joseph Chamberlain, the Foreign Secretary, appealed to Playfair to act as intermediary This was a telling compliment to a man who was at an age normally beyond that of active politics, and who was not of the same party as the Government in power Playfair opened up a channel of contact through the American Ambassador in London, with whom he was on good terms Feeling on both sides accordingly relaxed, and Chamberlain was later able to take over the personal contact made by Playfair, and ultimately a friendly solution was reached, in which the official arbitration confirmed the British claims

The 1851 Exhibition Scholarships

In 1890 Playfair's work with the Royal Commission of 1851 produced another result of lasting value Working on a scheme drawn up by Sir Norman Lockyer [first editor of *Nature*], Playfair's committee proposed to introduce a scheme of scholarships to be supported out of the remaining profits of the Exhibition Writing of this scheme, on his retirement from the office of chairman of the Science Scholarships' Committee in 1896, he emphasized the prospective benefits that these might bring "Suppose, for instance", he said, "by this system one great discoverer like Faraday or Stephenson were to appear among the young scholars thus encouraged by £150 annual grants, a gift would have been presented to this country which would have been cheap had the nation contributed a million sterling to his production" Even while he was writing, this prospect was being fulfilled for in 1895 one of the scholars so appointed was Rutherford Since that time, the record of the 1851 Exhibition Scholars has been one of the most remarkable in science

The Victoria and Albert Museum

The 1851 Scholarships were not quite the last mark that Playfair left on the welfare of Britain In the Diamond Jubilee Celebrations of 1897, there were many projects suggested as the best way of commemorating it Having first ascertained that the Queen was favourably inclined, he wrote to *The Times* suggesting that one last aspect of the 1851 Exhibition had not yet been fulfilled This was to set up a fitting and permanent museum in South Kensington to house the science and art collections inaugurated by the Exhibition itself Playfair had chosen the right moment to strike, and his letter produced a general response Parliament was induced to grant £800,000, and on May 17, 1899, the Queen laid the foundation stone of the new building, henceforth known as the Victoria and Albert Museum

Epilogue

But before this came about, Lyon Playfair had quietly died on May 29, 1898, eight days after his eightieth birthday. The place that he had made for himself in his country's service has never since been quite filled For although a few other men of science have played important and distinguished parts in Government, and although we are thinking earnestly about the part of science in Government to-day, no man of science other than Playfair has risen through regular political means to a position of public responsibility such as he had

It may be worth remarking here that Playfair might never have been in Parliament had it not been for the creation of the Scottish university seats, he might thus have been lost to Parliament had he lived to-day When we recall the distinguished service that he himself and others gave through entering Parliament in this way, we may well regret the abolition of the university seats in the British Parliament If it is considered undemocratic to give university graduates two votes, could we not re-establish the university seats, and give each graduate the option of using either his university or his residential vote ? In this way, we would make it easier for men to follow Playfair's example of transition from academic to parliamentary life, without offending the most doctrinaire democracy

Playfair's own notes about his life were written, he said, partly "that they may form some encouragement to others who, like myself, have had in early life few friends and no influence, to believe that their future position depends upon themselves, and not upon their surroundings" That he had great ability in science is obvious from the attitude of Faraday and other men of science towards him, he could, had he been less interested in humanity, have continued in a happy and distinguished research career But the humane personality that enabled him "to walk with kings nor lose the common touch", equally at ease either discussing the 1851 Exhibition with the Prince Consort or parleying with the rioters of Lancashire, led him to the view that science in isolation was not enough Although he longed for the laboratory, his sense of public service led him on to one Royal Commission and enquiry after another Universities, schools, margarine, postcards, vivisection, vaccination, water supplies, Buckingham Palace, herring fisheries, mine explosions, coal for the Navy, cattle plague, the Civil Service, the 1851 Exhibition, sanitation, famine in Ireland, the aged poor all these and others he tackled with energy, humanity, insight and common sense Invited arbitrator between men and management in the mines, he stood in the gap at a critical time between his own country and the United States

He was not the only man of his time to see what science and technology could do for civilization, nor was he the first He may be held fortunate to have encountered great men like Peel and Prince Albert, who recognized his own greatness early enough for his qualities to be used to the full in his country's service He himself modestly characterized the Playfairs as following "occupations requiring solid, but not brilliant brain power" But he had far more than that He broke through all the barriers of learning, society and politics to take a unique place in British history, and he used to the utmost the opportunities that he thereby earned for the advancement of science and the welfare of humanity. What he did has in many ways made better the fortunes of each one of us to-day What he stood for, a century ago, is still a fresh inspiration

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INVESTIGATION OF NON-RECURRING PHENOMENA

THE RESEARCH CINEMA FILM

By E. R. SORENSON and DR. D. C. GAJDUSEK

National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland

APERIODICITY is often a matter of crucial interest and concern to science Investigators in many disciplines, faced with the fact of non-recurrence, or their inability to recreate phenomena in which they are interested, are confronted with the problem of recording, preserving, and retrieving data on non-reproducible events for future study The astronomer studying unique celestrial events, the physicist turning his attention to the dynamics of Brownian movement, and the biologist happening on anomalous or passing speciation are no less concerned with the data of such events than the physician investigating individual patients or new diseases, the anthropologist investigating disappearing cultures, and the historian trying to reconstruct the past Similarly, the analysis of the flight of a costly rocket or satellite, or the critical testing of machines and structures to their breaking point, often pose problems which, because of expense in time, effort, and materials, are often best treated as though they were non-reproducible In our own work on the programming of the human nervous system of unique subjects, we often wish to review brief episodes of the programming process which cannot be fully re-created[1-3]

Essential to retrospective investigation of such events is an accessible, reliable record from which data of the original, not to be repeated, occurrence can be preserved and retrieved to re-create, in part at least, the lost phenomenon We have, therefore, turned to two potent recording tools, the motion picture camera and sound recorder, to devise a method of preparing such records, where light and sound recording are applicable Although the desirability of such preservation of original exposed film has been obvious to many, the cost, time, and urge to edit have repeatedly resulted in irredeemable loss of valuable data, through disorganization of reel and sequence identification, derangement of the original time sequence, and discarded footage We suggest here the possibility of avoiding these difficulties and of maximally preserving the basic data in the original filmed record for future investigative use We are reporting elsewhere the detailed method of handling raw footage to create the research cinema film[4]

Data from Non-recurring Phenomena

That preserved data from non-recurring phenomena cannot serve in lieu of the actual events for rigorous scientific study is obvious Neither manipulation of variables nor experimental verification is possible Yet, historical records have often been a vital source material for otherwise unobtainable data on past events and have served to initiate new avenues to scientific enquiry Hypotheses and ideas derived from such survey of historical records may direct systematization of observations and the discovery of new relationships, they may determine the course of experimentation, and their further elaboration may then proceed hand in hand with the controlled laboratory investigations Darwin's observations and impressions during the voyage of *The Beagle* inspired his theory, a concept making possible more definitive investigations By reference to accumulated records of past observed positions of the Sun and planets, Copernicus made his discoveries Similarly, perusal of records personally collected or held by libraries, archives, or museums provides the basis from which theoretical constructs and methods of their validation emerge in such diverse subjects as astronomy, geology, archæology, palæontology, ecology, anthropology, medicine, etc In particular, original tacks and new directions in experimental investigation can be the result of perusal and reflexion on a mass of accumulated data of the past In astronomy, accurate historical records of celestial observations, carefully identified in time and place, are the material *sine qua non* for inspiring and testing many theories Only the accumulation of a mass of such records for study permitted the discovery of the underlying periodicity in many celestial phenomena and the amassing of the body of interrelated data now comprising astronomy The inception of the theory of relativity ultimately depended on this same record

The lyophilized or frozen storage of microbial strains from past epidemics provide living tools for the study of virulence, pathogenicity, and other genetic traits Once these are lost, they are as definitive a loss as the extinction of species of higher animals One may affirm that the preservation of the virus responsible for the 1918 influenza pandemic or that of the infectious disease antecedent to von Ecconomo's encephalitis in Europe in 1918–20 would have been a more valuable contribution to modern knowledge than any examination of the agent performed then or in the interval Sero-epidemiological studies supplying the data for charting, investigating, and predicting disease patterns make use of a file of blood sera which cannot be reproduced by re-bleeding the populations in question at a later date The value of the serum file increases with time, the more so with increasingly accurate and detailed documentation of the subjects and their ecological setting. Uses not envisaged at the time of collection become possible with technical advances in the laboratory

Specific antigens, such as blood group antigens from mummies, may be identified in properly preserved dried or frozen biological materials Pollen grains may be identified in lake sediments or burial remains, and examination of ancient bones and teeth can reveal much about the nutrition, diet, and disease patterns of ancient peoples Thus, archæology, palæontology, and anthropology draw on records of excavations, explorations, and labelled museum collections without which much of our knowledge of the development and evolution of life and man would not be possible

Much of linguistic science depends on historical records of contacts and shifts of peoples, and on the written records of languages no longer spoken If most of the writings of the Mayas had not been destroyed by the zealous *conquistadores* and, instead, had been preserved, we would very likely long ago have been able fully to decipher the Mayaglyphs, with all the implications to the studies of linguistics, anthropology, and the human mind which the unravelling of this apparently independently originated written language would entail

Stored materials most useful are those which permit new analysis or measurement of specimens or re-analysis of data from the past The collections of libraries, archives, and museums provide much of the material needed for such investigation, and from them it is possible to create sufficiently precise new theoretical constructs to suggest further methods of validation, as in the use of dendrochronology or carbon-dating to verify the age of objects in museums or *in situ*

The Research Cinema Film

In our effort to record the language, movement, and other actions of children in primitive cultures, and the influences which determine this behaviour, we turned to the motion picture camera and sound recorder to capture and preserve data for later study We needed a method to permit review of brief episodes in the programming of the human nervous system and to provide data for the delineation of the various cultural and environmental forces producing this programming We have been very concerned with preservation of information on child development available from the now rapidly disappearing unique, isolated, and divergent primitive cultures, a concern confronting us dramatically with unreproducible events and non-recurring phenomena Our methods of handling cinema records are, however, applicable broadly[3,5]

From entertaining a fantasy of having cinema records from the travels of Xenophon, Marco Polo, Cortez, Captain Cook, and Darwin, among others, we have come to realize that we would want, in addition to the photographic records, extensive documentation of other kinds, such as associated field reports, journals, diaries, logs, tape-recordings, maps, studies, specimens, or other pictures Furthermore, we would like to have a way to relate the films to other occurrences and their preserved records The most convenient way to do this is by time, place, and subject identification, the precision of which largely determines the extent to which the filmed record can be related within itself and to other records We would certainly want to have preserved all the cinema footage exposed, rather than that selected and edited according to the interests or tastes of the time, and we would want to know what interests governed the use of the camera The names of the filmers of each section of the research film are also important, so that their filming habits can be recognized and taken into consideration when attempting to evaluate the filmed record

Film, more easily than most other types of records, can become the source of information and observation unforeseen by the filmer The camera, even when aimed at specifically chosen material, invariably picks up, unapprehended and unanticipated, incidental surrounding information which we may neither understand nor recognize at the time Review of such data can be the basis for uncategorized, intuitive impressions supplying the clues to fruitful avenues of enquiry Fleeting situations can be captured on film for leisurely review at a more propitious time, the speed of motion of the photographic record can be modified as needed, as in time-lapse photography or high-speed motion pictures, and random use of the camera can yield records of information which we have not yet attempted to apperceive, recognize, or evaluate

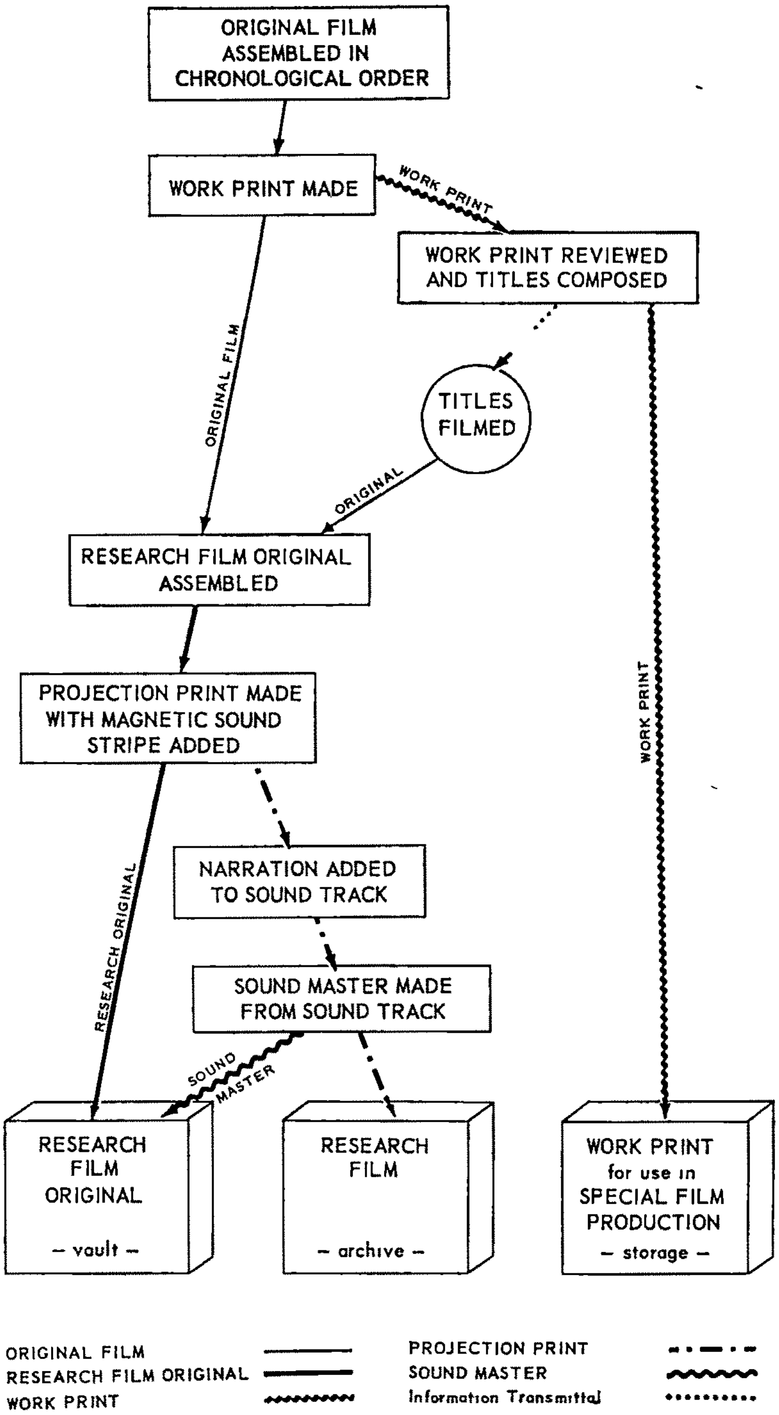
ORIGINAL FILM ASSEMBLED IN CHRONOLOGICAL ORDER
WORK PRINT MADE
WORK PRINT
WORK PRINT REVIEWED AND TITLES COMPOSED
ORIGINAL FILM
TITLES FILMED
ORIGINAL
RESEARCH FILM ORIGINAL ASSEMBLED
PROJECTION PRINT MADE WITH MAGNETIC SOUND STRIPE ADDED
NARRATION ADDED TO SOUND TRACK
SOUND MASTER MADE FROM SOUND TRACK
RESEARCH ORIGINAL
SOUND MASTER
WORK PRINT
RESEARCH FILM ORIGINAL – vault –
RESEARCH FILM – archive –
WORK PRINT for use in SPECIAL FILM PRODUCTION – storage –
ORIGINAL FILM
RESEARCH FILM ORIGINAL
WORK PRINT
PROJECTION PRINT
SOUND MASTER
Information Transmittal

Fig 1 Flow chart showing steps in preparation of research films

In order to exploit fully these virtues of the camera, we have developed the research film as a cinema record of discrete occurrences, cross-filed with related data, in which the original time sequence of the filming has not been disrupted or altered, the subject-matter of concern to the filmer has been identified in time, place, and person, and the professional bias and interests of the filmer and sponsor have been indicated

Technique for Research Film Production The beginning and end of each film are governed by the nature of the subject and the amount of film taken Large amounts of footage from a single time and place related study or survey are divided into parts not more than 1,600 ft in length (in the case of 16-mm film), for the sake of convenient storage, and natural divisions are used as much as possible in determining the beginning and end of each part The original exposed reels of film are developed, spliced together, and copied in a work print The work print is reviewed to check the accuracy of order, and technically unusable footage, such as that unexposed, totally light struck, or decomposed, is cut out.

The main title of the film includes the name of the event or subject under study, precise and detailed geographic identification, the purpose of the filming, and the dates or times covered by the film. Preliminary credit titles show the disciplinary bias, names of filmers, and sponsorship. Major subdivisions of the research film are indicated by appropriate titles which signal changes in time, place, or subject sought by the filmer; they are determined by the filmer while reviewing the work print, on which he marks their intended locations. When these titles have been composed and photographed, the work print is used as a guide for inserting them into the original footage.

Unusable footage, previously cut out of the work print, is also removed from the original, and corrections in time sequence are made, in the event that some sections were inadvertently out of order. A projection print is copied from the trimmed and titled original, and a magnetic recording stripe is put on to one edge. The original research film is then permanently filed in a film storage vault, and the copy for viewing is placed in a research film archive, after the filmer has narrated comments from memory or notes or transferred any synchronously recorded field sound on to its magnetic sound stripe.

Information giving background to, or revealing influences affecting, the behaviour of the specifically pictured subject are recorded on the sound track. The sound track may also serve to alert the viewer to imminent material which may otherwise pass before notice can be made. Unusual names or terms mentioned in the sound track also appear in the titles. Maximum identification is sought, and, whenever the identifying material is too long to be handled in titles or the sound track time is too brief to contain the needed additional information, appended typescripts or charts are keyed to the appropriate sections of the film by reference to the title covering the section or, when necessary, more precisely by the edge or frame numbers. In accompanying note-books, field journals, or logs, data relating to the filming and the subject-matter are preserved at greater length, and the interests guiding the camera are further revealed.

Special Films. One of the important uses of the research films will be to provide the material for special purpose documentary, scientific, demonstrative, or educational films, without disrupting the original research film document. Indexing and edge-numbering the research film make it possible to select and locate the needed sequences. If the originally made work print has not already been cut and used for such special showing, it may serve as the work print for the planned special film. When the value of the special film is such that many copies are desired, a printing master of the appropriate footage can be made from the original film and assembled with whatever other material might be required. From this printing master the desired number of special film copies can be made.

In the profession of motion pictures we discovered immense pressures stemming from the traditional uses of motion pictures for specific scientific investigation, educational purposes, or for promotion or entertainment, which tended to direct all film endeavours, including those of science, into diversionary, didactic, or documentary productions. The paraphernalia and methods of the industry were organized to demonstrate particular attitudes, beliefs, or desires from specially ordered observation, or to fabricate illusion through the reorganization of realistic, credible images. However, in spite of the greater coherence and smoothness of presentation of carefully edited and reorganized film, it became increasingly clear, as we developed our research film method, that no cutting, rearranging, abstracting, or correlating of scenes from original footage would be permissible in research films and, in particular, the time sequence of the original footage could not be disrupted. The research films were to be neither a summation of information, a demonstration of a conclusion, nor the imposition of an already structured idea or system of knowledge, but rather a source of material for viewers with investigative interests in events of the past. Contrary to the production of usual motion pictures, the work on preparing research films is principally that of extensive identification of subject-matter in its original time sequence and correlation of the filmed data with other associated material.

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EVIDENCE FOR LIFE ON MARS

By Dr. D. G. REA

Space Sciences Laboratory, University of California, Berkeley 4

IN 1954, de Vaucouleurs[1] wrote, "As for the idea, so often expressed, of a different kind of life adapted to such conditions (that is, on Mars)—what might be termed a 'generalized life'—it remains to a large extent beyond the realm of positive science".

This approach does not seem to have had much appeal, and most authors since then have stressed the positive aspects, generally overlooking contradictory observations. Indeed, the reader would undoubtedly conclude that various observations of Mars indicate considerably better than an even chance for the existence of life there. My opinion is that the evidence has been distorted and that a re-examination is required. An examination of the scope necessary will not be undertaken here, rather only a few observations intended to illustrate this point will be presented.

The observational arguments for the existence of Martian life are (1) the various colours, including green, exhibited by the dark areas; (2) the seasonal changes in the visual albedo and polarization of the dark areas; (3) the ability of the dark areas to regenerate after an extensive 'dust storm'; (4) the presence of 2,700–3,000 cm^{-1} 'absorption' bands, attributed to organic molecules.

It has been shown recently that the last, most recent, point is at least doubtful[2]. The argument on the basis of the colours is also inconclusive. Kuiper[3] demonstrated the absence of the near infra-red reflexion maximum, characteristic of most green plants, indicating that chlorophyll was not responsible for the colour. In this context Sinton has noted that this does not exclude chlorophyll since there may be present other pigments absorbing the near infra-red radiation. This would actually be expected since any Martian plants would probably evolve into forms reflecting the barest minimum of the sparse solar radiation available. It has been suggested by many workers that the colour is a contrast effect with the bright reddish continents The meagre quantitative data have been discussed by Öpik[4], who has reduced photometric observations of Kozyrev on the very dark area Syrtis Major to intrinsic reflectivities by factoring out the estimated atmospheric attenuation and reflectivity.

The result is that this area is bluer than a continent, but its colour is still reddish His reduction is questionable since he assumes the surface-scattering properties to be similar to the Moon in the sense that the disk would be uniformly bright if no atmosphere were present In fact, quantitative studies by Dollfus[5] show the surface to have reflecting properties in orange light intermediate between lunar and Lambert surfaces However, the qualitative conclusion that Syrtis Major has a red tint seems reasonable While these considerations all weigh against a chlorophyll-based plant life, the lichen systems suggested by Kuiper (but criticized by Salisbury) can satisfy the observed colours, real or imaginary The broad-leaved plants envisaged by Salisbury must have a chemical composition completely unlike any such terrestrial species, but this is to be expected The significant point is that there is no positive colorimetric evidence pointing to forms of vegetation with which we are familiar

The second and third arguments remain the most cogent, but the second loses some of its allure when one considers the severity of the Martian climate Focas[6] has photometered several dark regions on Mars from plates taken over a Martian year All reveal a seasonal change in the albedo with a distinct darkening wave moving from pole to pole with the seasons An area showing one of the greatest seasonal changes is Depressio Hellespontica, located at about 60°–65° S latitude Now the temperature at this location, according to the data of Lampland as reduced by Gifford[7], reaches a maximum of –23° C for the southern summer This temperature has an uncertainty due, among other factors, to the assumptions that the surface radiates as a black-body and the atmosphere is transparent The latter is reasonably valid for the 8–13-μ terrestrial window used for the observations, but the first has been questioned by Öpik, who believes the emissivity to be closer to 0 75 than to 1," by analogy with terrestrial substances" The net effect is to raise the Martian temperatures by about 5 per cent, that is, about 13° C, making the environment more hospitable to life Actually there are few data in the literature on the emissivity of terrestrial surfaces, but at least one report[8] indicates that the emissivities are higher Even accepting this low emissivity and possible systematic errors, it seems unlikely that the summer temperature of Depressio Hellespontica could be raised to 0° C Accepting this, and knowing the uninviting composition of the atmosphere, it appears highly improbable that any life, based on water as solvent, can exist in this region with the activity necessary to explain the darkening variations Schemes can no doubt be envisaged to circumvent these objections, but they must surely be very special If a non-life mechanism must be invoked to explain the seasonal change of the albedo for this one dark area, it is reasonable to assume it is applicable to the other dark regions and that this property cannot be used as an indication of life, even at the equator where the temperatures are more equable

Together with the seasonal change in the albedo a concurrent variation in the visual polarization has been observed by Dollfus[9–11] For the autumn and winter seasons in a particular hemisphere the polarization of the corresponding dark areas has a negative minimum of about –0 012 at a phase angle of 11°, and then increases with a value of 0 0 occurring at 25° In the spring and summer the dependence of the polarization on phase angle changes so that for the maximum difference, in late spring, the minimum polarization is –0 015 at a phase angle of 13°, and the zero point occurs at a phase angle of 30° The shift in the polarization curve cannot be due to atmospheric effects but must arise from changes in the surface Dollfus says this must be a change in the surface texture which in turn is most likely due to the proliferation of highly coloured micro-organisms when the water vapour from the subliming polar cap reaches a particular area Attempts to duplicate the Martian behaviour using terrestrial biological samples on inorganic substrata were unsuccessful, but this is not a strong counter argument since life-forms are highly adaptable and would presumably differ on Mars from terrestrial ones

However, there does not seem to be justification for relying so heavily on a life interpretation If the argument on the seasonal albedo variation is accepted then presumably an inorganic interpretation must also be found for the polarization variation Two possibilities based on the very extensive laboratory work of Dollfus[12] can be suggested First, it is not necessary to invoke a change of texture to explain the change in the polarization curve If the surface material becomes more absorbing in the spring then both the albedo and polarization will change in the manner observed Although no suitable mechanisms have been proposed to produce this darkening, the possibility should not be overlooked Secondly, a change in surface texture in which the surface becomes rougher will also explain the data Dollfus has demonstrated such a difference between samples consisting of the same small particles but where one has a smoothed surface and the other a surface roughened by stirring He concludes that the agglomeration of particles into larger entities increases the multiple reflexion of light in the surface and shifts the polarization curve In the case of Mars, the surface could be roughened by the removal of small loose particles by appropriate winds The material remaining would then be small particles partially cemented together into larger particles, perhaps similar to the "fairy castles" of Hapke and van Horn[13]

The rejuvenation property of the dark areas is one aspect which can be basically accepted as pertinent, although, as Salisbury has pointed out, a lichen cover would not be expected to behave in this manner Higher forms of plant life such as Salisbury favours would possess this property

While certain observations seem to favour life, others are very difficult to explain by this hypothesis The normally bright region Hellas was dark during the 1954 opposition but returned in 1956 to its position as the second brightest area on the planet, only the polar caps exceeding it in reflectivity[14,15]. Any form of plant life which, under Martian conditions, can so rapidly expand over and then retreat from an area of 290,000 square miles must be truly unique Other non-seasonal changes in the extent of the dark and bright areas are equally difficult to explain by a life hypothesis

As an alternative to the interpretation of life on Mars, I prefer an inorganic explanation based on ideas of McLaughlin[15] and Kuiper[16] Assuming volcanoes, both active and dead, and suitable winds, the phenomena can be interpreted in a reasonably consistent manner Thus the temporary darkening of Hellas would be ascribed to a film of volcanic ash produced by an active volcano just prior to the 1954 observations This volcano then became passive and the winds proceeded to scour the area (McLaughlin considers it to be a high plateau since "it is often snow-covered, and in winter forms a vast extension of the polar cap down to latitude 30°"), restoring it to its previous brightness Other non-periodic changes would be explained in like manner, involving appropriate combinations of volcanic ash, lava and winds

The regenerative feature according to Kuiper is then achieved by atmospheric circulation Presumably this involves dust being blown on to the dark areas (postulated by Kuiper to be lava and not ash) during the late summer, autumn and winter, and then removed during the spring Öpik objects to such an interpretation, his principal argument being that radiometric and polarimetric measurements indicate that "the soil of the dark areas seems to be of the same grainy nature as that of the bright areas" This is not convincing, since volcanic lava can have optical and thermal properties which are not dissimilar to those of the dark areas—similarity of certain optical and thermal properties for two materials does not guarantee that they are of the same nature Öpik also states that the presence of life is indicated by the permanence of the dark markings in spite of the numerous extensive dust storms. Without a regenerative property "during millions of centuries the dust storms would have covered the entire planet with a deep layer of dust " The significance of this statement is decreased by his acceptance of a surface which has a non-uniform elevation (also see Dollfus[14]) This is intended not as a complete treatment of the volcanic-wind model but as an indication of its real possibilities I am inclined to favour it, although not rejecting the interpretation of life on Mars

The question of life on Mars has intrigued scientists for decades Owing to the possibility of being able to verify directly the life hypothesis in the very near future it is becoming a subject of widespread study One approach is to determine if certain terrestrial organisms can exist, perhaps in a modified form, in the Martian environment Workers in this field have been encouraged by the recent positive identification of water vapour in the Martian atmosphere[17,18] The second approach is to investigate whether the various observed Martian phenomena suggest that life exists there. It is to this area that the present article is addressed On the basis of this brief discussion it is suggested that the life interpretation is very tenuous and that inorganic interpretations be given greater credence

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PROPOSAL FOR AN EDUCATIONAL RESEARCH COUNCIL AND SOME OF ITS IMPLICATIONS

By PROF R. D'AETH
Department of Education, University of Exeter

THE recent submission to the Minister for Science of a proposal for an Educational Research Council raises important questions about the future development of research in education, and also indirectly about the future relationships of research councils with the Government The memorandum making the proposal, which was supported by the Heads of Departments and Institutes of Education of the Universities of England and Wales, calls for a council with responsibilities similar to the three long-established research councils It is evident that the proposal will have to be considered on its own merits, and also as part of a movement to persuade the Government to provide money on a broader front for research of national importance

The Medical Research Council dates back to 1913, the Department of Scientific and Industrial Research to 1916, and the Agricultural Research Council to 1931 What is striking is that since then, in a period of more than thirty years, the only addition has been the Nature Conservancy Yet during this period there has been a growing appreciation of the need for more research in fields of national importance The budgets of the three large research councils have increased enormously, and there has been increasing pressure, especially in recent years, on the Government to give more support to research in education, and in economics and the social sciences generally

The three large research councils were started at the time of the First World War and of the subsequent severe economic depression The vital importance of scientific research was demonstrated even more sharply in the Second World War Since then, in two decades of intense economic competition, scientific research has proved to be just as crucial for the nation's survival and progress But there has been one very significant new realization that for the nation's prosperity it is equally important to develop its resources of human skill and vigour to the fullest possible extent Sir Eric Ashby, this year's president of the British Association, highlighted this point of view in his address when he made a searching analysis of Britain's "Investment in Man" at the level of higher education (*Nature*, 199, 877, 1963) In it he pointed out that, although more than £70 million a year is spent on universities, the expenditure on studying the process of teaching and learning in them is negligible Overall he estimated that expenditure on research in education is less than 1/20 of a penny in every pound spent on education This agrees with other estimates which have similarly put expenditure on educational research at about 0 02 per cent of expenditure on education. This would lead to rapid bankruptcy in most enterprises and education has become a large-scale enterprise on which the Government alone spent more than £1,000 million last year, far more than on any other public service except defence The consequence of this neglect of research is that decisions involving the expenditure of millions of pounds, and affecting the lives of generations of young people, are often taken on fragmentary evidence, or policy is shaped by a committee lacking information, or outmoded ways are carried on through engrained custom

Here the question naturally arises To what extent are educational problems susceptible to research of a scientific kind? Are not value judgments commonly involved? The answer is certainly that they are, and that a scientific approach cannot embrace education as a whole, even when the scope of this elastic word is restricted to the influences of 'organized' education, or of educational institutions, on children growing up But it is equally true that many aspects of education can be studied scientifically, as a few examples will show. For example, there has been much scientific research on the preparation of standardized mental tests for diagnosis, selection or measuring attainment Again, the psychological study of infants and young children has already cast rays of light into their mental growth and how they learn Or to take a more specific example, scientific research has transformed the teaching of deaf children Many more examples could be given, yet each of them brings to mind many associated problems which await investigation, and often projects have to be started empirically because there has been no underlying theoretical research The map of educational research is largely uncharted, not because of difficulty over techniques—it is significant that a number of research projects started in the past five years with grants from Foundations have begun to yield results quickly—but through lack of money and skilled research workers

Let us turn back, however, to the question of 'value judgments' for a moment They are inherent in educational thinking overall and there are obvious dangers if such things as '11 + tests', however admirable scientifically, are seen too much in isolation It is salutary also to remember that scientists cannot exclude 'value judgments' from their research, and are often entangled by it over the applications of research Are birth control, abortion, euthanasia morally right? Should the scientist whose research develops nuclear bombs feel any responsibility over their possible use ?

The growing realization of the need for more educational research has already led to more money being made available The Nuffield and other Foundations have given grants for a number of projects, and the Ministry of Education has also been able to make grants for research during the past two years Two of the new universities are going to study teaching at university-level, and the Robbins Committee is likely to stress the need for more educational research in its forthcoming report on the development of higher education The amounts of money available are still relatively very small, but they could be increased substantially If this were done, what would be the advantages in having an Educational Research Council ? Behind such questions there lurks the natural fear that pressures might lead to other research councils springing up like mushrooms, and this is presumably what the small committee set up by the Government in June, with Lord Heyworth as chairman, will look at, its terms of reference being "To review the research at present being done in the field of social studies in Government Departments, universities and other institutions, and to advise whether changes are needed in the arrangements for supporting and co-ordinating this research" The proposal for an Educational Research Council will no doubt be considered by this Committee in due course In the meantime, however, it is worth looking at the arguments in its favour

The main task of an Educational Research Council would be to encourage and give financial support to research in education, not simply to consider projects submitted by universities and other institutions of learning, but to assess critically the needs and opportunities for research judging the urgent needs of the present, deciding in the light of varied research experience which long-odds ideas or unorthodox investigations should be taken on as risk ventures, looking ahead to problems of the future This means watching the field as a whole, shaping not so much a clear programme as a continually growing strategy, looking not only at educational problems, but also at developments in other fields that might become significant—as has been proved to be necessary in medicine and agriculture and in the application of science to industry The province of the Council should be all of what might be termed 'organized education' not just schools, but also universities, technical colleges, the training of teachers, the use of radio and television and so on, seeing them as interrelated parts of an overall picture

The giving of grants for research projects will soon prove ineffective unless guided by a long-term and developing policy, taking account of both research needs and opportunities and also the training and career prospects of the required specialists It would be necessary to grant enough studentships for training, and also fellowships for the conduct of research, ensuring reasonable career prospects in research while retaining ease of transfer between teaching and research It is these linked functions, exercised over primary, secondary and higher education, which provide the main argument in favour of an Educational Research Council But there is also another aspect which would be important in the long run, namely, the need to keep the planning of research free from the day-to-day control of the executive departments of Government That is why the existing research councils were established under the Lord President of the Privy Council, and retained their independence when transferred in 1959 to the same person in his new capacity as Minister for Science and it was for the same reason that the proposal for an Educational Research Council was submitted to the Minister for Science There is, however, an additional reason in the case of education, in that a number of institutions, including the universities, do not come under the Ministry of Education The most valuable link between the Research Council and the Ministry of Education would be in the application of research findings, and here the Ministry has already taken a very significant step in proposing the formation of a Curriculum and Examinations Council

The nature of the responsibilities of the proposed Research Council, and the need for it to gain the respect —as the three large research councils have—of universities and other institutions of higher education, requires that its members should be of recognized standing, and that the Council should be small enough to be effective in action There would be little need to argue this point among the leaders in industry and commerce But there are sad examples within the sphere of education of committees so large as to be rather incoherent, so representative that they are almost restricted to compromises between set points of view, and which are sometimes only kept alive by the efforts of the chairman and secretary These are weaknesses which the three large research councils have avoided The oldest of them has now been established over half a century, and it is worth considering the reasons for their success They have had direct, not advisory, responsibilities They have had the services of the leading research experts The spheres of activity covered by them—medicine, agriculture and the application of science to industry—have been distinct enough to avoid confusion and overlapping, and they are of acknowledged national importance They have been answerable to the Government in broad terms for the proper discharge of their responsibilities, and not for the details of their programmes These are the conditions which have enabled them to function with vigour and imagination

It is a question for serious thought whether these conditions would apply to a research council for the social sciences, and one with which the Heyworth Committee will obviously be concerned A significant feature of the three large research councils is that the coherence of each comes from its field of application They are not related to single academic disciplines Rather the reverse, for the Medical Research Council is concerned with the various branches of medicine, biochemistry, physiology, bacteriology, psychology, demography, molecular biology and so on Likewise the Agricultural Research Council draws on the various branches of agriculture, botany, zoology, genetics, chemistry, economics and so on The Department of Scientific and Industrial Research also covers a wide academic spectrum An Educational Research Council would have a similar coherence of application, but it is difficult to see how a research council for the social sciences in general could do so

Universities draw Government money for research from two main sources from the University Grants Committee and from the three large research councils. The expenditure of the former was more than £70 million last year, while the budgets of the latter exceeded £30 million. By no means all the latter went to universities or technical colleges, for the three councils run large separate research institutions. Yet the tendency for money going to universities through the University Grants Committee to be closely tied to increased numbers of students makes it more difficult to get money for research, and is unlikely to be reversed. In consequence the research councils have a powerful influence on the development of research in universities, which is related to the policy for dividing advanced scientific research, especially that requiring very expensive equipment, between universities and specialized research institutions. Hence the two main sources from which universities receive money for research exercise quite different influences the University Grants Committee reaches a co-operative agreement on the form of the growth of each university, the three councils plan, in their respective spheres of application, the development of scientific research nationally. If the arguments outlined here carry weight—that there is an important need for more educational research, and that there are several linked functions which should be planned on a national basis—then the claim for an Educational Research Council is strong. The expenditure involved would be very small in comparison with the existing research councils. It would not by itself have any effect on the present situation as regards the importance of scientific research, and the growing expenditure by the Government on it. But these may lead, in any event, to a review of the machinery of Government which would affect the independence and responsibilities of universities, the University Grants Committee and perhaps even the research councils, which have shown how planning on a national basis can be combined with adequate freedom and scope for initiative for those engaged in scientific research.

NEWS and VIEWS

Bacteriology at Glasgow: Prof. R. G. White

Dr. Robert G. White, reader in bacteriology at the London Hospital, has been appointed to the Gardiner chair of bacteriology at the University of Glasgow in succession to Prof. J. W. Howie, who has been appointed director of the Public Health Laboratory Service Board (see *Nature*, 197, 749, 1963). Dr. White was educated at the Queen's College, Oxford, and qualified from the London Hospital Medical College in 1942. He gained his D.M. at Oxford in 1953. After a period of training in bacteriology under Sir Samuel Bedson in the Emergency Medical Service Laboratory at Epping (1943–44) and of supervising the Central Emergency Medical Service Laboratory in the University of Liverpool (1944–45), he spent two years of service with the Royal Navy as a specialist in bacteriology. On his return to the United Kingdom he joined the Department of Bacteriology at the London Hospital, first as registrar in pathology and later as Freedom Research Fellow. He was appointed reader in bacteriology under Prof. C. F. Barwell in 1954. Visits to the United States were made during this period when, in 1952, he spent a year with Dr. A. H. Coons at the Harvard Medical School as a Medical Research Council Travelling Fellow, and in 1961 when he was travelling professor to the University of Florida and worked there with Prof. E. Suter. Dr. White's research interests have been concerned with a wide range of experimental work bearing on such topics as cellular reactions in immunity, the adjuvant activity of mycobacterial peptido-glycolipids, the role of the thymus gland and auto-antibody responses.

The Rensselaer Polytechnic Institute: Prof. J. D. Mackenzie

Dr. J. D. Mackenzie has been appointed to the newly established professorship of materials science at Rensselaer Polytechnic Institute, Troy, New York. The appointment will strengthen the teaching and research of non-metallic solids in the Department of Materials Engineering at Rensselaer. Dr. Mackenzie graduated at Birkbeck College, London, in 1952 with honours in chemistry, and was awarded a Ph.D. and the Diploma of Membership of Imperial College of Science and Technology in physical chemistry by Imperial College of Science and Technology, London, in 1954. He was a Post-doctoral Fellow at Princeton University from 1954 until 1956. In 1956 he was elected to an Imperial Chemical Industries fellowship at Cambridge, and became a Fellow of the Royal Institute of Chemistry in 1958. Since 1957 he has been with the General Electric Research Laboratory, Schenectady, New York.

The Institute of Electrical and Electronics Engineers

A United Kingdom and Eire Section of the Institute of Electrical and Electronics Engineers (IEEE) has been established to serve the interests of the Institute's members in Great Britain and Ireland resulting from the merger of the Institute of Radio Engineers and the American Institute of Electrical Engineers to form the IEEE. The new Institute is a 'non-national' professional society of some 160,000 members, organized in nine Regions and with learned society activities designed to serve the expanding field of electrical and electronics engineering. Membership of the United Kingdom and Eire Section is made up of IEEE members of all grades who are living in Great Britain or Ireland and represents about half the present membership of the European Region of the IEEE, the territory of which is bounded by the Urals and the north coast of Africa and includes other sections in Benelux, Egypt, France, Israel, Italy, Norway and Switzerland.

The officers of the United Kingdom and Eire Section are *Chairman*, Dr. R. C. G. Williams, *Vice-Chairmen*, Sir Harold Bishop and Sir John Hacking, *Treasurer*, Dr. R. L. Smith-Rose, *Secretary*, Mr. F. S. Barton; *Assistant Secretary/Treasurer*, Mr. R. C. Winton. Until the Section Executive Committee is established, its functions are being carried on by the Advisory Committee of the IEEE in the United Kingdom and Eire which acts on behalf of the Board of Directors of the IEEE. The aim of the Section will be to achieve close co-operation with the Institute of Electrical Engineers, the British Institute of Radio Engineers and other professional societies, which between them already hold several hundred meetings, colloquia and conferences a year throughout Great Britain and Ireland covering all aspects of the electrical and electronic field. In these circumstances, the role of the Section will be primarily to join in or initiate joint meetings or conferences, and to carry out the special function of providing a two-way link with professional activities in the United States and elsewhere. In this way more opportunities will be created for lectures and papers by American engineers in Britain and by British engineers in the United States. Further information can be obtained from Mr. J. R. Smith, 2 Savoy Place, London, W.C.2.

British Computer Development Project

The first contract in a four-year £1½ million joint research project, which is planned to aid long-range British computer development, has been agreed between the Department of Scientific and Industrial Research and Mullard Research Laboratories This will extend Mullard's existing programme of research in low-temperature computer devices, and, spreading over two years, will cost a total of £100,000, to which the Department will contribute half The project is designed to support the British computer industry's already considerable research and development efforts in the face of future widespread technological change Twelve major British electronics firms are involved, as well as Government laboratories The National Physical Laboratory at Teddington will act as a co-ordinating centre for the programme as a whole in close association with the Ministry of Aviation's Royal Radar Establishment at Malvern About two-thirds of the total estimated cost will be borne by the Department of Scientific and Industrial Research, the remaining third being provided by those industrial firms which accept contracts About one-third of the total sum will be expended in the National Physical Laboratory and the Royal Radar Establishment, and the remaining £1 million will be spent in industry in the form of development contracts The first contract is concerned with low-temperature computer devices It is known already that superconducting stores are feasible, and, in particular, research will be carried out on one form of superconducting element—the cryotron—as a storage device It is most likely that the cryotron will also be used in the selection circuits of superconductive stores, and in regular arrays of switching circuits such as might occur in stores with special logical properties, sophisticated stores of this type may first become a practical proposition by the use of superconducting techniques The work to be done under the contract has three aspects First, the greater understanding of the physics of the cryotron, secondly, the further development of methods for laying down arrays of film with the desired purity and geometric form, and thirdly, the use of the devices in computer stores.

The Applied Scientific Research Corporation of Thailand

A National Scientific Research Organization is to be set up in Thailand under the leadership of Mr Frank Nicholls, of Melbourne, who has drawn up the plan for mobilizing Thailand's scientific resources to develop the country's industries

Mr Nicholls is international co-operation officer of Commonwealth Scientific and Industrial Research Organization (Australia) He has spent most of the past three years in South-East Asia, mainly investigating the scientific resources of developing countries He was a member of the Science Commission appointed by the President of Pakistan in 1959 to study that country's scientific work He has spent three periods in Thailand preparing the way for the establishment of the Applied Scientific Research Corporation of Thailand, and he drafted the Act recently passed by the Thai National Assembly for that purpose He has now arrived in Bangkok to head the team of some twenty international experts working under the United Nations, which is establishing the Research Corporation on the lines of the Commonwealth Scientific and Industrial Research Organization The Research Corporation of Thailand will be concerned with research in agriculture, industry and health It will examine ways of preparing for the growth of new industry, of supporting existing industries, exploiting mineral resources, finding new uses for plant products, and developing textile and building materials The Thai Government has taken the initiative for setting up the Research Corporation and has already earmarked ample funds for the work of the initial five-year period Aid will also be given from the U.N. Special Fund and several international foundations

Properties and Applications of New Ceramics

A booklet, *New Ways with Ceramics*, issued by the Department of Scientific and Industrial Research and written with the co-operation of the British Ceramic Research Association, outlines the properties and applications of the new and improved ceramics which have been developed to meet special industrial requirements, especially for work at high temperatures (Pp 12 London: Information Division, Department of Scientific and Industrial Research, 1963) Outstanding among these is boron nitride, which is easily machined, impermeable and has excellent electrical and corrosion-resistance properties up to 1,650° C Others described are alumina ceramics, boron carbide, silicon carbide and the cermets, which are complexes of metals and metallic oxides adjusted to bring out desired characteristics.

Bovey Valley Woodlands National Nature Reserve

The Nature Conservancy has declared the Bovey Valley Woodlands a National Nature Reserve One of the earliest National Nature Reserves to be acquired (1952) was Yarner Wood in the Bovey Valley, north-west of Bovey Tracey on the eastern edge of Dartmoor Recent plans for extensive conifer plantations, in some cases replacing scientifically interesting semi-natural woodlands, led the Nature Conservancy to review their Nature Reserve plans and to begin adding a number of further small woodlands to the Yarner nucleus Three such adjoining woodlands, Rudge (36 acres), Woodash and part of Houndtor Wood (61 acres), have been purchased as a step towards forming an enlarged Bovey Valley Woodlands National Nature Reserve within the Dartmoor National Park The upper reaches of the River Bovey between Lustleigh Cleave and the little valley including the well-known Becka Falls contain one of the best remaining series of Dartmoor native hardwoods Rudge Wood occupies one side of the steep slopes at the lower or southern end of the valley and extends through a short succession of wet and boggy fields, carrying much willow, alder and birch, almost to the southern extremity of Houndtor Wood The latter takes in the very steep northern flank of Houndtor Ridge, which shows the geological transition from the Culm through the metamorphosed Culm to the Granite, where it adjoins Woodash Wood Oak is the predominating tree throughout the Reserve, and the two species, pedunculate and sessile, are both well represented Ash is frequent mainly on the damper alluvial sites and also on granite-derived soils The woodlands are rich in the smaller forms of plant life, such as mosses, liverworts and lichens, especially along the river margins, where large strewn boulders and damp shade provide a favourable habitat for them. Access to the Reserve away from the public footpath will be by permit only Applications to visit the Reserve should be addressed to the Regional Officer (South-West), Furzebrook Research Station, Wareham, Dorset

Last Essays of R. E Snodgrass

Over a period of nearly forty years, R E Snodgrass published in the *Smithsonian Miscellaneous Collections* a series of masterly articles on the anatomy of insects At the time of his sudden death in September 1962, at the age of eighty-seven, Snodgrass was engaged in writing a kind of encyclopædia of insect anatomy. About a fifth of this had been completed and has now been edited by Dr A Glenn Richards and published as a final contribution in the *Smithsonian Miscellaneous Collections* under the title "A Contribution Toward an Encyclopedia of Insect Anatomy" (146, No 2 Pp v+48 Publication 4544 Washington, D C Smithsonian Institution, 1963) This was well worth publishing It takes the form of a succession of brief essays on most of the terms used in descriptions of the external anatomy of insects. Snodgrass wrote always a fine style of lucid English. He had a feeling for words, an interest in their etymology, and a gift for clear definitions. All these characteristics are evident in this contribution.

Prof. Eugène Pittard

THE *Bulletin de la Société Suisse d'Anthropologie et d'Ethnologie* for 1962–63 contains an excellent appreciation of the late Prof. Eugène Pittard of Geneva, who died a little while ago, aged ninety-five. Pittard was a delightful individual full of enthusiasm. One always looked forward to meeting him at archæological and anthropological congresses and similar functions. He started out as a zoologist, but soon found that anthropology was his real interest and vocation. He was no narrow specialist; everything concerning man, whether physical or cultural, interested him. Thus when prehistoric archæology blossomed towards the start of the present century Pittard was one of its foremost exponents. The University of Geneva eventually, in 1916, provided him with a professorial chair, and three years later with a suitable laboratory. In these days of narrow specialization it is pleasant to recall one who had wide interests and yet was always in the forefront of knowledge. He wrote many works and the 'appreciation' concludes with a list of his more important publications.

The Bowles Memorial Travel Scholarship

THE Bowles Memorial Travel Scholarship was established by the Royal Horticultural Society as a memorial to the late E. A. Bowles (1865–1954). The object of the Scholarship is to enable the holder to travel abroad for either or both of the following purposes: (*a*) the collection of plants for introduction to British gardens; (*b*) the study of practical horticulture in other countries. For the years 1964 and 1965 a sum of £500 will be made available for award. Applicants for the Scholarship must be of the male sex, not more than twenty-eight years of age on January 1, 1964, and be a holder of the National Diploma in Horticulture, or a student of, or graduate in, horticulture or botany at a British university, or a past or present student-gardener at the University Botanic Garden, Cambridge, the Royal Botanic Garden, Edinburgh, the Royal Botanic Gardens, Kew, the Royal Horticultural Society's Gardens, Wisley, or other horticultural educational establishment. Applications should be sent, by November 30, to the Secretary of the Royal Horticultural Society, Vincent Square, London, S.W.1, from whom further information can be obtained.

The Night Sky in November

FULL moon occurs on Nov. 1d. 13h. 56m. U.T., new moon on Nov. 16d. 06h. 51m. and full moon on Nov. 30d. 23h. 55m. The following conjunctions with the Moon occur: Nov. 18d. 04h., Venus 3° S.; Nov. 18d. 07h., Mars 3° S.; Nov. 23 05h., Saturn 2° N.; Nov. 27d. 00h., Jupiter 4° N. In addition to these conjunctions with the Moon, Mars is in conjunction with Antares on Nov. 6d. 16h., Mars being 4° N.; Venus with Antares on Nov. 12d. 11h., Venus being 4° N.; and Venus with Mars on Nov. 20d. 22h., Venus being 0·05° S. Mercury, Venus and Mars are too close to the Sun for easy observation. Jupiter sets at 4h. 30m., 3h. 25m. and 2h. 25m. on November 1, 15 and 30, respectively. It is in Pisces, its stellar magnitude is −2·4 and its distance from the Earth on November 15 is 385 million miles. Saturn sets at 23h. 10m., 22h. 15m. and 21h. 25m. at the beginning, middle and end of the month, respectively. It is in Capricornus, its stellar magnitude is +0·9, and its distance from the Earth on November 15 is 920 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Nov. 3d. 20h. 09·5m., 97 Tau (*R*); Nov. 4d. 4h. 11·1m., 106 Tau (*R*). *R* refers to reappearance. The Taurid meteors are active during the first fortnight of the month, but conditions for observation are unfavourable. The Bielid meteors are expected about November 15, the radiant being near R.A. 1h. 32m., Dec. +43°; conditions for observation are favourable. The Leonid meteors are active during November 14–17 with a maximum on November 16–17, the radiant is near R.A. 10h. 8m., Dec. +22°, and conditions for observation are very favourable.

Announcements

DR. J. H. HODGSON, chief of the Seismology Division of the Dominion Observatory, Canada, has been elected president of the International Association of Seismology and Physics of the Earth's Interior. Dr. Hodgson is a director of the Seismology Society of America as well as being a member of various international committees. He was assistant professor of geophysics in the University of Toronto from 1945 until 1949.

THE British Institute of Radiology is holding a meeting on October 17, at which the presidential address "Nothing New?" will be given by Prof. C. B. Allsopp. Further information can be obtained from the General Secretary, British Institute of Radiology, 32 Welbeck Street, London, W.1.

A MEETING to celebrate the centenary of Dr. L. H. Baekeland (1863–1944), the inventor of 'Bakelite', arranged by the Royal Flemish Society of Engineers and the Flemish Chemical Society, will be held in the State University of Ghent during November 22–23. Further information can be obtained from Dr. R. Thiers, Ingenieurshuis, Jan van Rijswijcklaan 58, Antwerpen I.

TWELVE public lectures on "The History of Medicine" will be delivered by Dr. Edwin Clarke in the Wellcome Historical Medical Library, London, on Tuesdays and Thursdays commencing October 15. Further information can be obtained from the Wellcome Historical Medical Library, the Wellcome Building, Euston Road, London, N.W.1.

A MEETING of the Biochemical Society will be held in the Department of Physiology and Biochemistry, the University of Southampton, on October 18. Included in the programme will be a colloquium on "Comparative Biochemistry and Physiology" (chairman, K. A. Munday). Further information can be obtained from H. R. V. Arnstein, National Institute for Medical Research, the Ridgeway, Mill Hill, London, N.W.7.

A SYMPOSIUM on "The Natural History of Aggression", arranged by the Institute of Biology, will be held at the British Museum (Natural History) during October 21–22. Topics under discussion will include: ritualized fighting in animals; aggression in societies of apes; the physiological background to aggression; aggression and mental illness; the origins of war; possible substitutes for war; the nature of aggression as revealed by the atomic age. Further information can be obtained from the Institute of Biology, 41 Queen's Gate, London, S.W.7.

A PRACTICAL four-week residential course in "Administration and Management of Research and Development", arranged by the Comparative Administration Trust, will be held at the Sundridge Park Management Centre, Bromley, during November 10–December 7. The aim of the course is to improve the practical effectiveness of those engaged in administration and management of technical work in industry, in industrial research associations or publicly sponsored research establishments. Topics to be considered will include: the nature of the individual and of the team; the teams in their wider context; special problems of science and technology. Further information and forms of application can be obtained from the Comparative Administration Trust, 30 St. George Street, Hanover Square, London, W.1.

THE ROCKEFELLER FOUNDATION

BESIDES discussing some major innovations in the Foundation's programme in 1962, the President's Review of the Rockefeller Foundation, 1962*, notes some features of the current and operating programmes In medical education substantial grants were made in 1962 to the medical schools of the University of Minas Gerais in Brazil and of the University of Cuyo in Argentina to assist in the establishment of a pre-medical curriculum and strengthen research and teaching The University of Khartoum was assisted in reorganizing its Department of Physiology and the King George's Medical School, University of Lucknow, the development of its Department of Pharmacology, while the Medical Training Centre of the Ministry of Health and Labour of Tanganyika received a grant towards a new training programme for rural physicians Help was also given to the Departments of Preventive Medicine and Pædiatrics, Makerere University College, in developing a field research and training centre in preventive medicine at Kasangati, to the Institute of Child Health of the University of Ibadan, and to the All-India Institute of Medical Science, New Delhi, in developing a teaching and research unit in rural medicine

In the humanities and social sciences a current grant is helping the International Press Institute, Zurich, to promote more effective use of the daily newspaper press in the countries of south and south-east Asia, and the Educational Broadcasting Corporation, New York, was assisted in the initial operation of an educational television channel Among research projects supported in the field of land economics, one at the University of Arizona, Tucson, relates the availability of water to social and economic growth in an arid environment, and programmes in agricultural economics are being supported at the Catholic University of Chili, Santiago In agricultural sciences, on June 30, 1962, 236 students from 28 countries held Foundation scholarships or fellowships for M Sc or Ph D courses at 40 different universities in the United States and Europe Continued support was given to an orientation programme for foreign students entering graduate schools of agriculture in the United States, the Texas Agricultural and Mechanical College System received a grant for studies with a mechanical device for measuring the nutritive value of forage crops during the digestive process in ruminants, and the University of Arizona a grant for investigation of the economic use of scarce water supplies, while establishment of an animal nutrition research centre at Nova Odessa, São Paulo, Brazil, was also assisted

For virus work in 1963 and its operating programme in the general development of professional education the Foundation appropriated some 1 5 million dollars, and the Review outlines the work which led to the discovery of the new virus, oropouche, and the investigation of arboviruses For co-operative projects in agricultural sciences in five countries, the Foundation has appropriated more than 2 6 million dollars in 1963 in addition to 515,000 dollars for the International Rice Research Institute These projects include the Mexican Agricultural Programme, now in its twentieth year, and similar projects in Colombia, at first concentrated on corn, wheat, potatoes and beans, but now yielding results from livestock research of great value in improving beef and dairy cattle, poultry, sheep and swine, Chile (concentrated on wheat and forage legumes and grasses), and India, where hybrid maize varieties adapted to all the major agricultural regions have been developed

During 1962, 723 persons from 58 countries held Foundation fellowships and scholarships, of which 265 were new awards, of the total, 298 were in agricultural sciences and 231 in medical and natural sciences; 3 2 million dollars were appropriated for these activities

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* The Rockefeller Foundation President's Review, 1962 Pp v+87 (New York The Rockefeller Foundation, 1962) See also p 97 of this issue of *Nature*

CONGENITAL ABNORMALITIES

THE decreasing rate for infant mortality in Great Britain and other parts of the world is bringing greater interest to bear on congenital abnormalities, which, apart from the effects on the children, cause so much distress to their parents The greater part of the August issue of *The Practitioner*[1] is taken up with a symposium on the subject

As C O Carter points out, it was not until 1956 that new cytological techniques confirmed that human beings have a normal complement of 46 chromosomes Non-disjunction may result in gametes being produced possessing 22 or 24 chromosomes Other abnormalities arise through fragmentations, with parts of separate chromosomes coming together in irregular ways, the remaining bits becoming lost About 2 per thousand babies are trisomic for the sex chromosomes, of the patterns XXX, XXY, and XYY Some of these arrangements produce no obvious defect, but all XXY persons suffer from Klinefelter's syndrome, with a eunuchoid body build and azoospermic testes In contrast to these, XO individuals exhibit Turner's syndrome, with dwarfism and abnormalities of the heart and kidneys About 2 per thousand babies are either trisomic for autosomes such as $G21$, $E18$, and $D15$, or they may have a compound of chromosomes $D15$ and $G21$, from which other parts may be lost All these are held to be suffering from Down's syndrome, exhibiting a great variety of derangements, such as deafness, polydactyly and mosaicism As Carter suggests, these findings provide some limited scope for genetic counselling

The most familiar case of abnormality associated with a chromosome derangement is that of the infant mongol This is discussed by A J Keay The affected children have 47 chromosomes, probably through the trisomy of $G21$ The clinical signs are easily recognized, and the intelligence quotients of the affected children range from about 25 to 68 These children are cheerful and responsive to affection, they all should be given some chance of responding to educational care The risk of producing a mongoloid child depends on the age of the mother, amounting to about 1 in 1,000 for young women and rising to around 1 in 50 for those of more than 40 years

The existence of inborn errors of metabolism inherited on a Mendelian basis was first noticed by Folling in 1934 That was a case of phenylketonuria The frequency of incidence is about 1 in 30,000, and the disease is now known to be due to a recessive gene which leads to an inability to metabolize phenylalanine properly, and the affected children show hypertonicity of the muscles and are on the verge of imbecility They respond favourably to a diet low in phenylalanine if it is started sufficiently early This and other inborn errors of metabolism are described by A Goldberg They include alkaptonuria, non-endemic familial cretinism with goitre, some forms of diabetes, galactosæmia, glycogen deposition disease, and many other abnormalities concerned with the metabolism of adrenal steroids, purines, metals, lipids, hæmoglobin and the clotting of blood, porphyrins, bile pigments and renal tubular transport In most cases the abnormalities are the result of enzyme irregularities dependent on recessive genes As Goldberg remarks, it would seem that no metabolic pathway is exempt from the occasional, and sometimes not so occasional, genetically expressed deviation

In their paper on congenital abnormalities of the limbs, J I P James and D W Lamb show that these may take many forms the absence of a hand, arm or fibula, for example, extra digits, syndactyly, 'club foot', etc , along with an extensive list of less-serious defects Some of them appear to be the result of chromosome derangements, others are known to be produced by agents of external origin, while congenital dislocation of the hip is now thought to be brought about by maternal hormones, especially relaxin Clubbed feet and related defects can be improved by gentle manipulation and strapping at an early age, a number of other abnormalities call for surgical treatment, but great care should be taken not to remove stumps of limbs which can be made use of in prosthetic replacement, with which, if in a simple form, the child should be allowed to grow familiar as soon as possible

A D M Jackson deals with congenital abnormalities brought about by agents of external origin, of which the most general are infections Leaving aside infections gaining entry at the act of birth, like gonococcal ophthalmia, and those passed on from mother to fœtus at a late stage in pregnancy, like chickenpox and Coxsackie *B* virus disease, Jackson draws on the investigation initiated by the Ministry of Health and General Register Office, in 1950, and a still larger one in Sweden These investigations show that the most serious abnormalities are due to infections of rubella during the first three months of pregnancy The effects are seen in higher rates than normal for abortions and stillbirths, low birth weight, cataract, heart defects, and deafness The risk that a child will be born with some affliction is about 30 per cent, with deafness as the commonest, deafness appears to be the only serious handicap if the mother becomes infected at later stages in pregnancy To avoid these defects the mother should be immunized against rubella before marriage, even passive immunization within a few days of exposure may be of some help

The British investigation also covered the risk of abnormalities produced by other infections, such as measles, poliomyelitis and influenza, but no clear association between infection and abnormality could be established

Among the congenital defects produced by external agents of an artificial kind, the tragedy of the thalidomide episode has directed attention to those resulting from drug treatment J M Robson deals with these Thalidomide itself is a mild sedative, even in large doses it produces scarcely any toxic effect on man and animals Why it should exert such a drastic influence on the fœtus is a question which still remains obscure There are other drugs, like sodium salicylate and nicotine, in wide clinical use which have not yet been shown to be teratogenic in man although in high doses they have serious effects on the fœtuses of experimental animals A number of other drugs seem to act in much the same way The action on man of 5-hydroxytryptamine still awaits elucidation, it is naturally present in the body, but in large doses it produces many malformations in animals, probably because of its interference with placental function Robson recommends that in introducing new drugs for clinical use great caution should be exercised over those which are teratogenic in experimental animals, particularly if there is a wide discrepancy between the dose which has a harmful effect on the fœtus and that which is toxic to the mother

The remaining paper in the symposium, by J W Millen, is on the effects of radiation He refers to the large amount of work which has been done on experimental animals many congenital abnormalities have been produced, affecting every organ system, and depending on the time, intensity and nature of the radiations, together with the stage in pregnancy Experiences with therapeutic irradiation have shown that similar defects can be produced in man The effect appears to be directly on the fœtus, not mediated by the mother Surveys of the results of the atomic bombs exploded in Japan have reported many cases of microcephaly, which is the only defect that can be linked directly with the intensity of exposure, but other effects may have been masked by fœtal deaths So far there is no convincing evidence that mutations can be produced by irradiation in man, and the risk of harmful mutations being caused by the background radiation of present-day living would seem not to be serious

R WEATHERALL

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[1] *The Practitioner*, **191**, No 1142 (August, 1963)

WORLD DIRECTORY OF VETERINARY SCHOOLS

A *World Directory of Veterinary Schools** has recently been published by the World Health Organization, Geneva, under the auspices of the Food and Agriculture Organization of the United Nations and the World Health Organization There has, in the past, been no available source of information about veterinary schools as a whole, and the *Directory* will be of great use to all those concerned with veterinary science

During the past decade and a half there have been great changes and extensions in veterinary schools The end of the Second World War led to considerable changes in certain European countries, in some instances the changes were merely the result of opportunity after hostilities had ended and there was return to normal life, a matter merely of delay Altered frontiers and other political matters resulted in changes The achievement of self-government in certain former British territories and in those of other European powers has been an important cause of development Whereas there were 6 schools prior to 1947 in India there are now 16 Pakistan has 2

The details for each country are set out according to a standard plan under the same sets of headings, beginning with tables showing the population of the country, the numbers of livestock and details of veterinary activity In the case of each country a table is given of the veterinary schools, date of foundation, numbers of teaching staff, total enrolment and tuition fees Information is given for each country concerning administration, conditions of admission, curriculum and examinations

Scrutiny of the *Directory* shows extraordinary disparity in the numbers of graduates, the numbers of veterinary schools and the numbers of livestock in a country It is of interest to compare essential figures given for livestock populations and the numbers of veterinarians The figures for the United Kingdom concern only the numbers resident in the country, and about 1,800 of the graduates are resident overseas A defect in the quotations of essential figures given in the first table for each country is that there has been some or even considerable delay in reporting them, those given for the United Kingdom refer to 1959 Unfortunately, the wording used in these tables is not very lucid

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* *World Directory of Veterinary Schools*, 1963 (Published under the auspices of the Food and Agriculture Organization of the United Nations and the World Health Organization) Pp 216 16 Sw francs, 26*s* 8*d* , 5 25 dollars

The following table is made up from data in the book It has been prepared by placing the order of the countries according to the numbers of the larger farm animals horses, cattle, camels and domestic water buffaloes, for the purposes of demonstration of animal wealth the smaller farm animals can be ignored This table shows the enormous farm animal wealth of India, with more than double the numbers of any other country It also brings out the very small numbers in many countries of great prominence

Farm livestock (large animals) (millions)	Veterinary schools	No of veterinarians	
207	16	5,583	India
96	18	20,861	United States
85	8	2,325	Brazil
83	23	× × ×	U S S R
44	3	2,000	Argentine
32	3	508	Mexico
32	2	490	Pakistan
20	3	4,379	France
18	1	185	Kenya
17	1	1,262	Turkey
16	2	1,200	Australia
13	2	456	Colombia
13	4	8,297	German Federal Republic
12	1	430	Union of South Africa
11	1	109	Thailand
11	3	4,500	Poland
11	6	4,660	United Kingdom
10	2	2,000	Canada
10	9	7,700	Italy
9	1	56	Sudan
8	2	191	Indonesia
7	1	478	Iran
7	1	445	Uruguay
7	1	300	Venezuela
6	4	2,947	Yugoslavia
5	1	133*	Romania
4	2	2,050	Czecheslovakia
4	2	750	Ireland
3	1	1,076	Denmark
3	14	18,589	Japan
3	1	1,379	Netherlands
2	1	1,601	Austria
2	2	948	Belgium
2	1	× × ×	Greece
2	1	2,550	Hungary
2	1	47	Iraq
2	1	896	Sweden
1	1	596	Norway
1	2	936	Switzerland
1	1	60	Viet Nam

* This figure would seem to be incorrect.
× × × No figure given

W. A Pool

NATURAL GAS IN THE NETHERLANDS

THE first natural gas field in Holland was discovered and developed by the N V Nederlandse Aardolie Maatschappij (N A M) in 1948 in the province of Drenthe, at Coevorden The gas-bearing formation is at a depth of more than 9,000 ft Coevorden proved to be a small but exploitable field Later, other small gas fields were discovered in the north-eastern provinces of Drenthe and Overijssel, also in the western province of South Holland, but the amount of gas available was relatively small, some 520,000 cubic yards per day, and at that rate a calculated twenty years' supply In terms of energy this quantity of gas per day is equivalent to about 150,000 tons of coal per year, in 1954 the coal production of the Netherlands amounted to 12 million tons, thus the quantity of natural gas available in that year did not represent more than 1 per cent of the total coal production

It was not until August 1959 that the natural gas situation was dramatically changed by the drilling of a well at Slochteren in the province of Groningen which proved the existence of an enormous gas-field, actually the third largest in the world, Panhandle, Texas, being the largest, followed by the Hassi R'Mel gas-field in the Sahara as second After Slochteren No 1 (as it was called), there followed successful strikes of gas at Delfzijl and Slochteren (No 2) in 1960, at Ten Boer in 1961, at Nordbroek in 1962 and at Schildmeer in February 1963 all in the province of Groningen

The total proved natural gas reserves in the Groningen province alone are so far estimated at some 520,000 million cubic yards, the largest in Europe Compared with the French gas-field at Lacq in France, with estimated reserves of 230,000 million cubic yards, the Groningen field is more than double the size A further factor is that the geographical spread of this natural gas deposit may not as yet have been completely determined, possibilities of extensions under the North Sea by off-shore drilling, equally eastwards into West Germany, are by no means unlikely

Production from the Gronginen region will be carried out by N A M , a joint venture of the Esso Petroleum and Shell Companies N A M and the State Mines Department of the Netherlands have formed a partnership "under which they will share the costs and the proceeds of the development of the natural gas on a basis of 60 and 40 per cent respectively" " N A M will deliver all the Groningen natural gas to a new company called N V Nederlandse Gasunie, which was formed on April 6 this year Forty per cent of the new company is owned by the State Mines, 25 per cent each by Esso and Shell and the remaining 10 per cent by the Dutch Government Its function is to purchase, transport and market natural gas in the Netherlands" Further information on these extremely important developments is given in an illustrated article in a recent issue of *Esso Magazine* (12, No 3, 1963) H B Milner

SEARCH FOR PETROLEUM IN AUSTRALIA

IN 1900 there was an accidental discovery of natural gas at Roma, some 300 miles west-north-west of Brisbane, Queensland Since that time there have been many short-lived and unsuccessful attempts to locate oil and gas deposits in commercial quantities in that area But prospecting for oil still continues with persistent enthusiasm and cautious confidence not only in this region but also in other potential places in Australia As recently as 1959 the Commonwealth Government enacted the Petroleum Search Subsidy Act enabling exploration companies to carry out geophysical or bore-hole surveys for new sub-surface stratigraphical information or in prospecting for petroleum The Act provides for subsidy for the cost of operations subject to approval of projects by the Minister of National Development On behalf of the Minister, the Bureau of Mineral Resources, Geology and Geophysics must examine and approve applications made under the Act, maintain surveillance of the operations and, in due course, publish the results Thus has been provided a new stimulus in the time-long search for oil in this continent and four publications recently issued by the Department of National Development (Bureau of Mineral Resources) clearly demonstrate that the provisions of the 1959 Act are working in a most practical manner*

Publication No 8 deals with Frome Rocks No 1 and No 2 Wells, located about 100 miles east-south-east of Broome on the north-west coast of Western Australia, the operations being carried out by West Australian Petroleum Pty , Ltd , No 1 penetrated 734 ft of Recent deposits, thence through normal Jurassic sequence to the Frome Rocks Salt Dome No signs of hydrocarbons were seen No 2 was drilled to more than 7,500 ft , but results were not significant This area does not appear very promising

Publication No 35 takes us back to Roma once again, it deals with the East Roma Seismic Survey, carried out in 1959–60 by Austral Geo Prospectors Pty , Ltd , on behalf of Associated Australian Oilfields N L The conclusion is reached "The complex geology of the East Roma area is such that there are a multitude of positions which are favourable for the accumulation of oil and gas . . The East Roma area should be given first priority for continued drilling and further seismic exploration programmes It seems certain that there is gas in commercial quantities"

Publication No 36 describes the Blue Hills–Logue Seismic Survey, Western Australia, also made in 1959–60 by West Australian Petroleum Pty , Ltd The survey area is some 50 miles south-east of Broome The results clarify the delineation of known anticlinal structures in this area, but petroleum prospects are so far not disclosed

Publication No 39 concerns the Longreach–Silsoe Seismic Survey, Queensland, carried out by Cree Oil of Canada, Ltd , in 1960 This again resulted in fresh structural interpretations in this area, but beyond some oil and gas at certain horizons previously known, there appears to be little encouragement to anticipate commercially valuable petroleum deposits here.

Despite all these results, however, the search for oil and gas still proceeds in Australia, and if the vast gas-fields of the Netherlands and the Sahara (traditionally considered unlikely regions for hydrocarbon accumulations in commercial quantity) are anything to go by, then there is ample reason for prosecuting that search

H B MILNER

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* Commonwealth of Australia Department of National Development Bureau of Mineral Resources, Geology and Geophysics Publication No 8 *Frome Rocks No 1 and No 2 Wells, Western Australia of West Australian Petroleum Pty , Limited* Pp 55+4 plates Publication No 35 *East Roma Seismic Survey, Queensland, 1959–1960, by Associated Australian Oilfields N L* Pp 18+6 plates Publication No 36 *Blue Hills–Logue Seismic Survey, Western Australia, 1950–1960, by West Australian Petroleum Pty , Limited* Pp 24+3 plates Publication No 39 *Longreach–Silsoe Seismic Survey, Queensland, 1960, by Cree Oil of Canada Limited* Pp 10+5 plates (Canberra Department of National Development, Bureau of Mineral Resources, Geology and Geophysics, 1962)

DETERMINATION OF THE NON-ZONAL HARMONICS OF THE GEOPOTENTIAL FROM SATELLITE DOPPLER DATA

By DR. W. H GUIER

Applied Physics Laboratory, Johns Hopkins University, Silver Spring, Maryland

A LEAST squares determination of values for the coefficients, C_n^m, S_n^m, of the non-zonal harmonics of the geopotential·

$$U(r,\varphi,\lambda) = \frac{K}{r}\left\{1 + \sum_{n=2}^{\infty} \sum_{m=0}^{n} \frac{P_n^m (\sin \varphi)}{r^n} \left[C_n^m \cos m\lambda + S_n^m \sin m\lambda\right]\right\} \quad (1)$$

has been made through $n=m=4$ using Doppler data from the three Satellites 1961 o1, 1961 $\alpha\eta$1, and 1962 $\beta\mu$1 In equation (1)

r = geocentric radius from the Earth's centre of gravity, φ = geocentric latitude, λ = geocentric longitude from Greenwich meridian,

and $P_n^m(z)$ are the associated Legendre polynomials

$$P_n^m(z) = (1-z^2)^{\frac{m}{2}} \frac{d^m}{dz^m} P_n(z) \quad (2)$$

The values of C_2^1 and S_2^1 were constrained to be identically zero in the determination

Table 1 presents a brief summary of the Doppler data which were chosen for the determination The data were chosen as being the best available from the point of view of (1) quality with respect to noise-level, timing errors, presence of ionospheric refraction errors, and stability of the satellites' transmitted frequencies, (2) uniform distribution of station locations over the Earth's surface with particular care taken to avoid an overweight of data from stations located within the continental United States, (3) uniform coverage of the satellite orbit by the data with respect to the angle between the ascending node and the Greenwich meridian

The principal ionospheric refraction contribution was removed by coherently combining two frequencies received simultaneously from the satellite in the Doppler receiving equipment[1,2] To ensure further minimum refraction errors, data were considered only if the maximum elevation of the pass was less than 83° and greater than 20° Finally, data points which corresponded to instantaneous elevations below 13° were deleted, and the remaining data points corrected for tropospheric refraction[3] A typical noise-level for that data finally chosen was roughly 5 parts in 10^{10} of the transmitted frequency Timing errors were judged to be below 0 003 sec (3-sigma value)

The data which survived the foregoing criteria were then grouped by days, one group containing all passes for a given satellite which occurred between two successive midnights (G M T) After grouping, only those were selected which contained ten or more high-quality sets of data from at least five separate stations with at least three being outside the continental United States From Table 1 it can be seen that the fewest groups of data for any one satellite occurred for Satellite 1961 $\alpha\eta$1 This is due to the low inclination of the satellite precluding reception of data from high-latitude stations While considerably more groups of data satisfying the foregoing criteria were available for the other two satellites, the total number of groups chosen for each satellite was limited to being roughly equal to avoid overweighting the data corresponding to a particular inclination In the final choice of groups care was taken to ensure a reasonably even distribution of angles between the ascending node and the Greenwich meridian at the time of the first pass in each group In addition, groups for two different satellites occurring on the same day were avoided to increase the *a priori* probability that the effects of undetected station instrumentation errors and refraction errors were random

Table 1. SUMMARY OF DOPPLER DATA USED

Satellite designation	Inclination (deg)	Semi-major axis (km)	Eccentricity	No. individual satellite passes	No distinct station loc.	No data groups (satellite orbits)	Average No. passes per group	Average time span per group (h)
1961 $\alpha\eta 1$	32 4	7,410	0 010	87	9	8	11	15 9
1962 $\beta\mu 1$	50 1	7,510	0 007	199	15	9	22	22 7
1961 o1	66 8	7 320	0 008	155	13	10	16	23 3
			Totals	441	18	27		

Having made the final choice of groups, a least-squares determination of a satellite orbit for each group was made, resulting in no computed arc of the satellite motion lasting more than 24 h Orbit parameters spanning data over such short time-intervals were chosen because (1) the satellite trajectory could be integrated numerically with negligible error and made unnecessary the very tedious task of solving the perturbed equations of motion to high orders, (2) unknown fluctuations in atmospheric drag and errors in the values for the zonal harmonic coefficients introduce negligible errors

The computer programme used to determine the orbits[4] includes the Sun and Moon forces, although they should introduce negligible errors for these short data spans The resulting differences between the Doppler data points and the corresponding computed values for the Doppler shift (based on fixed initial values for the harmonic coefficients of the geopotential) were used as the final data for the least-squares determination of the differential changes in the values of the non-zonal harmonic coefficients

Very briefly, the least-squares procedure used for determining the non-zonal harmonic coefficients was the following The differences between the theoretical and experimental values for the Doppler shift were assumed to arise wholly from errors in the locations of the tracking stations and values for the non-zonal harmonic coefficients It was further assumed that because of these errors there existed errors in the orbit parameters associated with each group of data Perturbation equations were then derived to express these residuals as a function of differential changes in the three co-ordinates of each tracking station represented in the totality of data, differential changes in each of the six orbit parameters for each group of data, and differential changes in the values of the C_n^m and S_n^m of equation (1) A special IBM 7094 computer programme was designed and coded in which the differential values of the station positions and orbit parameters are continually adjusted to yield an extremum of the mean-square data residuals with respect to these parameters (irrespective of whether the resulting values are reasonable) for any given values for the differential harmonic coefficients Consequently, at any point in the computing process, changes in the values of the differential station and orbit parameters can produce negligible reduction in the mean-square data residuals In this way the station co-ordinates and orbit parameters are effectively eliminated from the least-squares determination of the harmonic coefficients The programme is then iterated until stable values for the differential non-zonal harmonic coefficients are obtained

Table 2 presents the results of the determination using the data summarized in Table 1 Without new data at a different inclination, it is very difficult to estimate the true error in these values, and no attempt has been made to ascribe a realistic error to each coefficient The fact that the station parameters were effectively eliminated from the determination ensures that the principal error in the resulting values for the coefficients arises from correlated errors in the experimental data and not from erroneous values for the station locations Consequently, the efficacy of this method depends principally on there being sufficient experimental data of sufficient accuracy that the build-up of computer error combined with experimental errors does not destroy the sensitivity of the data residuals to the harmonic coefficients once the station and orbit differential parameters are eliminated Several numerical experiments were performed with the amount of data intentionally reduced to the point where a realistic solution would not be expected The objective of the experiments was to obtain criteria for the number of harmonic coefficients which could be evaluated with the data available As expected, these experiments indicated that if stable values for the coefficients were produced with successive iterations, and if their resulting values did not change markedly with changes in the relative weighting of the data residuals, the final values should be trustworthy

Table 2 VALUES OF THE NON-ZONAL HARMONIC COEFFICIENTS

n	m	C_n^n	S_n^m
2	2	$1\ 68 \times 10^{-6}$	$-0\ 64 \times 10^{-6}$
3	1	$1\ 77 \times 10^{-6}$	$0\ 19 \times 10^{-6}$
3	2	$2\ 86 \times 10^{-7}$	$-0\ 25 \times 10^{-7}$
3	3	$1\ 48 \times 10^{-7}$	$1\ 41 \times 10^{-7}$
4	1	$-5\ 69 \times 10^{-7}$	$-4\ 60 \times 10^{-7}$
4	2	$0\ 60 \times 10^{-7}$	$2\ 66 \times 10^{-7}$
4	3	$7\ 90 \times 10^{-8}$	$-0\ 28 \times 10^{-8}$
4	4	$-7\ 85 \times 10^{-9}$	$6\ 56 \times 10^{-9}$

Recognizing that the Tesseral harmonic, $n=2$, $m=1$, should have negligibly small coefficients, a final check on the validity of the values in Table 2 was made by performing a new determination with C_2^1 and S_2^1 added to those coefficients listed in Table 2 The resulting values for those coefficients in Table 2 were not materially altered and the values for the new coefficients were found to be

$$C_2^1 = 1\ 78 \times 10^{-8},$$

$$S_2^1 = -\ 3\ 48 \times 10^{-8} \qquad (3)$$

The fact that they are negligibly small (compared with the values in Table 2 for $m=1$) lends considerable credence to the values given in Table 2

A comparison between the values given in Table 2 and the present literature[5-8] indicates agreement of roughly the same character as the agreement between any two previously published sets of values Consequently, such comparisons add little additional information on the accuracy of the values presented in Table 2

Clearly the overall effort involved in obtaining these results was very large, and it is impossible to acknowledge all the people who made valuable contributions However, I wish to acknowledge especially the contributions of R R Newton and S Yionoulis for their aid in developing the satellite perturbation equations, G Worsley, G C Weiffenbach, P E P White, and their colleagues in connexion with obtaining the experimental Doppler data, and H D Black, C Weisert, R Henderson, and their colleagues for their help in coding and running the complex computer programmes involved

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EXACT INTEGRATION OF THE SPACE-CHARGE EQUATION FOR A PLANE DIODE: A SIMPLIFIED THEORY

By DR. D. H. PARSONS

Department of Mathematics, University of Reading

THE relationship between current and potential difference in a planar vacuum diode has been investigated theoretically in two different ways. Child[1] and Langmuir[2] first supposed that all electrons are emitted from the cathode with zero velocity, and that the potential gradient is zero at the cathode. These hypotheses led to the well-known 'three halves power law'. Schottky[3], Epstein[4], Fry[5], Adams (unpublished), Langmuir[6,7] and Blodgett[7] assumed a Maxwellian distribution of velocities of the emitted electrons, and thereby obtained a satisfactory theory. But the latter hypothesis has the drawback that exact integration of the differential equation is impossible, so that a new function has to be calculated numerically.

We shall generalize the simple Child–Langmuir case, making two hypotheses. We suppose (1) that all electrons are emitted from the cathode with the same velocity, this velocity being independent of the anode potential, and small compared with the final velocity attained on reaching the anode, and (2) that the ratio of the (negative) potential gradient at the cathode to the (positive) gradient at the anode is independent of the anode potential.

By using a technique different from that of Child and Langmuir, we shall integrate the differential equation exactly, and obtain a simple relationship between current and potential difference which agrees extremely well with that calculated by Fry[5], and, independently, by Langmuir[6] for a specific example.

Using C.G.S. and electrostatic units, let a be the distance between the electrodes, u the initial velocity of an emitted electron, v its velocity at a distance x from the cathode. Let the potential of the cathode be zero, and let V, V_a be respectively the potential at a distance x from the cathode, and the anode potential. Let m be the mass, and $e(>0)$ be the magnitude of the negative charge of an electron, let I be the current per unit plate area, and $\rho(<0)$ the charge density at a distance x from the cathode. Then we have, from the equations of energy and continuity, and Poisson's equation

$$\left.\begin{aligned} \tfrac{1}{2}mv^2 &= \tfrac{1}{2}mu^2 + eV \\ I &= -\rho v \\ \mathrm{d}^2V/\mathrm{d}x^2 &= -4\pi\rho \end{aligned}\right\} \qquad (1)$$

Let

$$u = 2^{1/2}(e/m)^{1/2}\varepsilon > 0 \qquad (2)$$

Then from (1) and (2) we have

$$v = 2^{1/2}(e/m)^{1/2}\sqrt{(V + \varepsilon^2)} \qquad (3)$$

$$\mathrm{d}^2V/\mathrm{d}x^2 = 4\pi I/v$$

and thus

$$\frac{\mathrm{d}^2V}{\mathrm{d}x^2} - \frac{4\pi I}{2^{1/2}(e/m)^{1/2}\sqrt{(V+\varepsilon^2)}} = 0 \qquad (4)$$

The second-order equation (4) admits two integrating factors, one less obvious than the other. Thus we have

$$-\frac{2\mathrm{d}V}{\mathrm{d}x}\left\{\frac{\mathrm{d}^2V}{\mathrm{d}x^2} - \frac{4\pi I}{2^{1/2}(e/m)^{1/2}\sqrt{(V+\varepsilon^2)}}\right\}$$

$$\equiv \frac{\mathrm{d}}{\mathrm{d}x}\left\{\frac{16\pi I}{2^{1/2}(e/m)^{1/2}}\sqrt{(V+\varepsilon^2)} - \left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)^2\right\} = 0$$

and

$$3\left\{\left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)^2 - \frac{8\pi I}{2^{1/2}(e/m)^{1/2}}\sqrt{(V+\varepsilon^2)}\right\}\left\{\frac{\mathrm{d}^2V}{\mathrm{d}x^2} - \frac{4\pi I}{2^{1/2}(e/m)^{1/2}\sqrt{(V+\varepsilon^2)}}\right\}$$

$$\equiv \frac{\mathrm{d}}{\mathrm{d}x}\left\{\left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)^3 - \frac{24\pi I}{2^{1/2}(e/m)^{1/2}}\frac{\mathrm{d}V}{\mathrm{d}x}\sqrt{(V+\varepsilon^2)} + \frac{48\pi^2I^2x}{(e/m)}\right\} = 0$$

Integrating, we therefore have

$$\frac{16\pi I}{2^{1/2}(e/m)^{1/2}}\sqrt{(V+\varepsilon^2)} - \left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)^2 = A \qquad (5)$$

$$\left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)^3 - \frac{24\pi I}{2^{1/2}(e/m)^{1/2}}\frac{\mathrm{d}V}{\mathrm{d}x}\sqrt{(V+\varepsilon^2)} + \frac{48\pi^2I^2x}{(e/m)} = B \qquad (6)$$

where A and B are independent of x. Since $V = 0$ when $x = 0$, and $V = V_a > 0$ when $x = a$, it follows that if $\mathrm{d}V/\mathrm{d}x < 0$ when $x = 0$ (which we suppose to be the case), there must be a value of x $(0 < x < a)$ for which $\mathrm{d}V/\mathrm{d}x = 0$. At this point, the velocity $v > 0$, that is, $\sqrt{(V+\varepsilon^2)} > 0$, and thus we see, in (5), that $A > 0$, so that the left-hand side of (5) is positive for all values of x $(0 \leqslant x \leqslant a)$.

The boundary conditions are $V = 0$ when $x = 0$, $V = V_a$ when $x = a$. Let the ratio of the potential gradient at the cathode to that at the anode be $-\lambda(1-\lambda)$ $(0 < \lambda < 1)$, so that we may write

$$\left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)_{x=0} = \frac{-4\pi^{1/2}I^{1/2}\lambda p}{2^{1/4}(e/m)^{1/4}}$$

$$\left(\frac{\mathrm{d}V}{\mathrm{d}x}\right)_{x=a} = \frac{4\pi^{1/2}I^{1/2}(1-\lambda)p}{2^{1/4}(e/m)^{1/4}}$$

λ and p being unknown functions of V_a. Putting these values, together with the above boundary conditions, in (5) and (6), we obtain, after a little reduction

$$\left.\begin{aligned} &\sqrt{(V_a+\varepsilon^2)} - (1-\lambda)^2p^2 = \varepsilon - \lambda^2p^2 \\ &2(1-\lambda)^3p^3 - 3(1-\lambda)p\sqrt{(V_a+\varepsilon^2)} + \\ &\qquad \frac{3\pi^{1/2}I^{1/2}a}{2^{1/4}(e/m)^{1/4}} = -2\lambda^3p^3 + 3\lambda p\varepsilon \end{aligned}\right\} \qquad (7)$$

and thence

$$(1-2\lambda)p^2 = \sqrt{(V_a+\varepsilon^2)} - \varepsilon \qquad (8)$$

$$I = \frac{2^{1/2}(e/m)^{1/2}}{9\pi a^2}p^2\{(1-3\lambda)p^2 + 3\varepsilon\}^2 \qquad (9)$$

Since we have shown that the left-hand side of (5) is positive for all values of x, we see from (7) that

$$\lambda^2p^2 < \varepsilon \qquad (10)$$

while, from (8), we also have

$$\lambda < \tfrac{1}{2}$$

Eliminating p^2 between (8) and (9), we obtain, after some reduction

$$I = \frac{2^{1/2}(e/m)^{1/2}V_a{}^{3/2}}{9\pi a^2}\left[\begin{array}{l}\{1 - \lambda^2(3 - 8\lambda)/(1 - 2\lambda)^3\}(1 + \varepsilon^2/V_a)^{1/2} \\ +\{3(1 - \lambda)(1 - 3\lambda)/(1 - 2\lambda)^3\}(\varepsilon/V_a{}^{1/2}) \\ +\{1/(1 - 2\lambda)^3\}(\varepsilon^2/V_a)(1 + \varepsilon^2/V_a)^{1/2} \\ -\{1/(1 - 2\lambda)^3\}(\varepsilon^2/V_a)^{3/2}\end{array}\right] \quad (11)$$

Up to this point we have made no hypothesis concerning the initial velocity, or the potential gradients at cathode and anode We shall now suppose that, except possibly for very small values of V_a, the initial velocity of an electron is independent of anode potential, depending only on cathode conditions, and being small compared with the velocity attained on reaching the anode That is, from (2) and (3) we shall suppose that ε is constant, and that $\varepsilon/V_a{}^{1/2}$ is small

From (10) and (8) we have

$$\begin{aligned}\lambda^2 < \frac{\varepsilon}{p^2} &= \frac{(1 - 2\lambda)\,\varepsilon}{\sqrt{(V_a + \varepsilon^2)} - \varepsilon} \\ &= (1 - 2\lambda)\{\sqrt{(V_a + \varepsilon^2)} + \varepsilon\}(\varepsilon/V_a) \\ &< (\varepsilon/V_a{}^{1/2})\{(1 + \varepsilon^2/V_a)^{1/2} + \varepsilon/V_a{}^{1/2}\}\end{aligned}$$

Thus, if $\varepsilon/V_a{}^{1/2}$ be small, λ^2 is small of the same order Neglecting ε^2/V_a in (11), writing μ for $\lambda^2(3 - 8\lambda)/(1 - 2\lambda)^3$, and ν for $3(1 - \lambda)(1 - 3\lambda)\varepsilon/(1 - 2\lambda)^3$, we obtain

$$I = \frac{2^{1/2}(e/m)^{1/2}}{9\pi a^2}\ \{(1 - \mu)V_a{}^{3/2} + \nu V_a\} \qquad (12)$$

or, expressing I and V_a in amps and volts

$$I = (2\ 33 \times 10^{-6}/a^2)\{(1 - \mu)V_a{}^{3/2} + \sigma V_a\} \qquad (13)$$

where μ and σ are small and positive, μ being dimensionless and σ having the dimensions of the square root of a potential

We now come to our second hypothesis We suppose that the ratio of the potential gradient at the cathode to that at the anode (namely, $-\lambda\ \ (1 - \lambda)$), is constant That is, we suppose λ constant, and therefore that μ, ν, σ here are all constant Equation (13) therefore expresses I in terms of V_a

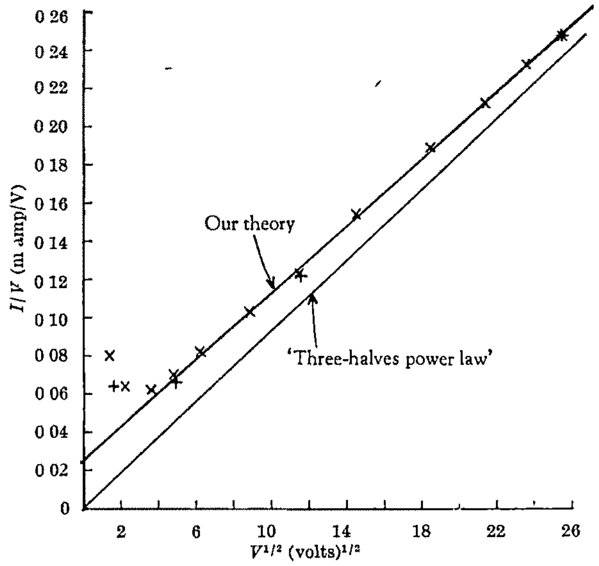

Fig 1 Plot of I/V against $V^{1/2}$ ×, Fry's figures, +, Langmuir's figures $\frac{I}{V_a} = \frac{2\ 33 \times 10^{-6}}{a^2}\{(1 - \mu)V_a{}^{1/2} + \sigma\}$ (μ, σ small positive)

Table 1

I (m amp)	V (V)	I/V (m amp/V)	$\sqrt{V}$ (V)$^{1/2}$
		Fry's figures	
0 16	2	0 080	1 4
0 32	5	0 064	2 2
0 8	13	0 062	3 6
1 6	23	0 070	4 8
3 2	39	0 082	6 2
8	78	0 103	8 8
16	130	0 123	11 4
32	208	0 154	14 4
64	338	0 189	18 4
96	453	0 212	21 3
128	550	0 233	23 5
160	645	0 248	25 4
		Langmuir's figures	
0 16	2 5	0 064	1 6
1 6	24 4	0 066	4 9
16	131 6	0 122	11 5
160	645 0	0 248	25 4

To demonstrate the great accuracy of the results obtained by the simple expression (13), with our hypothesis that μ and σ are small positive constants, we consider an example calculated, on the hypothesis of the Maxwellian velocity distribution, by Fry[5] and by Langmuir[6] This is the case of a cathode of tungsten at 2,400° K, the plate separation being 0 5 cm Noticing that (13) may be written in the form

$$I/V_a = (2\ 33 \times 10^{-6}/a^2)\{(1 - \mu)V_a{}^{1/2} + \sigma\}$$

we plot I/V_a against $V_a{}^{1/2}$ The excellent straight line obtained confirms our hypothesis that μ and σ are constants The slight deviation from the straight line for the smallest values of V_a is due to the error in neglecting ε^2/V_a at this end of the range, in deriving (12) from (11)

The intercept on the axis of (I/V_a) and the slope of the line give, for this example, the values $\mu = 0\ 05$, $\sigma = 2\ 7$ (volts)$^{1/2}$

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FREE-ENERGY DIFFERENCE BETWEEN THE ALPHA AND BETA STATES IN KERATIN

By M FEUGHELMAN

C S I R O Division of Textile Physics, Wool Research Laboratories, Ryde, Sydney

WHEN a keratin fibre is extended, the high-angle X-ray diffraction pattern shows a disappearance of α-keratin, as indicated by the 5 1 Å meridional repeat Bendit[1] has shown, and Skertchly and Woods[2] have confirmed, that this disappearance of α-keratin and the appearance of the β-keratin pattern are continuous over the extension range of 0–70 per cent for wool fibres in water This indicates that the opening up of the α-crystallites and the formation of the β-crystallites occur continuously, and Bendit[3] has suggested that, at any fixed extension, equilibrium is established between α-crystallites ⇌ liquid phase ⇌ β-crystallites Estimates from mechanical

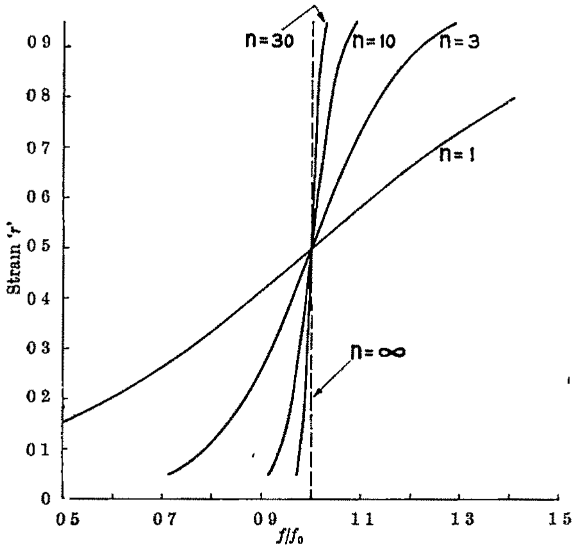

Fig. 1. Plot of the equilibrium strain 'r' in equation 1 (see text) against the stress ratio f/f_0 for different values of 'n', the number of molecular units acting co-operatively in the unfolding and folding of α- and β-crystallite units

measurements on single fibres of the free-energy difference between the units in the α- and β-crystallites have been made by a number of authors[5,6,11]. However, because their measurements are not continued until mechanical equilibrium was obtained, their values were high. Values of the free-energy difference are estimated in this article from mechanical data obtained at mechanical equilibrium.

Let the free-energy difference between the individual units in the α- and β-crystallites be ΔF^* calories/mole. The 'liquid' phase intermediary between the α- and β-states will be assumed to be at a free-energy level above that of the α- and β-states. In these circumstances at stress equilibrium the strain 'r', expressed as a fraction of the maximum strain (when all the structure is in the 'β'-state), may be shown, from the work of Burte and Halsey[4], *et al.*[5,6], to be

$$r = \frac{1}{e^{n\left(\frac{\Delta F^*}{RT} - 2af\right)} + 1} \qquad (1)$$

where f is the force acting on the individual molecular chain, n is the number of molecular units acting co-operatively, in the sense that they must fold or unfold simultaneously, R is the universal gas constant, T is the absolute temperature in degrees, and a is a quantity dependent on the temperature and the volume swept out by the unfolding structure (see equation (2)).

For a large value of n (as might be expected in a crystalline structure), the value of r varies rapidly from zero to unity for values of f close to $f_0 = \Delta F^*/2aRT$. For all values of 'n', $f = f_0$ at $r = 0{\cdot}5$. In Fig. 1 the relationship between the strain 'r' and the force 'f' expressed as a fraction of f_0 demonstrates how with increase of the value of 'n' rapid changes of 'r' occur for values of 'f' close to 'f_0'. Hence for most values of strain at which both α- and β-crystalline phases are present the value of f is almost constant. The approach here has been on the basis of the rate process theory. The same value of 'f' may be obtained by application of the analogue of the Clausius-Clapeyron equation to the equilibrium between two crystalline phases[7].

However, the latter approach only applies for $n = \infty$ and does not indicate, for smaller values of 'n', the deviation from constancy of the force 'f' as the ratio of the two crystalline fractions varies. The stress-strain curve for a wool fibre in water at room temperature has a region between about 2 and 30 per cent strain (in Fig. 2, AB the yield region) in which little change in the stress-level occurs over a large strain range. Several authors[5,6] have analysed this region of the stress-strain curve in terms of an $\alpha \rightleftharpoons \beta$ transformation. With extension into the post-yield region (BC), where there is a rapid increase in stress with extension, there is no discontinuity in the graph of X-ray intensity at 5·1 Å against extension for fibres extended in water. However, the normal stress-strain curve, carried out over periods of minutes, is not an equilibrium curve. Stress measurements on Corriedale wool fibres held extended in water at 45° C at 15 and 45 per cent strain indicated that stress equilibrium was reached after about 1,300 min (Fig. 3). The mean value of the stress (based on the wet diameter) for fibres extended 15 and 45 per cent was $1{\cdot}7_3 \times 10^8$ dynes/cm^2, and $1{\cdot}5_0 \times 10^8$ dynes/cm^2 respectively. As pointed out by Ciferri[7], a similar value for the equilibrium stress was obtained in Wood's[8] stress relaxation measurements. This suggests that the value of the critical stress f_0 to maintain equilibrium in a structure including both the α- and β-forms is approximately $1{\cdot}5 \times 10^8$ dynes/cm^2. A further estimate of the equilibrium stress was obtained in which the stress was approached from a lower value. Fibres were swollen and contracted in 8M lithium bromide solution at room temperature to the first stage of contrac-

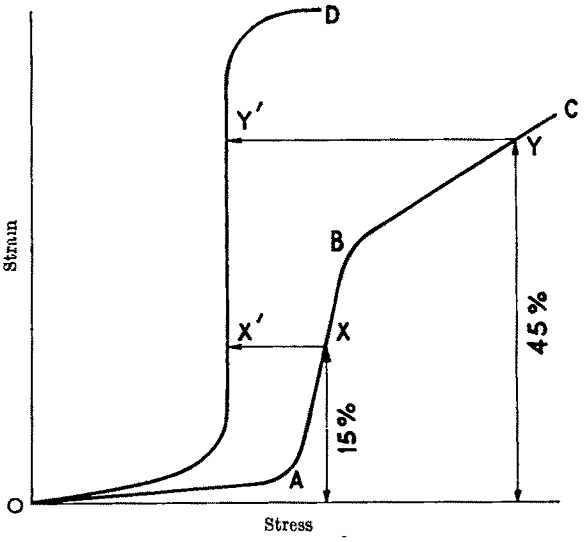

Fig. 2. A typical stress-strain curve of a wool fibre in water ($OABC$) showing the three distinct regions, the Hookean (OA), yield (AB) and post-yield regions (BC). Also shown is the equilibrium stress-strain curve OD

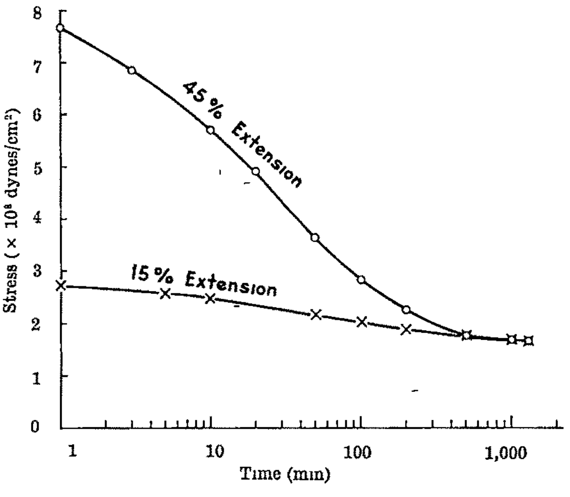

Fig. 3. The stress relaxation of two typical Corriedale wool fibres, one at 15 per cent extension, the other at 45 per cent in water at 45° C. The fibres initially are at different stress-levels (as indicated by points X and Y in Fig. 2) and come to equilibrium after about 1,300 min to the same stress-level (points X' and Y' in Fig. 2)

tion and then extended to 15 per cent above their original native length The lithium bromide was then washed out with distilled water, a treatment which has been shown to restore the fibre to its native state[9] The stress in the extended fibres rose from a lower value in the lithium bromide solution to an average value of 1 $3_1 \times 10^8$ dynes/cm^2 in the water X-ray diffraction patterns of these fibres in their extended state showed that the structure had returned to that of a normal fibre in water The process of washing out a swollen, extended fibre would not be expected to introduce any temporary stress, such as XX' and YY' in Fig 2, the introduction of such stress would mean formation under tension of bonds and entanglements[10] which are unstable when under tension It follows that the stress of 1 $3_1 \times 10^8$ dynes/cm^2 represents the stress necessary to maintain the α- and β-crystalline phases in equilibrium, and as such is in reasonable agreement with the previous figure of 1 5×10^6 dynes/cm^2

If the energy barrier between the α- and β-crystalline phases is assumed to be symmetrical, then the value of a in equation 1 is given by[4]

$$a = \frac{V}{2kT} \tag{2}$$

where V = volume of the molecular flow unit, and k = Boltzmann's constant, therefore

$$\Delta F^* = 2f_0 RT$$
$$= f_0 V \frac{R}{k} = f_0 VN \tag{3}$$

where N = Avogadro's number

The α—β translation distance is 1 8_4Å(3 33Å – 1 45Å) in the fibre direction Assuming hexagonal packing of the α-helices, resulting in the X-ray diffraction pattern having the 9 8 Å equatorial reflexion, the value of V is 200 Å^3 If the force f_0 is due only to the crystalline part of the keratin structure, then, with f_0 = 1 5×10^8 dynes/cm^2 based on a wet fibre diameter, a flow volume of 200 Å^3, a diametral swelling dry to wet (in water) of 16 per cent, and for an assumed crystalline fraction-range of 0 2–0 5, it follows that the value of ΔF^* ranges from about 3 to about 1 kcal/mole Previous values[5,6,11] obtained for the difference in free energy between the folded and unfolded states of the polypeptide chains in keratin range from 4 5 to 10·5 kcal/mole These higher values are due to the fact that equilibrium measurements of f_0 were not obtained In these circumstances the values of f_0 included a large component of force which disappears with time (Fig 3)

It should be further noted that, with the reduction of disulphide content of wool fibres[10] the value of f_0 is reduced Whether removal of disulphide reduces the free-energy difference between the α- and β-states or reduces the effective crystalline fraction of the fibre is not clear

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SOIL MOVEMENT ON SLOPES

By Dr. ANTHONY YOUNG
University of Sussex, Falmer, Brighton

IN a previous communication[1] I described techniques developed to measure the rate of movement of soil on slopes, and reported preliminary results Measurements of movement over a 4-year period have now been obtained

The technique is illustrated in Fig 1 On each of 5 sites a trench was dug through the soil and 6 reference pegs were firmly embedded in the rock beneath, 3 near the up-slope and 3 near the down-slope end of the trench The distances from each rod to the reference pegs were measured twice, thus for each rod the distances AB and AC were the means of 6 measurements The positions of the rods were obtained by solving the triangle ABC The plots were established in October 1958, and the trenches filled in, in October 1962 they were re-excavated and the measurements repeated The movements of the rods were derived from changes in the mean lengths of AB and AC

The plots were sited on the east side of Alport Dale, North Derbyshire (Nat Grid Ref *SK*/133921), on slopes of 25°–30°, at 350 m altitude The soil is a yellowish-brown sandy loam, passing into Millstone Grit sandstones (Upper Carboniferous) at approximately 50 cm Above the roots and 5-cm humus There is a dense grass cover, with mineral soil are horizons of 5-cm undecomposed grass *Deschampsia flexuosa* dominant The mean monthly temperature is 1 8° C in January and 13 7° C in July, and the mean annual rainfall is 1,400 mm

After rejecting rods which showed inconsistent movements, assumed to be caused by disturbance during re-excavation, results from 57 rods were obtained These results are shown in Table 1 and Fig 1 The mean movement in the humus horizon was 3 3 mm, while in the horizon of undecomposed organic matter irregular movements of up to several centimetres occurred In the mineral soil most movements were of approximately 2 mm, and were in directions intermediate between vertical and perpendicular to the ground surface There was no systematic variation in the amount of movement from 50 to 350 cm depth, in the upper 50 cm movements were slightly more than 2 mm, whilst below 350 cm they were of the order of 1 mm

The unexpected feature of the results is that the components of movement perpendicular to the ground surface substantially exceed the parallel (down-slope) components. This is difficult to explain in terms of soil creep, in which material is removed from a slope by transportation to the slope foot The perpendicular movement could be accounted for by a loss from the soil of material released by weathering and carried away in solution or suspension by ground-water, thereby causing a direct removal of material from the slope The results suggest that this

Table 1 MOVEMENT OF RODS BURIED IN THE SOIL ON 25°–30° SLOPES DURING FOUR YEARS

Depth below surface of mineral soil (mm)	No of rods	Mean movement		Components of movement parallel to, and perpendicular to, ground surface	
		Distance (mm)	Angle, relative to perpendicular to ground surface (θ in Fig 1)	Parallel (mm)	Perpendicular (mm)
Humus horizon	11	3 3	23°	1 3	3 0
0–50	9	2 3	25°	1 0	2 1
51–100	7	1 9	18°	0 6	1 8
101–150	6	2 1	12°	0 4	2 0
151–200	4	1 8	4°	0 1	1 8
201–250	7	1 8	11°	0 3	1 8
251–300	4	1 9	20°	0 6	1 8
301–350	4	2 0	0°	0 0	2 0
351–550	5	1 2	5°	0 1	1 2

process is more important in slope retreat than is the down-slope movement of soil creep

The unit for measuring volumetric down-slope movement of soil is the volume of soil moved annually across a plane perpendicular to the ground surface and parallel with the contour of the slope. for unit horizontal distance along the plane, expressed in cm³/cm/yr From Table 1 a down-slope movement of 0 46 cm³/cm/yr is obtained, this compares with 0 6 cm³/cm/yr previously obtained from observations over 2 years[1] If a soil depth of 50 cm is assumed, this may be extrapolated to give a mean down-slope movement of the soil layer of the order of 1 cm in 100 years

The amount of slope retreat corresponds to the movement of the mineral soil perpendicular to the ground surface Taking a mean value of 2 0 mm over four years, this may be extrapolated to give a slope retreat of 5 cm in 100 years Sources of error in measurement are such that the precise values obtained are not significant, but if the order of movement is correct, it may be concluded that under present climatic conditions, on soil-covered slopes of 20°–30°, slope retreat occurs at a rate of the order of 500m per million years This relatively high value is in accord with the recent trend towards attributing the evolution of land-forms to shorter periods of time than was previously believed[2] Under colder climatic conditions in Sweden, and for rock cliffs, Rapp[3] has measured the present rate of slope retreat at 0 04–0 15 mm/yr, equivalent to 40–150m per million years

I acknowledge with thanks permission to site these experiments on Forestry Commission land The work was assisted by a grant from the Research Fund of the University of Sheffield

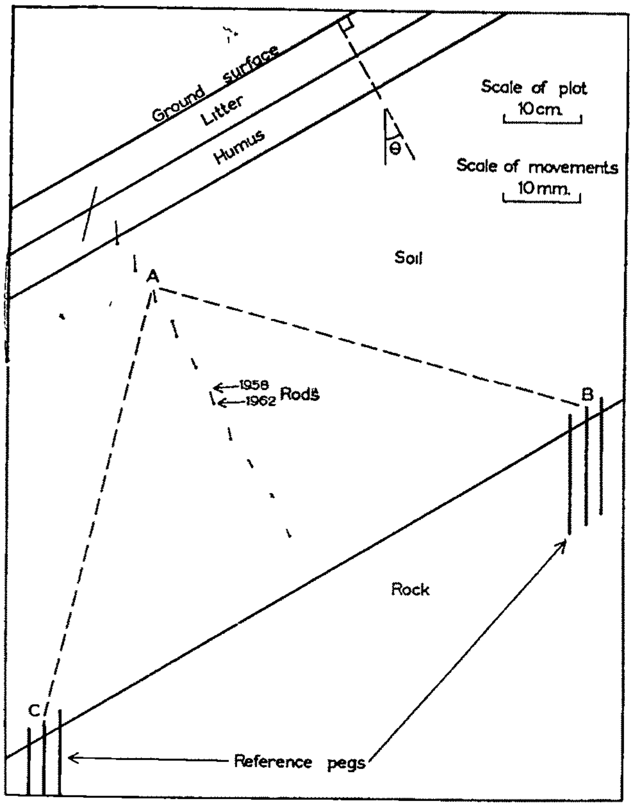

Fig 1 Technique of measurement of rods buried in the soil, and mean movements obtained from five plots over a four-year period θ is the angle given in Table 1

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CONFORMATION OF OCTACHLORONAPHTHALENE

By Dr G GAFNER and Dr F H HERBSTEIN

National Physical Research Laboratory, Council for Scientific and Industrial Research, Pretoria

OCTACHLORONAPHTHALENE is severely overcrowded, as the distance between α-substituents in the hypothetical regular and planar molecule would be 2 4 Å, whereas the closest approach found between chlorine atoms in different molecules in crystals is about 3 5 Å Furthermore, the overcrowding of the α-chlorines cannot be effectively reduced by in-plane splaying apart because of buttressing by the β-chlorines Larger deviations from planarity are thus expected than have been found for 1 4 5 8-tetrachloronaphthalene[1,2]

We have recently analysed the crystal structure of octachloronaphthalene, and the available results give the molecular conformation unequivocally The structure was determined by Patterson methods and a three-dimensional least-squares refinement was carried out on an IBM 704 computer using the *ORXLS* programme of Busing and Levy The R factor is 21 per cent at the present stage of refinement of the 2160 visually-measured intensities Separate scale factors for $h0l$ to $h6l$ layers have been used and separate isotropic temperature factors for all atoms The crystallographic data[3] are

$$a = 19\ 48 \pm 0\ 03,\ b = 7\ 30 \pm 0\ 04\quad c = 9\ 76 \pm 0\ 01\ \text{Å}$$

$$\beta = 111°33' \pm 5'\quad \text{Space group } P2_1/a\quad Z = 4$$

$$\rho_{meas} = 2\ 08,\ \rho_{calc} = 2\ 07\ \text{gm/cc}\quad \text{Needle axis } [010]$$

The atomic parameters and their estimated standard deviations are given for one molecule (the asymmetric unit) in Table 1 The results derived from these values are shown in Fig 1

Table 1 Atomic Parameters (Fractional Co-ordinates and Isotropic Temperature Factors) and their Estimated Standard Deviations

Atom	*u*	*v*	*w*	*B* (Å²)
Cl 1	0 1262	0 3128	−0 0198	2 68
	0 0002	0 0006	0 0004	0 09
Cl 2	0 0594	0 3764	−0 3481	3 10
	0 0003	0 0007	0 0005	0 10
Cl 3	0 1557	0 4851	−0 5232	3 24
	0 0003	0 0007	0 0005	0 10
Cl 4	0 3231	0 4925	−0 3750	3 26
	0 0003	0 0007	0 0005	0 10
Cl 5	0 4325	0 2454	−0 1576	3 03
	0 0003	0 0007	0 0005	0 09
Cl 6	0 5087	0 2636	0 1762	3 45
	0 0003	0 0007	0 0006	0 10
Cl 7	0 4228	0 3777	0 3716	3 23
	0 0003	0 0007	0 0005	0 10
Cl 8	0 2585	0 4270	0 2423	2 79
	0 0002	0 0006	0 0005	0 09
C 1	0 1864	0 363	−0 106	2 4
	0 0009	0 003	0 002	0 3
C 2	0 1534	0 390	−0 260	2 2
	0 0009	0 002	0 002	0 3
C 3	0 1983	0 424	−0 338	2 1
	0 0008	0 002	0 002	0 3
C 4	0 2727	0 419	−0 273	2 0
	0 0008	0 002	0 002	0 3
C 5	0 3819	0 320	−0 059	2 2
	0 0008	0 002	0 002	0 3
C 6	0 4166	0 318	0 096	2 4
	0 0009	0 002	0 002	0 3
C 7	0 3773	0 356	0 182	2 4
	0 0009	0 002	0 002	0 3
C 8	0 3007	0 382	0 123	2 0
	0 0008	0 002	0 002	0 3
C 9	0 2636	0 370	−0 035	1 7
	0 0007	0 002	0 002	0 2
C 10	0 3076	0 370	−0 122	1 6
	0 0008	0 002	0 002	0 2

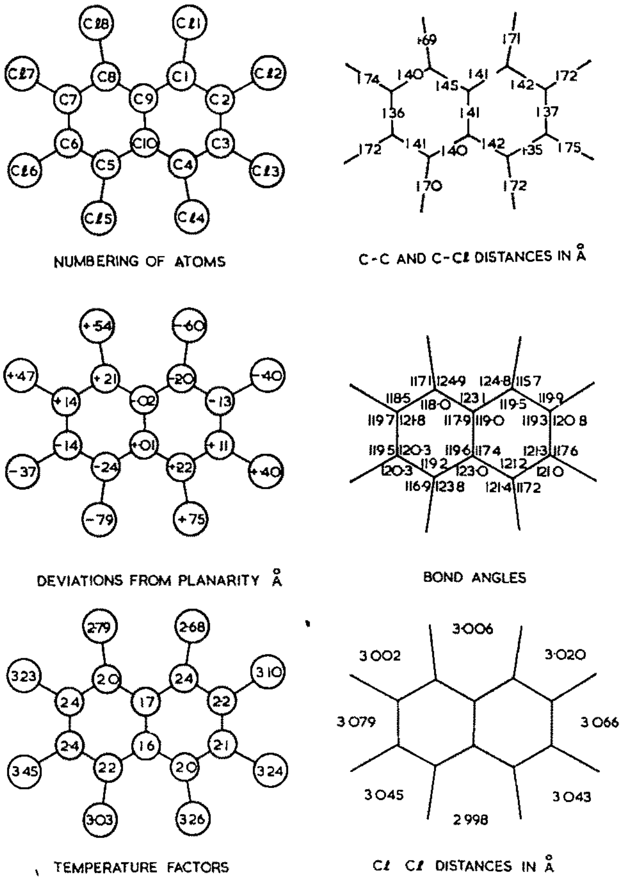

Fig 1 Numbering of atoms, molecular dimensions and isotropic temperature factors The estimated standard deviations are σ(Cl ··· Cl) = 0.007, σ(C–Cl) = 0.018, σ(C–C) = 0.024 Å, σ(CĈCl) = 1.3°, σ(CĈC) = 1.5°

The mean C–C distance is 1.40 Å and the mean C–Cl distance is 1.72 Å, both these averages are as expected and none of the differences between the individual distances and the appropriate mean, nor between the bond angles within the carbon rings and 120°, is statistically significant The deviations of the atoms from the mean molecular plane (calculated by the method of Schomaker *et al* [4], giving Cl and C atoms relative weights of 10 and 1 respectively) are highly significant and establish the conformation of the molecule The overcrowding of α-substituents is relieved by out-of-plane displacements, in different directions within a pair, of between 0.54 and 0.79 Å and an increase of the angles of type Cl 1Ĉ1C9 to a mean of 123.7° The β-substituents are displaced by between 0.37 and 0.47 Å from the mean molecular plane The most interesting feature of the molecular conformation is that adjacent α- and β-chlorine atoms (for example, Cl 1 and 2) are displaced in the same direction The carbon atoms follow the chlorines to which they are bonded with displacements about one-third as large as those of the chlorines

The free molecule of octachloronaphthalene would be expected to have D_2-222 symmetry Fig 1 shows that highly significant deviations from this symmetry occur for the molecule in the solid state The main deviations from D_2 symmetry are

(1) The smaller out-of-plane displacements and temperature factors of Cl 1 and Cl 8 compared with those of Cl 4 and Cl 5

(2) The shorter Cl 1 ··· Cl 2 and Cl 7 ··· Cl 8 distances compared with Cl 3 ··· Cl 4 and Cl 5 ··· Cl 6

These deviations are a consequence of packing forces and can be qualitatively explained in terms of the molecular arrangement shown in Fig 2 The crystal is composed of stacks of molecules with stack axes parallel to [010] The molecules within any one stack are related by a screw axis parallel to [010] and are successively displaced by about 2.4 Å in positive and negative directions parallel to [$\bar{2}$01] This results in one half of each molecule being positioned between the molecules above and below it, the other half being essentially free The packing forces thus cause Cl 1 and Cl 8 to have smaller out-of-plane displacements than Cl 4 and Cl 5 The lower temperature factors of Cl 1 and Cl 8, compared with those of Cl 4 and Cl 5, and the shorter Cl 1 ··· Cl 2 and Cl 7 ··· Cl 8 distances as compared with Cl 3 ··· Cl 4 and Cl 5 ··· Cl 6 are also consistent with the operation of compressive packing forces on Cl 1 and Cl 8 However, it should be noted that some of these latter differences are not yet securely established statistically

The closely analogous molecule octamethylnaphthalene has been investigated by Donaldson and Robertson[5] and found to possess exact D_2-222 symmetry in the solid state by virtue of its position in the unit cell It can thus have either of the following conformations

(I) As found for octachloronaphthalene in the present work, that is, with the adjacent substituents within each α- or β-pair displaced in different senses from the mean plane and with adjacent α–β substituents displaced in the same direction

(II) With adjacent substituents displaced in opposite senses independent of whether they constitute an α- or β-pair or not

Donaldson and Robertson pointed out that the two-dimensional data obtained by them did not permit a direct choice to be made between these two conformations, as II was considered to be the more plausible, their subsequent discussion was based on the assumption that this was in fact the true molecular conformation In the light of the present results there seems little doubt that conformation I, and not II, is adopted by octamethylnaphthalene

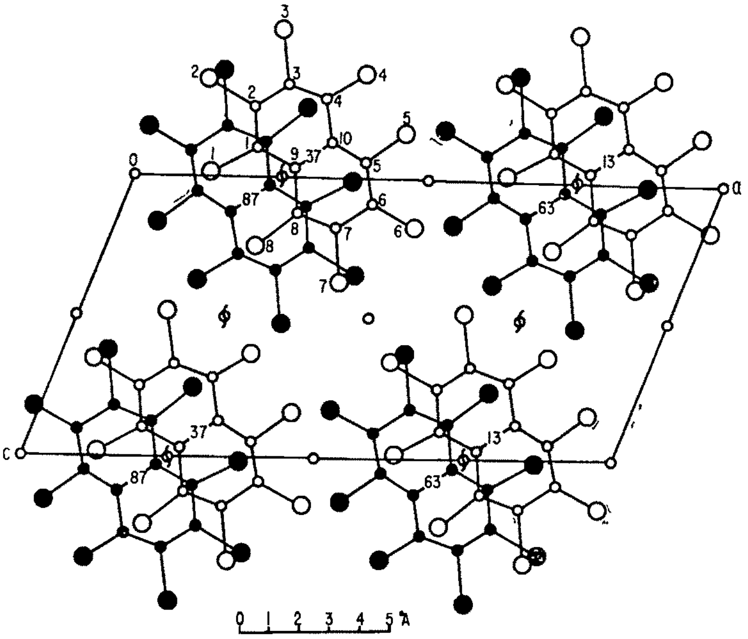

Fig 2 Packing of molecules in the crystal, seen in projection on (010) The numbered atoms are those the parameters of which are listed in Table 1 The v co-ordinate of the mean molecular plane is denoted at the centre of C9–C10 for each molecule, the upper (puckered) layer of molecules has full circles, the lower open circles The intermolecular distances are those expected for normal van der Waals contacts between different molecules

An explanation for the preference for conformation I over conformation II can be given in terms of the molecular strain energy which can be considered to consist of two parts (*a*) the energy associated with close approaches between adjacent substituents, (*b*) the skeletal strain energy

Conformation I minimizes the skeletal strain energy at the expense of increased substituent interaction energy In contrast, conformation II does the opposite as in this conformation the carbon atoms would form chair-shaped rings by following the chlorine deviations from planarity Chair-shaped rings would only be expected to be energetically preferred when bonding is tetrahedral, for example, in *cyclo*hexane A diminution in skeletal strain energy would thus be expected on going from the hypothetical double chair-shaped naphthalene nucleus to the twisted (propeller-shaped) naphthalene system found in the present work This diminution must be numerically greater than the increase in repulsive energy associated with type Cl 1 Cl 2 chlorine pairs, on going from conformation II to I

Another naphthalene derivative which is probably deformed in the same way as octachloronaphthalene is tetrabenzonaphthalene, for here coupling of adjacent α- and β-carbon atoms of the naphthalene nucleus by the substituent rings precludes the adoption of conformation II A partial analysis of the crystal structure of tetrabenzonaphthalene has been reported[6], but the only definite conclusion that could be drawn from the one projection obtained was that the molecule was not centrosymmetric The naphthalene ring system in 1 4 5 8-tetrachloronaphthalene also shows a tendency towards the twisted conformation, but the deviations from planarity (less than 0 05 Å) are much smaller than in octachloronaphthalene

The novel features established by this analysis are the propeller-like conformation of octachloronaphthalene and the appreciable influence of the crystal packing-forces on its symmetry in the solid state Detailed information about the naphthalene ring system and the thermal vibrations of the molecules in the crystal will require more accurate intensities, and it is hoped to re-measure these by counter techniques

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SOLVENT RETENTION AND GEL FORMATION IN POLYVINYL CHLORIDE

By M. A. CROOK*

British Insulated Callender's Cables, Ltd , Research Organization, London

IT is generally recognized that cross-linking with gel formation, or degradation with decrease in molecular weight, can occur in polyvinyl chloride depending on the irradiation conditions[1–3] and on the presence of additives in the polymer[4,5] It has also been shown that gel formation in polyvinyl chloride follows closely the form required for a chain reaction[6] and that considerable differences in gel formation may be observed between powdered polyvinyl chloride and that cast in the form of a film

It is proposed to give an account of the work which has been done in this laboratory using the normal technique of casting films from a solvent, and to discuss the effect of the residual solvent on the cross-linking process The preliminary results apparently indicated that the percentage gel obtained on irradiation is proportional to the thickness of the film, however, infra-red examination of polyvinyl chloride cast from tetrahydrofuran (THF) shows in fact that in spite of prolonged pumping under high vacuum to apparently constant weight, such films may contain a considerable amount of solvent in them Films cast from chlorobenzene and methyl ethyl ketone show similar behaviour , a film pumped under high vacuum to apparently constant weight may contain 2–4 per cent solvent The variation of gel fraction with percentage solvent depends on the radiation dose given, Fig 1 shows this variation for two films of thickness 0 20 and 0 05 mm cast from chlorobenzene, the percentage solvent was determined by infra-red absorption measurements

Unfortunately, in spite of prolonged pumping of the film (> 100 h) at a pressure of less than 1μ and at a temperature of 60° C, it has not been possible to obtain samples of the required shape and thickness with less than 0 25 per cent solvent without incurring some degradation Attempts to remove solvent by solvent exchange with either methyl alcohol or carbon disulphide were also unsuccessful Films of thickness 0 05–0 1 mm thick cast from chlorobenzene, immersed in carbon disulphide for 28 days, still contained 0 3–0 4 per cent chlorobenzene Similar films cast from tetrahydrofuran also contained residual solvent[7]

Examination of the solvent-containing films before and after irradiation shows no substitution of the solvent molecules by chlorine nor hydrogen atoms, and extraction of the sol fraction almost completely exchanges the solvent present in the film with that used for extraction This shows that the solvent molecules are not chemically attacked nor are they attached to the cross-linked material This is contrary to the experience of Alexander and Toms[8], who showed that naphthalene was incorporated in films of polymethyl methacrylate on irradiation These polyvinyl chloride films also obey the relationship log $S = \gamma(R - R_0) + 2$, where S is the percentage sol fraction at dose R megarads and R_0 the gel dose, both γ and R_0

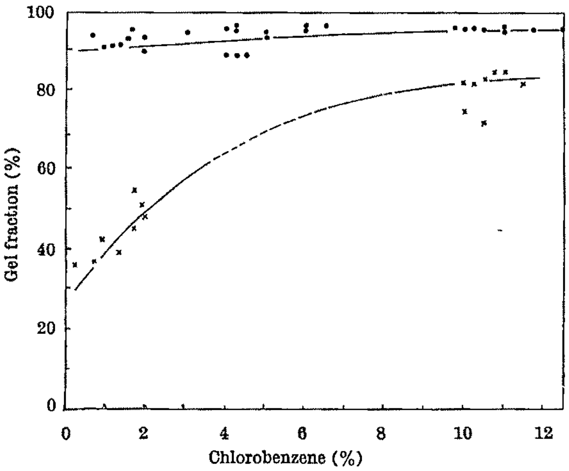

Fig 1 Variation of gel fraction with percentage chlorobenzene in a sample of polyvinyl chloride ('Corvic 65/8' supplied by Imperial Chemical Industries, Ltd) ●, 54 Mrads, ×, 27 Mrads

* Present address Department of Chemistry, Borough Polytechnic, London, S E 1

Table 1 VARIATION OF PERCENTAGE OF CHLOROBENZENE IN POLYVINYL CHLORIDE ON IRRADIATION*

Dose (Mrad)	% Chlorobenzene before irradiation	% Chlorobenzene after irradiation	% Chlorobenzene after extraction†	% Gel determined by extraction with THF
27	10 4	10 0	0	82 7 ± 1 5
54	10 9	10 1	0	95 1 ± 0 3
108	10 6	8 5	0	96 9 ± 0 3

VARIATION OF PERCENTAGE OF METHYL ETHYL KETONE (MEK) IN POLYVINYL CHLORIDE ON IRRADIATION‡

Dose (Mrad)	% MEK before irrad	% MEK after irrad	% MEK after extraction	% Chlorobenzene from extraction	% Gel determined by extraction with chlorobenzene
54	3 0	2 7	0 2§	8	71 6 ± 2 2
108	3 0	2 0	0 5	11	95 4 ± 1 4
216	1 5	1 0	00	16	102 ± 1 3

All the samples were irradiated at 60° C and under high vacuum, the cell was opened to air 2 min after irradiation and immediately extracted with solvent These results are the average of at least four separate samples.

* The results fit the equation log $S = 2 - 1\ 22 \times 10^{-2}\ (R - 34\ 5)$, with a standard deviation of ± 0 18

† The infra-red absorption of THF in the extracted samples was always too large to measure

‡ The results fit the equation log $S = 2 - 1\ 46 \times 10^{-2}\ (R - 16\ 5)$ when the solvent content is 3 per cent

§ After irradiation considerable broadening of the peak of the MEK absorption spectra was observed due to interference of oxidation products formed during the extraction process, hence the values quoted for percentage MEK after extraction are upper limits only Antioxidant added to prevent this oxidation was preferentially absorbed by the polyvinyl chloride and gave even greater interference of the spectra

depend on the amount of solvent and its nature The results are given in Table 1

Hence any free radical chain mechanism proposed for irradiation processes in polyvinyl chloride must allow for the presence of small quantities of solvent molecules which markedly affect the radiation properties without themselves of necessity being involved chemically Such a possible mechanism may be described as follows If the primary process of irradiation results in the production of free radicals[9] either directly or through intermediate ions[10], then such radicals are probably formed in an excited state with a considerable quantity of excess energy which presumably is present as vibration energy In any polymer this vibrational energy is available to overcome steric factors for cross-linking between adjacent molecules In the case of polyvinyl chloride, however, some of this energy may be removed by the process of 'splitting off' a hydrogen chloride molecule, such a process would be favourable as the free radical would then become resonance stabilized, but would have less energy available for cross-linking This accounts for the exceptional stability of some polyvinyl chloride radicals[11], and for the loss of hydrogen chloride which occurs after irradiation and on heating[12]

Now it is known that when polyvinyl chloride is irradiated in solution, even in very dilute solution, it cross-links with a high degree of efficiency[2], and this is explained as being due to the mobility of the polymer molecules Therefore, if the local presence of a solvent molecule enables part of a polyvinyl chloride chain to acquire more freedom of movement, this solvent molecule will have increased the cross-linking efficiency without itself having taken part in the reaction This idea is further supported by the work of Shinohara *et al* [12], who showed that treatment of polyvinyl chloride with tetrahydrofuran after irradiation but under vacuum increases the amount of cross-linking

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ABNORMAL GLUCOSE TOLERANCE IN REPEATEDLY BRED RATS WITH ARTERIOSCLEROSIS

By PROF BERNARD C. WEXLER and DR CHARLES T. FISCHER

May Institute for Medical Research, Jewish Hospital of Cincinnati, and Department of Pathology, University of Cincinnati College of Medicine, Ohio

PREVIOUS work in this laboratory has demonstrated that male and female rats which have been repeatedly bred will develop arteriosclerosis[1-4] The early arterial lesions consist of subintimal deposition of mucopolysaccharides which are capped by fibrous tissue More advanced lesions consist of marked alterations in medial mucopolysaccharides, elastosis and dystrophic calcification In addition to the arteriosclerosis, these animals also develop a fatty liver, hyperplasia of the islets of Langerhans with beta cell degranulation and hypertension All these degenerative changes become more pronounced with each successive breeding These changes are also accompanied by progressive adrenal hyperplasia and thymic involution The gravimetric and histologic changes in the adrenal and thymus glands coupled with *in vitro* investigations of the adrenal steroid production of these animals indicated that a state of hyperadrenocorticism existed There was reason for believing that the fatty liver and the islet hyperplasia could be related to either the hyperadrenocorticism or to the existence of a diabetes-like condition Further, the mucopolysaccharide nature of the arteriosclerotic lesions in these breeder rats, the high incidence of arteriosclerotic complications in diabetic patients and the concomitant existence of diabetes, arteriosclerosis and hyperadrenocorticism in Cushing's syndrome served as a general background to provide some basis for interrelating the variety of degenerative changes observed in breeder rats Therefore, it was decided to investigate the carbohydrate tolerance of these animals Glucose tolerance tests were performed on arteriosclerotic breeder rats and non-arteriosclerotic virgin rats to determine whether the conditions of hyperadrenocorticism, fatty liver, islet hyperplasia and arteriosclerosis observed in breeder rats were also accompanied by any abnormal carbohydrate metabolism

One-hundred-and-forty virgin Sprague–Dawley rats, free of any arteriosclerosis (80 females and 60 males), served as controls These virgin rats were 10–12 months old and were approximately the same age as the breeder rats The virgin control animals were divided into equal groups so that blood-sugar determinations could be made at the following intervals 0 h (fasting-level), 0 5, 1, 2, 3, and 5 h A total of 215 female and 70 male Sprague–Dawley breeder rats were used. The male breeders were arranged so that there were 15, 15, 10, 10, 10, and 10 animals in the six groups investigated As in the controls, the male breeders were killed at 0–5 h intervals The male breeders were arbitrarily divided in this fashion because

Table 1 BLOOD GLUCOSE-LEVELS AT TIMED INTERVALS FOLLOWING A SINGLE INTRAPERITONEAL INJECTION OF GLUCOSE IN VIRGIN FEMALE RATS WITH NO ARTERIOSCLEROSIS COMPARED WITH BREEDER FEMALE RATS HAVING VARYING DEGREES OF GROSS ARTERIOSCLEROSIS

		Time					
Breeders		Fasting (0 h)	0 5 h	1 h	2 h	3 h	5 h
Arterio-sclerosis	Clear $M \pm S D$ (n)	94 ± 3 (22)	376 ± 18 (21)	212 ± 12 (19)	154 ± 5 (15)	150 ± 6 (15)	150 ± 6 (13)
	Minimal $M \pm S D$ (n)	88 ± 3 (15)	327 ± 42 (6)	240 ± 16 (10)	157 ± 6 (10)	160 ± 10 (6)	141 ± 7 (5)
	Moderate $M \pm S D$ (n)	91 ± 2 (17)	365 ± 28 (7)	224 ± 18 (12)	156 ± 7 (6)	139 ± 0 (1)	181 ± 9 (3)
	Severe $M \pm S D$ (n)	85 ± 0 (1)	— (0)	246 ± 13 (2)	155 ± 22 (3)	150 ± 24 (4)	134 ± 0 (1)
Average of all breeders $M \pm S D$ (n)		91 ± 2 (55)	365 ± 14 (34)	224 ± 8 (43)	155 ± 3 (34)	152 ± 5 (26)	151 ± 6 (22)
Virgins $M \pm S D$ (n) P		114 ± 2 (13) 0 001*	402 ± 25 (14) 0 1	179 ± 5 (12) 0 005*	181 ± 7 (12) 0 001*	163 ± 6 (14) 0 1	166 ± 5 (14) 0 05

M = mean, $S D$ = standard deviation from the mean, (n) = No of animals, $P = P$ value (Those marked with an asterisk are considered to be statistically significant)

Table 2 BLOOD GLUCOSE-LEVELS AT TIMED INTERVALS FOLLOWING A SINGLE INJECTION OF GLUCOSE IN VIRGIN MALE RATS WITH NO ARTERIOSCLEROSIS COMPARED WITH BREEDER MALES HAVING ARTERIOSCLEROSIS MICROSCOPICALLY

	Time					
	Fasting (0 h)	0 5 h	1 h	2 h	3 h	5 h
Breeders, males $M \pm S D$ (n)	119 ± 4 (15)	296 ± 23 (14)	272 ± 19 (9)	158 ± 6 (10)	190 ± 10 (10)	158 ± 10 (10)
Virgin males $M \pm S D$ (n)	100 ± 0 5 (10)	324 ± 30 (10)	177 ± 8 (9)	166 ± 8 (9)	163 ± 13 (9)	152 ± 6 (9)
P	0 001*	0 5	0 001*	0 2	0 2	0 5

M = mean, $S D$ = standard deviation from the mean, (n) = No of animals, $P = P$ value (Those marked with an asterisk are considered to be statistically significant)

their arterial lesions cannot be determined grossly, thus obviating any attempt to correlate abnormal carbohydrate metabolism with gross arterial abnormality On the other hand, female breeders do develop arteriosclerosis which can be easily detected by gross examination Therefore, the female breeders could be categorized according to the degree of gross arterial pathology exhibited at autopsy Female breeders with no grossly visible arteriosclerosis were classified as 'clear', those with thin annular streaks in the abdominal aorta as 'minimal', those with discrete plaques in the arch of the aorta in addition to having lesions in the abdominal aorta as 'moderate', and those with plaques in all portions of the aorta as 'severe' These animals were killed after the test dose of glucose at the same time-intervals as the controls and male breeders A record was kept of the degree of gross arteriosclerosis in female breeders found at autopsy and this was later correlated with the blood sugar values

Prior to the time of the glucose tolerance test, all the animals had been stabilized on a regular 'Rockland Rat Chow' diet

All animals were starved for 18 h before autopsy During this time they were permitted water *ad libitum* A test dose of 10 per cent glucose (350 mg/100 g body-weight) was injected intraperitoneally Animals were killed by decapitation to avoid the stress of anæsthesia Blood sugar determinations were carried out by a modification of the anthrone method of Kahan[5] for the determination of glucose using trichloroacetic acid as a serum protein precipitant

Pancreatic tissue for histological examination was fixed in either Zenker's solution or neutral formalin (Lillie), cut at 3μ, and stained with either hæmatoxylin and eosin or a modification of Gomori's aldehyde fuchsin with trichrome counterstain as described by Lazarus[6] to demonstrate alpha and beta cells in the islets of Langerhans

Islet size was determined by counting the total number of islets larger than 25μ in any one diameter, in each section, and determining the average Individual samples of pancreas were taken from 6 virgin male and 6 female control rats Separate samples of pancreas were taken from 12 male and 12 female breeder rats The measurements were made with the aid of a calibrated ocular micrometer

Comparison of the glucose tolerance curves of breeder rats with virgin control rats revealed the presence of a significant hyperglycæmia in the breeder rats (cf Tables 1 and 2) at the critical 1-h period after the introduction of a glucose load

The mean fasting blood sugar-level of female breeders was significantly lower than that shown by female virgins virgins = 114 ± 2 0 versus breeders = 91 ± 2 0 mg per cent The female breeders showed marked hyperglycæmia compared with the female virgins at the first hour, that is, breeders (43 rats) = 224 ± 8 0 versus virgins (12 rats) = 179 ± 5 0 mg per cent This condition was reversed after the second hour and all blood sugar-levels in the arteriosclerotic rats returned to levels slightly lower than the controls in the third and fifth hours No differences were seen in the glucose tolerance curves between female breeders with varying degrees of gross arteriosclerosis (Table 1)

In contrast to the female breeders, the male breeders showed significantly higher fasting blood sugar-levels than controls under similar fasting conditions (Table 2) The male breeders also showed a significantly greater glycæmia than controls at the 1-h period, that is, male breeders = 272 ± 19 0 versus male virgins = 177 ± 8 0 mg per cent

Finally, female breeders showed a significantly lower fasting blood sugar-level than male breeders, that is, male breeders = 119 ± 4 0 versus female breeders = 91 ± 2 0 mg per cent ($P = 0\ 001$) This was the reverse of the finding in fasting virgin male and female rats, that is, male virgins = 100 ± 0 5 versus female virgins = 114 ± 2 0 mg per cent ($P > 0\ 001$)

Pancreatic tissue taken from both male and female breeders showed that the islets of Langerhans were greatly increased in both size and number as compared to virgins (Figs 1 and 2) The average size of the islets sampled in the virgin female and male control rats was 138μ and 130μ, respectively The average size of the islets in female and male breeders was 160μ and 176μ, respectively Also, in breeder rats the ratio of beta to alpha cells in the islets was increased However, the beta cells were also degranulated The degree of islet hypertrophy and hyperplasia corresponded with the intensity of the arteriosclerosis That is, the largest islets were found in rats with severe arteriosclerosis The details of the histology and cytology of the islet tissue in these animals have been described in a separate publication[7]

The results of these glucose tolerance tests indicated to us that there was some defect in the carbohydrate metabolism in the repeatedly bred rats The degree of

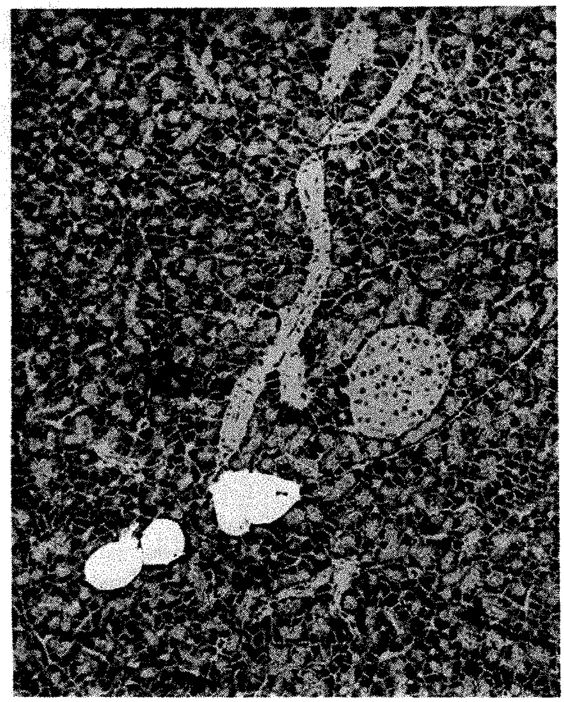

Fig. 1. Pancreas of 9-month-old female virgin rat. Note size of islet in centre of photo. Compare with giant-sized islet in Fig. 2. (Hæmatoxylin and eosin, × 90)

hyperglycæmia, however, was not markedly abnormal. This condition of hyperglycæmia would best fit the known physiological facts concerning these animals. That is, the mild hyperglycæmia would be consonant with a steroid type of diabetes, which is in keeping with the hyperadrenocorticoid condition of these animals[8-12]. In this connexion, a large percentage of patients with atherosclerosis given glucose tolerance tests are found to have a mild, stable diabetes[12]. It has frequently been suggested that there may be an ætiological connexion between carbohydrate metabolism and atherosclerosis[12-20]. A similar mechanism may be applicable to the development of atherosclerosis in the case of these breeder rats, either by virtue of their disturbed carbohydrate metabolism or because of abnormal fat metabolism which is a frequent concomitant of deranged carbohydrate metabolism.

Comparison of the glucose tolerance curves of animals with graded gross arterial pathology (female breeders) indicated that there was no relationship between the severity of hyperglycæmia with ascending degrees of arteriosclerosis ('clear' to 'severe'). The male breeders displayed a more abnormal glucose tolerance curve than the females, yet the arteriosclerosis in male breeders was mostly of microscopic proportions, rarely appearing grossly. This sex dichotomy in the severity of arteriosclerosis and hyperglycæmia in the breeder rat is interesting in that, among human diabetics, female diabetics compared with non-diabetic females show a definite increase in the appearance and severity of arteriosclerosis, hypertension, and in the incidence of myocardial infarction[13-15]. The morphology of the arterial lesions seen in breeder rats is highly reminiscent of the type of arterial lesion which is alleged to be characteristic of diabetes in the human, that is, endothelial hyperplasia, and intense accumulation of acid mucopolysaccharides[13-15].

The spontaneous nature of the mildly disturbed carbohydrate metabolism in these breeder rats resembles the diabetes seen in women during pregnancy. It is well known that latent diabetes may be unmasked during the stress of pregnancy[16-18]. This diabetic tendency has been linked with the gluconeogenic effects of the increased adrenal steroid secretion during pregnancy. Campbell and McKechnie[19], of South Africa, have found spontaneous diabetes developing in both husband and wife among Natal Indians now residing in Africa. Although the diabetes is mild, it is particularly productive of severe and lethal diabetic vascular disease. These Natal diabetics have buffalo-hump obesity, hypertension, striæ, are resistant to and are not dependent on insulin, and seldom become ketotic. The Natal diabetic females are often very hirsute. The inhabitants of Natal pride themselves in having large families, the female being kept practically in a constant state of pregnancy[20]. These workers believe that the diabetes which develops in Natal husbands and wives may be linked to parity and to the overproduction of adrenal steroids and certain sex steroids. Although it is highly speculative to suggest that the phenomena described in the Cushingoid Natal inhabitants and the hyperadrenocorticoid repeatedly bred rats are the same, none the less the similarity between the two is so striking that it is provocative and bears mention and investigation.

The heightened activity of the pituitary-adrenal system which accompanies repeated breeding in these animals could be responsible for the hyperglycæmia. This physiological interrelationship has both medical and biological counterparts. It has been amply demonstrated that administration of ACTH or the adrenal steroids can predispose towards and produce a diabetic state in the normal man or animal[11,21-24], that is, steroid diabetes. It should be borne in mind, also, that patients with Cushing's disease often have a mild diabetes accompanied by islet hyperplasia, adrenal enlargement, fatty liver and severe arteriosclerosis[15,25]. This same set of conditions has already been described in these breeder rats[1-4]. A similar syndrome of hyperadrenocorticism, islet hyperplasia, diabetes and arteriosclerosis has been described in spawning salmon[26-28] and in mice with transplanted ACTH-secreting tumours[29,30].

Finally, in separate cytological investigations of the alpha and beta cells of the islets of these breeder rats, we

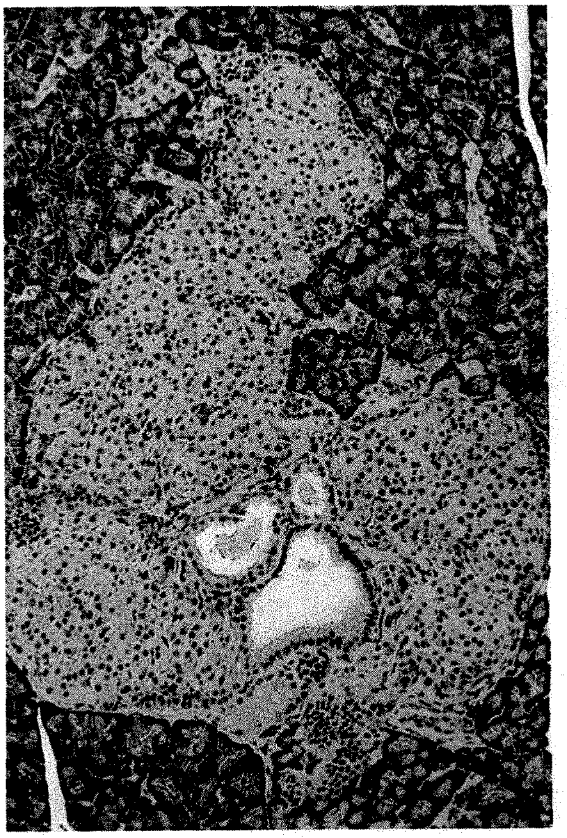

Fig. 2. Giant-sized islet in 9-month-old female breeder rat (4–5 litters). Contrast with normal-sized islet in virgin rat (Fig. 1). (Hæmatoxylin and eosin, × 90)

found that islet hyperplasia was accompanied by beta cell degranulation[7]. In addition, beta cell degranulation was more marked in the more enlarged islets. It has been suggested that the islets of Langerhans through their capacity for compensatory increase in insulin production (or functional reserve) are able to maintain normal levels of blood glucose[31]. Islet hyperplasia may be produced by the injection of ACTH or adrenal steroids and is believed to be due to increased need for insulin because of the hyperglycæmia induced by the potent gluconeogenic effects of the adrenal steroids[30-32]. When the islets lose their resiliency for increased insulin production, overt hyperglycæmia becomes evident. Thus, the vitality of the insular cells plays an essential part in offsetting diabetes[31]. It is felt that the increased adrenal activity which accompanies repeated breeding stimulates islet hyperplasia by way of response to the heightened gluconeogenic activity induced by the increased production of adrenal steroids which accompanies repeated breeding.

In conclusion, it is felt that repeated breeding leads to the development of a disturbed carbohydrate metabolism. The degree of hyperglycæmia reflected by the glucose tolerance curves indicates that the carbohydrate metabolic disturbance is of mild degree. The mildness of the hyperglycæmia, the islet hyperplasia and beta cell degranulation, and the heightened adrenal activity are believed to be indicative of a Cushingoid condition existing in these breeder rats. Future investigations will be directed towards understanding the possible connexion between the hyperglycæmia, the hyperadrenocorticism and the arteriosclerosis observed in these breeder rats.

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BIOCHEMICAL EFFECTS OF X-IRRADIATION AND THE SULPHYDRYL HYPOTHESIS: A RE-APPRAISAL

By Dr. MARGERY G. ORD and Dr. L. A. STOCKEN

Department of Biochemistry, University of Oxford

THE radiosensitivity of dividing cells has been apparent from the earliest investigations of the biological consequences of X-irradiation[1]. One of the first attempts to provide a unified biochemical basis for irradiation damage was that of Barron *et al.*[2-4], who showed from experiments *in vitro* that sulphydryl (SH) groups in such compounds as cysteine, glutathione, proteins and enzymes could be oxidized as a result of exposure to X-rays, and who postulated that such an oxidation, if occurring intracellularly, would produce serious metabolic disturbances within the cell. Unfortunately many detailed investigations of SH enzymes in tissues following whole-body irradiation failed to detect any significant inhibition, so that a generalized inhibition of SH enzymes could not be established[5,6]. Since that time work on the biochemical effects of X-irradiation has been focused on the reduction in DNA synthesis[5,6], although reports have been available of reduced sulphydryl contents of tissues and nuclear extracts[7,8].

In the course of many experiments on DNA synthesis, it has been shown that the nucleus itself is directly affected by X-irradiation[9-13], and attempts have been made to find biochemical reactions associated with the nucleus which might explain its radiosensitivity. One such reaction is nuclear phosphorylation[14], and in an attempt to determine the site(s) at which X-rays interfere with nuclear generation of energy[15-17] it has been shown[18] that sulphydryl compounds present in thymus nuclei (Table 1, series 1–3) can not only be oxidized by exposure (series 4, 6, 8) but also made more reactive towards sulphydryl reagents

Table 1. SULPHYDRYL CONTENT OF THYMUS NUCLEI. SH AS MμMOLES/MG DNA PHOSPHORUS (NO. EXPERIMENTS)

Series	Time of incubation with dithiobis (2-nitrobenzoic acid) (min)	Control	Experimental
1 Nuclei isolated in ionic medium (15)	30	308	—
2 Nuclei isolated in sucrose medium (7)	5	375	—
	30	469	—
3 Nuclei isolated in sucrose and suspended in ionic medium (6)	30	632	—
4 Nuclei isolated in sucrose medium. 1,000 r. *in vivo* or *in vitro* (5)	5	381	329
5 As above, no Zn (2)	30	489	489
6 As above, with Zn (4)	30	528	421
7 Nuclei isolated in ionic medium after 1,000 r. *in vivo* (2)	30	285	351
8 Ionic medium extract from nuclei isolated in sucrose. With Zn, 1,000 r. *in vitro* (2)	30	276	214

Nuclei were isolated from thymus glands of 5 or 6 rats[18]. The sulphydryl (SH) content was measured by the method of Ellman[34] after 5 or 30 min incubation with dithiobis (2-nitrobenzoic acid). Where present, Zn^{2+} was 10^{-5} M zinc sulphate.

(series 7) The oxidation of sulphydryl groups is reversed by glutathione reductase, a normal component of the isolated nuclei, so that the fall in SH content is maintained only if glutathione reductase is inhibited by 10^{-5} M Zn^{2+} (ref 19) (series 5, 6). The increase in SH content, which has only been detected after irradiation *in vivo*, is probably due to alteration of the nucleoprotein complex and it can also be brought about in normal nuclei prepared in sucrose by suspension in a medium of increased ionic strength (series 2, 3)

Reduced glutathione was detected only in small amounts in nuclei isolated in sucrose medium and was not found in nuclei obtained in ionic medium About 70 per cent of the total SH content of the nuclei was associated with the arginine-rich histones[20], which contain small amounts of cystine or cysteine[20,21] Since sulphydryl and disulphide groups of proteins and glutathione interact[22] the lowered content of nuclear SH groups following X-irradiation will lead to the temporary formation of oxidized glutathione (GSSG) within the nucleus

As a result of these findings it seemed important to attempt to relate directly the alterations in SH content of the nuclei to the known changes in nuclear metabolism post-irradiation GSSG was used to reproduce the oxidative effects of X-irradiation on nuclear sulphydryl groups To increase the number of reacting SH groups, nuclei which had been isolated in 0 25 M sucrose/5mM calcium chloride/5mM *tris* *p*H 7 2 were suspended in ionic medium[18]

Oxidized glutathione reduced the activity of glucose-6-phosphate dehydrogenase and inhibited the formation of labile phosphate (Table 2) The mechanism of nuclear phosphorylation is still uncertain and especially its dependence on the presence of oxygen The glutathione reductase system offers a means by which NADPH can be re-oxidized but does not explain the need for oxygen Moreover, GSSG is usually present in only very small amounts within cells, so that added GSSG might be expected to increase the rate of phosphorylation if glutathione reductase was the preferred enzyme for re-oxidation of NADPH The inhibitory effect of GSSG on nuclear phosphorylation therefore suggests either that the formation of ATP in thymus nuclei is associated with an oxygen-dependent oxidation of NADPH, or that the disulphide has reduced phosphorylation by interaction with some SH groups essential for the reaction

Table 2 EFFECT OF OXIDIZED GLUTATHIONE ON REACTIONS OF THYMUS NUCLEI

Reaction	Medium	Control activity	Conc (M)	+ GSSG activity as % control
Glucose-6-PO_4 dehydrogenase[18]	*I*	1 35 ± 0 485 (12)	10^{-3}	10, 32, 46 5, 33
			10^{-4}	71, 65, 62
mμmoles NADPH/min/ mg DNA P	*S*	10 46 (2)	10^{-4}	74
Nuclear phosphorylation[18] labile phosphate	*I*	20 4	10^{-3}	36 3
formed in 30 min at 0° mμmoles/mg DNA P	*I*	19 7	10^{-3}	7 1
^{23}P incorporation into	*S*	100 (ref 35)	10^{-3}	11 4, 20 6
nuclear RNA fraction	*S*	100 (ref 36)	10^{-3}	8 0
arbitrary units			10^{-4}	13 8
Cytidylic acid	*S*	100 (ref 37)	10^{-3}	2 6

Nuclei were isolated from rat thymus gland in either ionic (*I*) or sucrose (*S*) medium[15] Reactions were performed in the presence or absence of oxidized glutathione (GSSG)

Interpretation of the experiments in which the nuclear sulphydryl content was increased by suspending the preparation in ionic medium is complicated by the specific effect which some of the ions have on nuclear metabolism Na^+ particularly is known to increase the incorporation of amino-acids into nuclear protein[14], and this effect is a likely explanation of the results (J A Smit, unpublished) on the uptake of leucine where the rate in ionic medium is about twice that of nuclei in unsupplemented sucrose medium The rate of phosphorylation by nuclei isolated in sucrose medium and finally suspended in ionic medium was not diminished Phosphorylation, therefore, is not reduced by treatment of the nuclei with ionic medium, although from the increased SH content in parallel experiments some disorganization of nuclear structure must have occurred Allfrey and his colleagues have shown[14] that, although deoxyribonuclease treatment prevents phosphorylation, protein synthesis could be restored by the addition of DNA, suggesting that radical changes in structure do not cause cessation of all synthetic functions in the nucleus

When incorporation of phosphorus-32 into nucleic acids was examined, unequivocal uptake into DNA was not obtained Incorporation into the RNA fraction was unaffected by suspending nuclei in ionic medium but was profoundly reduced by 10^{-3} M GSSG (Table 2) The effect of oxidized glutathione was confirmed by isolation from the RNA hydrolysate of cytidylic acid X-irradiation *in vivo* is known to inhibit precursor uptake into nuclear RNA in rat thymus as well as into DNA[23]

From these experiments it is clear that agents which produce alterations in the nuclear SH/SS equilibrium produce metabolic effects which are paralleled by X-irradiation The biochemical changes associated with the formation of the mitotic apparatus are not completely established, but SH/SS interactions are involved and the process is profoundly affected by exogenous SH compounds[24] The radiosensitivity of spindle formation is well known It seems possible, therefore, that the changes in sulphydryl groups which can be detected in nuclei after X-irradiation may represent a morphological change at the macromolecular level and be responsible for some of the cytological effects and for the early biochemical lesion

The importance of the glutathione reductase system in reversing the oxidative effects of X-irradiation is obvious, and the presence of this enzyme in cell fractions other than the nucleus[25] may account for the failure to detect any reduction in the activity of tissue SH enzymes after exposure In the thymus, nuclear SH groups account for about 20 per cent of the SH content of the cell, but glutathione appears to account for a comparatively small fraction of the nuclear sulphydryl compounds[18] The response of all cell fractions will presumably depend on the relative availability of glutathione reductase, NADPH and glutathione, the protection afforded by sulphydryl compounds against X-radiation damage is well established[5,26,27] It is known that mitochondrial function may also be disturbed following irradiation *in vivo*[28,29]; the role of SH groups in maintaining structure/function relationships in mitochondria has been considered[30].

While the presence of glutathione reductase may account for the difficulty in detecting changes in SH groups after irradiation, it is not certain that the original conformation of the sulphydryl groups will be restored The increase in nuclear SH groups which is detectable after X-irradiation *in vivo*, especially in nuclei isolated in ionic medium, suggests that disorientation of the nucleoprotein structure is also produced When normal nuclei are suspended in ionic medium protein loss into the aqueous phase is more marked than with suspension in sucrose medium[31] and total SH reactivity is increased Loss of nuclear catalase[12] and increased extractability of DNA[32] following irradiation *in vivo* may be correlated with the disturbance to nucleoprotein structure, and this disorganization seems likely to be irreversible and to be associated with the less-sensitive phase of the irradiation response[33]

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Note added in proof The Russian workers[38] have now extended their findings on changes in sulphydryl content of rat liver nuclei after 1,500 r total-body irradiation 30 min after exposure the SH content of two nuclear protein fractions is decreased, and in a third fraction, thought to be associated with the nucleolus, the SH-level rises Their data are discussed in relation to the effects of X-irradiation on mitosis and cell division

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INHIBITION OF THE AGGREGATION OF BLOOD PLATELETS BY NUCLEOSIDES

By SONIA CLAYTON, PROF G. V R. BORN and DR M J CROSS

Department of Pharmacology, Royal College of Surgeons of England, London, W C 2

ONE of the immediate causes of thrombosis is the aggregation of blood platelets It has been found that adenosine diphosphate (ADP), out of a large number of nucleotides tested, was the only one which brought about the aggregation of platelets in human plasma[1] We have used a turbidimetric method to follow the aggregation of platelets quantitatively, and shown that the aggregation induced by ADP is partially prevented by adenosine triphosphate, by adenosine monophosphate (AMP) and by adenosine, the last being much the most effective[2] It was suggested that ADP combines with specific sites on the surface of the platelets To find out more about this reaction, and because inhibitors of aggregation may prove to be of therapeutic value, we have now tested the effects of more compounds related to adenosine on the aggregation of platelets

The methods for preparing human plasma rich in platelets and for measuring their aggregation have been described previously[2] In each experiment ADP was added at a final concentration of 10^{-6} M, and the optical density of the plasma was recorded at short intervals for about 15 min When a compound was tested it was mixed with the plasma 5 min before the ADP was added and readings of the optical density were made during this period also This was done for two reasons (1) to see if the compound could itself bring about the aggregation of platelets, (2) although the inhibitory effect of adenosine increased the longer it was in the plasma, the effect was evident in less than 5 min[2]

The plasma was stored in a 'Perspex' cylinder at room temperature During the day the extent to which the platelets aggregated after adding ADP often decreased This decrease persisted even when the plasma was kept at 37° C or, at room temperature or at 37° C, in a dialysis bag suspended in Krebs solution through which was bubbled a mixture of 95 per cent oxygen and 5 per cent carbon dioxide (v/v) Because of this, the effects of inhibitors were always assessed by bracketing an experiment in which an inhibitor was present between two control experiments in which ADP alone was added

The activities of other compounds were compared with that of adenosine in the following way A concentration of adenosine (usually 5×10^{-6} M) was chosen which reduced the maximal change in the optical density brought about by ADP (10^{-6} M) by about 60 per cent The potential inhibitor was then tested at a concentration one hundred times greater than that of adenosine If it had less effect than the adenosine its activity was recorded as less than 1 per cent of that of adenosine and no further tests were made (Table 1) If, however, its activity was greater than that of adenosine a more exact comparison was made by finding the concentration of inhibitor which had the same effect as (i) 5×10^{-6} M adenosine, and (ii) 10^{-6} M adenosine In each case the activity of the compound was expressed as a percentage of the activity of adenosine, that is,

$$\frac{\text{concentration of adenosine selected for the comparison}}{\text{concentration of inhibitor required to produce the same inhibition as the chosen concentration of adenosine}} \times 100$$

Though the absolute effects of the inhibitors varied from plasma to plasma their relative activities did not alter very much This is illustrated in Fig 1, in which are compared the activities of adenosine and isoguanosine at different concentrations and in samples of plasma from three different individuals

The average of the two results for each inhibitor is shown in Table 1 They emphasize that the adenosine moiety is a very important component of the nucleosides and nucleotides which influence the aggregation of platelets The activity of adenosine as an inhibitor was considerably reduced by small changes in the purine ring itself, as in 6-amino-8-azapurine riboside, in the substituents on the purine ring, as in purine riboside, 6-methylaminopurine riboside and isoguanosine, or in the ribose fraction, as in deoxyadenosine and 6-amino-1′-hydroxymethylpurine riboside It was particularly striking that cyclic-AMP not only had less than 1 per cent of the activity of adenosine but also less than 1 per cent of the activity of AMP, with which, in one experiment, it was directly compared

Table 1 INHIBITORY ACTIVITIES OF COMPOUNDS RELATED TO ADENOSINE COMPARED WITH THAT OF ADENOSINE ITSELF

Compound	Activity % of the activity of adenosine
2-oxy, 6-amino purine riboside (isoguanosine)	5
Diphosphopyridine nucleotide (DPN)	2
6-methyl amino purine riboside	2
Flavin adenine dinucleotide (FAD)	2
Purine riboside*	Less than 1
2 6 Diamino purine riboside	
2-amino, 6-oxy purine riboside (guanosine)*	
2-oxy, 6-amino pyrimidine riboside (cytidine)*	
6-mercapto purine riboside	
6-oxy purine riboside (inosine)*	
Adenine*	
6-amino purine, 2-deoxy-D-riboside (deoxyadenosine)	
6-amino, 8-aza purine riboside (azaadenosine)	
6-amino-9-β-D-fructopyranosyl purine	
6-amino-9-α-D-fructofuranosyl purine	
6-amino-1′-hydroxymethyl purine riboside (psicofuranine)	
Adenosine 3′,5′-cyclic phosphate (cyclic AMP)	
Triphosphopyridine nucleotide (TPN)	
Ribose*	

* Tested by Born and Cross (ref 2)

Only two of the compounds tested, deoxyadenosine diphosphate (deoxy-ADP) and adenosine tetraphosphate, brought about aggregation of platelets Their activities were compared with that of 10^{-6} M ADP by finding the concentrations at which they produced the same response. Deoxy-ADP had 0 3 per cent of the activity of ADP Gaarder *et al* also found that, apart from ADP deoxy-ADP was the only nucleotide which induced aggregation and in their system its activity was exactly the same as in ours[1] Adenosine tetraphosphate had 12 5 per cent of the activity of ADP This result is difficult to interpret, as the compound is unstable and may have been broken down to ADP

The observations suggest that derivatives of adenosine are in some way bound to the platelet through the adenosine part of the molecule When ADP is bound, pyrophosphate groups are introduced at the platelet surface, these may provide bridges between one platelet and another, perhaps through the formation of complexes with calcium which is necessary for aggregation by ADP[3]

Adenosine and AMP presumably inhibit aggregation by occupying sites normally available to ADP However, neither adenosine nor AMP provide pyrophosphate groups Therefore, it might be expected that derivatives of ADP in which its pyrophosphate group was 'blocked' would act as inhibitors of platelet aggregation In fact, both DPN and FAD have inhibitory activity

The specificity of the adenosine derivatives, when compared with other nucleosides and nucleotides, is even more remarkable in view of the recent observation that 5-hydroxytryptamine and noradrenaline induce aggregation of platelets[4] We have found that adenosine inhibits aggregation by 5-hydroxytryptamine and by noradrenaline just as it does aggregation by ADP Aggregation brought about by thrombin is also inhibited by adenosine (unpublished results, see also ref 5) It is likely, therefore, that adenosine derivatives influence some fundamental step common to all these ways of bringing about aggregation of platelets

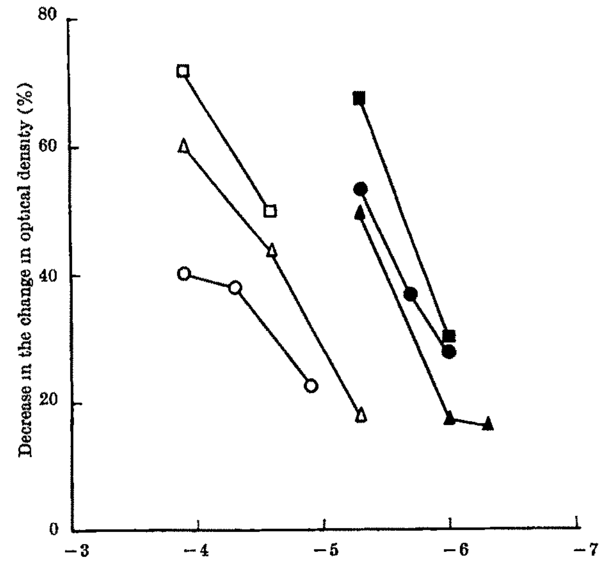

Fig 1 Relation between the logarithms of the concentrations of isoguanosine and adenosine and the inhibition of platelet aggregation Ordinate, reduction in the change in optical density expressed as a percentage of the change in optical density which followed the addition of ADP alone, abscissa, logarithm of concentration of isoguanosine (open points) and adenosine (closed points) Plasmas from three different individuals were used (□—□, △—△, ○—○) In each experiment the inhibitor was added 5 min before the addition of ADP (10^{-6} M)

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IN VITRO EFFECT OF SALICYLATE ON AMINO-ACID ACCUMULATION BY KIDNEY CORTEX SLICES

By Dr STANTON SEGAL and ALBERTA BLAIR

Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

ANDREWS *et al* [1] contributed to our knowledge of the biochemical changes in salicylate intoxication with the finding of amino-aciduria in several affected patients Because of increased amounts of urinary cystine, they postulated the mechanism to be due to 'overflow' of plasma amino-acids elevated as a result of salicylate-induced liver damage Their hypothesis received support with the later observation of elevated serum α-amino-nitrogen levels in these patients[2] Later, Elliott and Murdaugh[3] demonstrated that the oral ingestion of 1 6 g of acetylsalicylic acid by normal subjects increased urinary amino-acid excretion two-fold, the increase representing a generalized amino-aciduria Although the latter investigators did not measure plasma amino-acids, they suggested that salicylate produced a defect in tubular transport by interfering with oxidative metabolism Amino-aciduria has also been observed in salicylate-intoxicated rats[4]

It is known that amino-acids labelled with carbon-14 are actively accumulated against a concentration gradient in rat kidney cortex slices, and that the processes involved are dependent on oxidative metabolism and conform to Michaelis–Menten kinetics[5] This, therefore, seemed an appropriate system in which to ascertain whether a direct effect of salicylate on the kidney tubular cells might explain the salicylate-induced amino-aciduria This report presents results concerning the influence of this drug in the rat kidney slice system. In addition, results are presented of investigations performed using human kidney tissue obtained at surgery

Male Sprague–Dawley rats weighing 120–160 g were used in all experiments and were fed a 'Purina' chow diet and water *ad libitum* until killed by stunning and decapitation The techniques utilized for the preparation of kidney cortex slices, aerobic incubation in Krebs–Ringer bicarbonate buffer, *p*H 7 4 at 37° C and assessment of intracellular accumulation of the labelled amino-acids have been described previously[5] The concentrative uptake of amino-acid has been expressed as a distribution ratio which is expressed

$$\text{distribution ratio} = \frac{\text{counts/min/ml intracellular fluid}}{\text{counts/min/ml medium}}$$

and reflects the ratio of the concentration of amino-acid inside to that outside the cell A distribution ratio higher than one indicates concentration against a gradient and generally indicates an active transport process

All radioactive samples were counted in a liquid scintillation spectrometer at 57 per cent efficiency Ninety-minute incubations were carried out except when noted Sodium salicylate, as well as the radioactive amino-acids, were obtained commercially All amino-acids were chromatographically pure After incubation, aliquots of the boiled aqueous tissue extract of the slices were chromatographed and more than 90 per cent of the radioactivity was recovered at the appropriate R_F for the specific amino-acid investigated

The distribution ratios obtained in rat kidney slices for the several amino-acids tested are shown in Table 1 A concentration of 40 mg per cent salicylate was used since this approximated the blood levels of salicylate-intoxicated children shown to have amino-aciduria Salicylate significantly inhibited the accumulation not only of the model amino-acid α-amino-isobutyric acid (AIB), but also that of the natural amino-acids, glycine, L-phenylalanine, L-histidine and L-lysine This concentration of the drug had no influence on the size of the inulin space, 25 7 ± 1 1 per cent of the wet tissue weight[6], or the amount of total tissue water, 80 2 ± 1 per cent of the wet tissue weight

Table 1 EFFECT OF SALICYLATE ON ACCUMULATION OF AMINO-ACIDS BY RAT KIDNEY CORTEX SLICES

	Distribution ratio		
	Control	Salicylate 40 mg %	*P*
AIB (0 065 mM)*	4 45 ± 0 31† (13)	2 70 ± 0 17 (13)	< 0 001
Glycine (0 015 mM)	9 10 ± 1 15 (4)	5 25 ± 0 86 (4)	< 0 05
L-phenylalanine (0 15 mM)	2 72 ± 0 32 (4)	1 67 ± 0 11 (4)	< 0 05
L-histidine (0 035 mM)	2 75 ± 0 13 (6)	1 65 ± 0 07 (6)	< 0 01
L-lysine (0 065 mM)	3 99 ± 0 25 (6)	2 87 ± 0 22 (6)	< 0 01

* Initial medium concentration
† Mean ± *S E* All data are paired
No of determinations in brackets

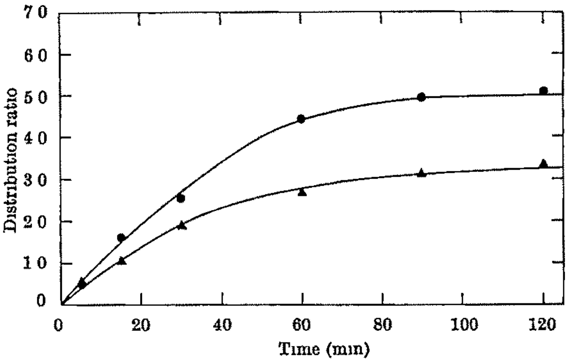

Fig 1. The effect of salicylate on the accumulation of AIB by rat kidney cortex slices ●, Control, ▲, salicylate, 40 mg per cent

The values obtained for distribution ratio after ninety-minute incubation (Table 1) represent the accumulation by the cells after a steady state has been reached[7] That is, the distribution ratio is at a constant level dependent on the relation of the rate of entry of the amino-acid into the cell and the rate of egress from the cell To demonstrate the findings prior to attainment of the steady state, the effect of salicylate on the time course of accumulation of α-amino-isobutyric acid (AIB) is shown in Fig 1 Each time point represents paired data obtained from three different animals It may be observed that the salicylate has an inhibitory effect on the accumulation as measured by the distribution ratio after fifteen minutes of incubation, with the attainment of a final distribution ratio about 35 per cent lower than the control

Data such as shown in Fig 1 may be analysed kinetically, using the mathematical model approach of Rosenberg, Berman and Segal[7] We have thus determined the fractional rate of influx and efflux of AIB for the kidney slice as influenced by salicylate The fractional rate constants were 0 00247 min^{-1} and 0 0216 min^{-1} for influx and efflux respectively in the controls The values were 0 00192 min^{-1} and 0 0294 min^{-1} for influx and efflux respectively for the salicylate-treated tissue This represents a 22 per cent decrease in influx and a 36 per cent increase in efflux fractional rate constants According to this analysis, the effect of salicylate is to decrease the transport of amino-acid into the cell while at the same time accelerating the rate at which the amino-acid already accumulated leaves the intracellular pool and returns to the medium

Salicylate was found to have an inhibitory effect on accumulation of amino-acid by rat kidney cortex slice at drug concentrations lower than 40 mg per cent, a significant inhibition being found at a concentration of 10 mg per cent At 20 mg per cent the inhibition appeared to be maximal with little further inhibition beyond this point up to the 80 mg per cent investigated An attempt was made to overcome the inhibition of amino-acid accumulation caused by salicylate by the addition of various substrates to the incubation buffer None of the following compounds at 5 millimolar levels were able to accomplish this glucose (90 mg per cent), pyruvate, fumarate and acetate

The salicylate-induced defect in amino-acid accumulation was also present when rat hemidiaphragm was investigated No such effect could be induced, however, in rat small-intestinal segments, using glycine as the test amino-acid With regard to the structural specificity of the salicylate molecule to produce its effect, we were unable to show a similar inhibition with the isomer *p*-hydroxybenzoic acid

Slices of human cortex weighing 5–20 mg were examined in a fashion identical to that utilized for the rat using a single 90-min incubation point The results are shown in Table 2 The effect of salicylate in human tissue paralleled that in rat tissue and caused a decreased intracellular accumulation of amino-acid in each of the patients examined Cystine was included in the experiment in one patient to see if the cystinuria seen in salicylate intoxication could be reflected in a direct kidney action of the drug

Table 2 ACCUMULATION OF AMINO-ACIDS BY HUMAN KIDNEY CORTEX SLICES

	Distribution ratio							
	AIB (0 065 mM)		Glycine (0 015 mM)		L-lysine (0 065 mM)		L-cystine (0 020 mM)	
Diagnosis	Control	Salicylate*	Control	Salicylate	Control	Salicylate	Control	Salicylate
Normal	13 00†	11 94	21 71	16 57			8 17	6 76
Ischæmic	18 87	13 37	20 32	15 02	21 77	14 16		
Ischæmic	18 40	16 07	24 19	18 23	16 44	12 08		

* 40 mg per cent
† Each value is an average of triplicate determinations.

The physiological mechanism of the salicylate effect on amino-acids appears to be an inhibition of transport of the amino-acid into the cell and prevention of maintenance of the intracellular amino-acid pool by causing an acceleration of the loss of amino-acid from the pool The biochemical effect can only be speculated on. The similarity of the effect of salicylate to the effect of metabolic inhibitors such as dinitrophenol[7] and maleic acid[8] in the same kidney slice system prompts the suggestion, similar to that of Elliott and Murdaugh[3], that salicylate interferes with the oxidative metabolism so vital to the formation and maintenance of intracellular amino-acid pools[5] That salicylate uncouples oxidative phosphorylation has been well established[9]

The conclusion by Andrews *et al* [1] that the amino-aciduria of salicylate intoxication is entirely explainable as an 'overflow' phenomenon does not seem to be entirely justified Their conclusion was partly based on the presence of cystine in the urine We have shown that salicylate can interfere with cystine accumulation (Table 2) The present findings recorded here suggest that a major factor in salicylate-induced amino-aciduria, though perhaps not the sole factor, is a direct effect of salicylate on the renal tubule, causing a decreased reabsorption of filtered amino-acids The recently reported shedding of renal tubular cells caused by salicylates in humans[10] indicates that a structural alteration may be induced in the tubule The association of the latter phenomenon to the amino-aciduria is open to speculation

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PHYTOHÆMAGGLUTININ: SEPARATION OF HÆMAGGLUTINATING AND MITOGENIC PRINCIPLES

By Dr. P. BARKHAN and Dr A. BALLAS

Medical Research Council Experimental Hæmatology Research Unit, Wright-Fleming Institute, and Department of Hæmatology, St Mary's Hospital Medical School, London, W 2

THE existence of hæmagglutinins (phytohæmagglutinins) in the seeds of some plants, particularly Leguminosae, has been known for many years[1,2] Li and Osgood[3] used an extract of red kidney beans (*Phaseolus vulgaris*) to sediment red cells and then isolate leucocytes for culture Later, Rigas and Osgood[4] described a method for the preparation and concentration of the bean phytohæmagglutinin (PHA) Nowell[5] then made the important discovery that PHA induced mitosis in human peripheral blood leucocytes, and his observation led to the development of a simple culture method which has been widely used for the examination of human chromosomes The cellular and biochemical changes in the leucocytes cultured with PHA have been examined by Cooper, Barkhan and Hale[6,7], Carstairs[8], Elves and Wilkinson[9] and Quaglino, Hayhoe and Flemans[10] It seems that PHA induces synthesis of DNA in the lymphocyte population of the leucocyte cultures Enlargement of certain lymphocytes with morphological transformation to cells that resemble blast cells is a striking feature of these cultures These transformed cells then undergo mitosis

It is of fundamental interest to know more about the plant mitogen and how it works, since this information may be valuable during investigations on the general problem of cellular proliferation An important preliminary step is to find out whether the hæmagglutinating activity is separable from the mitogenic activity In this communication we present evidence which favours the existence of two separate principles

Normal venous blood obtained from clinically healthy subjects was mixed with preservative-free heparin (0 25 mg heparin/ml. blood) The container was then centrifuged at 1,500 r p m for 10 min and about 0 5 ml buffy coat was removed and set aside in a separate bottle containing culture medium *TC* 199 The leucocytes in this buffy coat suspension were used for culture (see following) To the remainder of the blood 0 2 ml (2 mg) PHA was added The blood and PHA were carefully mixed and then left to stand at room temperature for 30–60 min Thereafter the container was centrifuged at 3,500 r p.m. for 20 min The supernatant cell-free plasma (3–4 ml) was then removed, great care being taken not to disturb the buffy coat This plasma was then well mixed with a fresh volume of red cells from which the plasma had been removed by prior centrifugation After standing for 30–60 min at room temperature the bottle was centrifuged at 3,500 r p m for 20 min and the supernatant plasma was carefully removed The plasma was then absorbed a third time with fresh red cells This thrice-absorbed plasma was added to the buffy coat suspension to produce a leucocyte concentration of 0 5–1 million cells/ml , the total volume of the culture was about 10 ml and the plasma concentration was 25–50 per cent The culture was incubated at 37° C for about 65 h Colchicine to a final concentration of 10^{-6} M was then added to the culture, which was incubated for a further 2 h. Thereafter the bottle was centrifuged and a drop of the cellular deposit was mixed with a drop of 1 per cent orcein in 45 per cent acetic acid and overlaid with a coverslip This preparation was examined for cell transformation and mitotic activity

The agglutinin titre was estimated by a tube method, using a 5 per cent suspension of red cells in normal saline

The phytohæmagglutinin was prepared from red kidney beans (*Phaseolus vulgaris*) at the Wellcome Research Laboratories, Beckenham, England The dry material was dissolved in normal saline to a concentration of 10 mg/ml

Removal of agglutinin Group A, B and O red cells were equally well agglutinated by the PHA preparation, and it was assumed, therefore, that they adsorbed the PHA equally well

Most of the agglutinating activity of the PHA was removed from the plasma by the first absorption with red cells (titre reduced from 1 32 to 1 · 4) The second absorption removed most of the remaining agglutinins (agglutination present only in undiluted plasma) After the third absorption plasma samples still showed a trace of agglutinating activity Even with six absorptions careful examination showed that traces of agglutinin remained Three absorptions removed more than 95 per cent of the original agglutinating activity

Cell transformation and mitotic activity The cultures prepared with absorbed plasma showed excellent cell transformation and mitotic activity (Fig 1) In terms of these two parameters they were at least as active as the

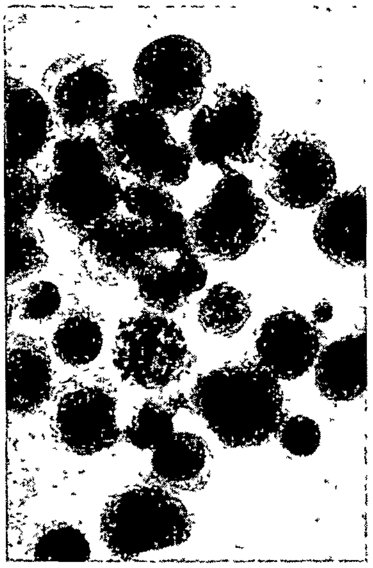

Fig. 1. Photograph of transformed cells and mitoses in a leucocyte culture prepared with PHA in plasma after absorption with red cells. The culture was treated with 0·5 per cent sodium citrate to partially separate the chromosomes in the dividing cells. The photograph shows two cells in metaphase (× 800)

control cultures which were set up with plasma and 0·2 ml PHA. Equally good results were obtained with A, B and O group blood. In drawing off the buffy coat for culture, relatively large numbers of red cells accompanied, and, in fact, outnumbered the leucocytes. The homogeneous unagglutinated appearance of the red cell suspension in the 'absorbed' culture was a striking macroscopic and microscopic feature and contrasted sharply with the control which showed gross red cell agglutination. These appearances persisted throughout the culture period.

Absorption from culture medium. During the course of this investigation it was found that if PHA was added to *TC* 199 medium a single absorption with washed red cells was sufficient to remove all the agglutinating activity, that is, in the absence of plasma the agglutinin was more easily removed. This agglutinin-free absorbed supernate produced both cell transformation and mitoses. Although these changes were less marked than those found with absorbed plasma, they were clearly evident and unequivocal.

Electrophoresis of PHA. We have attempted to separate the mitogenic and agglutinating principles by starch block electrophoresis (barbital buffer, *p*H 8·6, ionic strength 0·05). Agglutinating activity was found on the cathodal side of the origin in a zone stretching between and overlapping the β- and γ-globulin bands of the human plasma marker. However, none of the fractions showed mitogenic activity.

The PHA was electrophoresed on cellulose acetate paper (barbital buffer *p*H 8·6, ionic strength 0·05) which was then stained with ponceau *S*. Three bands were seen: two major ones corresponding in mobility to γ-globulin and a minor one with the mobility of α-2-globulin of the human plasma marker.

This investigation shows that agglutinating activity can be removed from PHA without loss of the mitogenic factor. It would seem, therefore, that the agglutinating and mitogenic activities are different entities. The removal of the agglutinin by absorption with red cells offers a simple and rapid way of separating the two principles. Although it is difficult to remove the last traces of agglutinating activity from plasma, removal is complete from culture medium.

Punnett and Punnett[11] have attempted to separate the mitogen (leucocyte growth factor) from the agglutinin with the use of a rather drastic and extensive chemical fractionation procedure. They concluded that either the growth factor and agglutinin are different molecules or separable portions of one macromolecule. However, they pointed out that the preparative procedure used was so extensive that it was not possible to distinguish between these two possibilities, and stated that it would not be certain that the leucocyte growth factor and phytohæmagglutinin are different components until a less-drastic procedure has been worked out. Absorption with red cells is probably the least-drastic procedure for this kind of separation. Another point in favour of two different substances is the result of fractionation by starch-block electrophoresis, which is also a relatively mild procedure; agglutinating activity was easily recovered, but the mitogenic activity appeared to have been lost. Failure to recover the mitogen might have been due to its intrinsic instability under the conditions used, or to dissociation from an active complex or to very fast migration causing it to run off the block. Thus, after absorption with red cells, mitogenic activity could be obtained with little or no agglutinating activity, and after starch-block electrophoresis agglutinating activity could be found without mitogenic activity.

It has recently been reported that tuberculin[12] and diphtheria toxoid[13] are mitogenic in leucocyte cultures from appropriately immunized subjects. Their mitogenic activity appears to be similar to that of phytohæmagglutinin, but, so far as is known, neither of these substances has hæmagglutinating activity. These findings show that hæmagglutinating activity is not a necessary requirement for mitogenic activity.

When PHA was put into *TC* 199 medium, which was then adsorbed with washed red cells, mitogenic activity persisted although the agglutinin was completely removed. However, there seemed to be less mitotic activity than that found with the absorbed plasma, and this would suggest that some mitogen and agglutinin were removed together. Nevertheless, it was quite clear that mitogenic activity was still present in the complete absence of agglutinating activity. It might be argued that the mitogenic activity of the absorbed plasma was dependent on the remaining trace of agglutinating activity. While this argument cannot be directly refuted at present, we have found that certain batches of commercial phytohæmagglutinin preparations (obtained from abroad) possess no mitogenic activity, although they have agglutinating activity which is greater than that present in the adsorbed plasma.

TC 199 medium is a synthetic, non-protein mixture which is clearly very different, both chemically and physically, from plasma. It may therefore be that the conditions for absorption of PHA from plasma and from *TC* 199 medium are different, and that this could produce both quantitative and qualitative differences in the effects of absorption. Two explanations might be considered: mitogen complexes with some component in plasma, which prevents its adsorption by red cells, or mitogen reacts with one or more constituents in *TC* 199 medium which allows partial adsorption on to red cells. An attempt is being made to investigate these possibilities.

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EARLY EFFECTS OF GLUCOCORTICOIDS ON PRECURSOR INCORPORATION INTO GLYCOGEN

By Prof HAROLD L SEGAL and Miss CONCEPCIÓN GONZÁLEZ LÓPEZ

Department of Pharmacology, St Louis University School of Medicine, St Louis 4, Missouri

THE activity in liver of a number of enzymes concerned with amino-acid metabolism has been shown to be increased consequent to administration of glucocorticoids to the whole animal These include tryptophan pyrrolase[1], glutamic-tyrosine transaminase[2], and glutamic-alanine transaminase[3] The first two of these reach a peak of activity a few hours after hormone administration, while the last increases in activity more slowly, requiring about five days to reach a maximum The question has been raised whether the increases in activity of these enzymes are causally related to the glucocorticoid-induced increase in glycogen synthesis Although the elevation in liver glycogen content in starved animals is clearly evident about four hours following hormone administration[4,5], it is not known whether all precursors flow into glycogen at an accelerated rate according to the same time sequence, or whether there is a stimulation in the conversion of diverse precursors into glycogen each according to a characteristic time course, perhaps as a consequence of the induction of separate enzyme activities A decision on this point would be expected to have a bearing on considerations of the mechanism whereby the glucocorticoid-induced increase in glycogen synthesis is brought about

As an approach to this question the incorporation of labelled alanine, pyruvate, glutamate, fructose and glucose into liver glycogen has been measured in normal rats and in rats treated 2 h previously with prednisolone acetate The results are presented in Table 1 It can be seen that in the period from 2–3 h after hormone administration there was a marked increase in the incorporation of all the precursors examined into glycogen, all to about the same extent

Since there is little or no detectable change in the activity of liver glutamic-alanine transaminase until several days after glucocorticoid administration[3]; it can be concluded that the glucocorticoid-induced alterations in the activity of this enzyme do not account for the prompt increase in the conversion of alanine into glycogen noted here In further support of this conclusion, in rats pre-treated with prednisolone acetate for five days, in which liver glutamic-alanine transaminase was elevated 3–5-fold, there was no further augmentation in alanine incorporation over that found under the conditions described in Table 1

Table 1 EFFECT OF PREDNISOLONE ON THE INCORPORATION OF PRECURSORS LABELLED WITH CARBON-14 INTO LIVER GLYCOGEN

Precursor	C p m in glycogen per g liver per 10^6 c p m injected* *N*	*P*	*P/N*
Alanine	139 ± 56 (7)	1,270 ± 289 (8)	9 2
Pyruvate	43, 56	359, 366	—
Glucose	129 ± 27 (10)	1,008† ± 162 (8)	7 8
Fructose	284 ± 74 (6)	2,004 ± 371 (6)	7 2
Glutamate	27, 88	1,163, 664	—
Metabolite	Concentration *N*	*P*	
Glycogen (mg/g liver)	45 1 ± 3 3 (29)	51 3 ± 4 4 (29)	
Glucose 6-phosphate (μmoles/g liver)	0 607 ± 0 029 (10)	0 796 ± 0 059 (8)	

200–250 g male rats were injected with 1 8–13 0 × 10^6 c p m of the compound shown They were killed by decapitation and a portion of the livers dropped into hot 30 per cent potassium hydroxide 1 h later for glycogen isolation Another portion was homogenized in perchloric acid for determination of glucose-6-phosphate with the use of glucose-6-phosphate dehydrogenase Glycogen was isolated and prepared for counting as described by Hassid and Abraham[6] Chemical assay of glycogen was by the anthrone method[9] Data are expressed as means ± the standard error, except for pyruvate and glutamate where each number is an individual measurement The number in parentheses below each mean value is the number of animals in the group *N* refers to normal animals *P* refers to animals injected subcutaneously with 2 mg of prednisolone acetate 2 h previous to administration of the labelled precursor * Corrected for self-absorption † In addition to the values represented in this calculation, 3 animals exhibited little or no increase in glucose incorporation consequent to prednisolone administration (97, 75, and 164 per cent of normal, respectively)

The results in Table 1 suggest that a reaction common to the pathways of conversion of all the precursors into glycogen is stimulated after administration of the glucocorticoid Recently, Hilz *et al* [7] reported a marked elevation in the glucose-6-phosphate levels of starved, adrenalectomized rats within a few hours after glucocorticoid administration, and drew the conclusion that the increase in glucose-6-phosphate levels was a primary effect of the hormone, leading to an increase in the rate of the glycogen synthetase reaction, on which the increase in glycogen formation was dependent However, in our experiments, under conditions where a several-fold increase in incorporation of precursors into glycogen was obtained in fed rats, there was little change in the liver glucose-6-phosphate concentration (Table 1) (Livers of fed adrenalectomized rats contained as high a level of glucose-6-phosphate as did those of fed normal animals, and glucocorticoid administration, as with the normals, had little effect on this parameter On the other hand, in livers of animals deprived of food for 17 h, the glucose-6-phosphate levels fell to values more than an order of magnitude less than those in fed animals, and glucocorticoid administration in this case produced a detectable increase within 2 h, as reported by Hilz *et al* [7] An important factor in the effect of starvation on the liver glucose-6-phosphate levels is probably the marked decrease in liver glucokinase activity under these conditions, as recently reported by Viñuela *et al* [8] In our experiments, prednisolone acetate administration 2 h prior to killing produced no effect on liver glucokinase activity in either fed or starved animals, although the levels in the latter were about 0 25 those in the former, as expected from the data of Viñuela *et al* [8] Administration of the hormone was also without effect on the activities of glucose-6-phosphatase and glucose-6-phosphate dehydrogenase under these conditions) It is possible that there are two pools of glucose-6-phosphate, a relatively small one in the pathway of glycogen synthesis and a second one in the pathway of glycogen breakdown An increase in the pool in the synthetic pathway might be masked in the fed animals, where the pool from glycogen breakdown is large, but apparent in starved animals, where the latter pool has all but disappeared

It would be useful to determine whether modification of the rate of synthesis of relevant enzymes is involved in the glucocorticoid control of glycogen synthesis Puromycin inhibited the hormonal stimulation of alanine incorporation into glycogen (Table 2). However, the results are somewhat ambiguous since the level of glycogen was also greatly reduced by puromycin administration, as previously reported by Hofert *et al* [10] On the basis of the rapidity of the puromycin effect, these workers have

Table 2. EFFECT OF PUROMYCIN AND PREDNISOLONE ON THE INCORPORATION OF ALANINE INTO GLYCOGEN AND PROTEIN

Compound injected	Glycogen		Protein	
	c.p.m./g liver per 10^6 c.p.m. injected	mg/g liver	c.p.m./g liver per 10^6 c.p.m. injected	mg/g liver
None (7)	168 ± 57	41.7 ± 5.7	1,810 ± 155	169 ± 7
Puromycin (5)	42 ± 8	12.2 ± 2.2	390 ± 109	170 ± 7
Prednisolone (7)	1,540 ± 320	43.9 ± 6.0	2,070 ± 350	161 ± 5
Prednisolone + puromycin (6)	76 ± 37	7.3 ± 1.4	295 ± 58	167 ± 8

Counting and assay of glycogen, timing of prednisolone and alanine administration, and expression of data are as described in Table 1. Puromycin dihydrochloride was injected intraperitoneally in 15 mg doses 3, 2, and 1 h before killing. For protein counting and assay, a portion of liver was homogenized in trichloroacetic acid, the sediment washed with alcohol-ether and hot trichloroacetic acid and dissolved in formic acid. An aliquot was plated for counting and another aliquot evaporated to dryness for protein assay by the method of Lowry *et al.*[9] All counts were corrected for self-absorption. Number in parentheses is the number of animals in the group. There is some overlap in the individual measurements from which means were calculated in Tables 1 and 2.

concluded that the reduction in glycogen-level cannot be accounted for primarily on the basis of even a complete block of glycogen synthesis, and therefore appears to indicate a stimulation of glycogen breakdown. Thus the lower level of counts from alanine appearing in glycogen may reflect an acceleration of the breakdown of newly-formed glycogen, rather than, or in addition to, a decrease in the rate of incorporation. (This ambiguity also exists in the interpretation of the data of Greengard *et al.*[11], who found that liver glycogen-levels in starved rats administered cortisone six hours before killing were greatly reduced when puromycin was also given.) The exceedingly short time interval between the administration of the hormone and the effect observed on precursor incorporation noted here would also appear to be an argument against modification of protein synthesis as an important factor.

The results in Table 2 also permit an assessment of the possible role of increased transport into the liver as a factor in the stimulation of precursor incorporation into glycogen. As can be seen from col. 3, there was at best only a minor effect of the hormone on the incorporation of alanine into protein, of the order expected from the results of Noall *et al.*[12] on the effect of glucocorticoids on α-aminobutyrate transport in liver. If a single pool of amino-acids exists as a precursor of both protein and glycogen, this result seems to rule out increased transport as a significant factor, at least in so far as alanine incorporation is concerned.

If the activity of an enzyme controlling the rate of conversion of precursors into glycogen is affected by the administration of glucocorticoids, it might be expected that the increased incorporation would be demonstrable in liver homogenates from hormone-treated animals. However, a two-hour previous administration of prednisolone acetate was without effect on the incorporation of alanine or pyruvate into glycogen in homogenates in our experiments thus far.

The primary site of action of glucocorticoids in their stimulatory effect on glycogen synthesis still eludes definition. The marked increase in the flow of precursors into glycogen reported here precedes the increase in glycogen-level and is the earliest effect of glucocorticoids on glycogen synthesis yet observed. The fact that precursors which enter the pathway of glycogen synthesis both at the 3-carbon-level and at the hexose-level are affected indicates that the site of stimulation is at a step late in the sequence. The rates of a number of the reaction steps of glycogen metabolism are under the complex control of components of the catalysed reaction as well as other compounds present in liver[13,14]. It may well be that the key to the glucocorticoid effect on glycogen formation lies in the interaction of the hormone with these control systems.

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ACIDIC POLYSACCHARIDE ANTIGENS OF A NEW TYPE FROM *E. COLI* CAPSULES

By IDA ØRSKOV and FRITS ØRSKOV

International Escherichia Center, Statens Seruminstitut, Copenhagen

AND

BARBARA JANN and KLAUS JANN

Max-Planck-Institut fur Immunbiologie, Freiburg-Zahringen

DURING recent years, Westphal and Luderitz, in collaboration with Kauffmann, have carried out extensive immunochemical analyses of polysaccharide antigens from various genera of Enterobacteriaceae[1-3]. As a logical continuation of these studies, during the past two years, in our laboratories in Freiburg and Copenhagen a more detailed examination of cell-wall and surface antigens of many *Escherichia* strains was undertaken. Until now, about one hundred serologically classified *E. coli* strains have been extracted and analysed. In earlier communications[1-3] the main emphasis was placed on the lipopolysaccharides (*O antigens*) of *Salmonella* serotypes. It is known that many *E. coli* strains carry *K antigens* in addition to their specific O antigen. We therefore extended our studies to include capsular antigens also. Results of the analysis of the sugar constituents of *Escherichia* O antigens (see also ref. 4) will be published later. In this article a new type of acidic polysaccharides corresponding to known *E. coli* K antigens is described. From two *E. coli* strains these polysaccharides were isolated, purified and analysed. By routine analysis of extracts, acidic polysaccharides were found to be present in about 20 per cent of all investigated *E. coli* strains.

The first acidic polysaccharide was isolated from strain *RVC* 2907(0141 *K*85 *ab→ac H*4). The notation of the K antigen of *RVC* 2907 as *K*85*ab→ac* indicates that quite frequently this strain splits off colonies which agglutinate in *K*85*ac* and not in *K*85*ab* serum. Changes in the opposite direction from *K*85*ac* to *K*85*ab* have not yet been observed. The common factor *K*85*a* is only poorly developed. It is possible to produce pure factor sera corresponding to the factors *b* and *c*. The K antigen of this strain was designated as a B antigen, even though it was found to be more thermo-resistant than ordinary B antigens. With respect to thermostability it should be placed between the A and B types of *E. coli* K antigens[5]. The second acidic polysaccharide originates from strain *A*295*b*(08 *K*42(*A*) —), which is the test strain for *E. coli* K antigen 42. This K antigen belongs to the A type which renders the parent strain inagglutinable in 08 serum in the living state, but also after boiling for 1 h. Two hours at 120° C will render it agglutinable to some extent in the homologous O serum.

E. coli strains were grown on ox heart infusion agar and the acetone-dried bacteria were extracted with 45 per cent phenol at 65° C for 5 min (ref. 6). The aqueous phase was dialysed, concentrated and ultracentrifuged at 105,000*g* to give a sediment of the high molecular weight lipopolysaccharide (O antigen). The supernatant was freeze-dried and the resulting material dissolved in 0·25 molar sodium chloride. Addition of 'Cetavlon'[7,8] led to the precipitation of nucleic acid–'Cetavlon' salts which were removed by centrifugation. By dilution of the supernatant with water the acidic polysaccharide was precipitated as the 'Cetavlon' salt. After separation by centrifugation the 'Cetavlon' salt was transformed into the sodium salt which was dissolved in water. By ultracentrifugation at 105,000*g* trace amounts of lipopolysaccharide, carried along with the procedure, were removed as sediment. After freeze-drying of the supernatant the purified acidic polysaccharide was obtained as a white powder. Recently, Srivastava and Adams[9,10] have applied a similar procedure for the isolation and purification of acidic polysaccharides from *Serratia marcescens*.

The procedure, when applied to *E. coli RVC*2907(0141 *K*85(*B*) *H*4) and to *E. coli A*295*b*(08 *K*42(*A*) —), yielded 5·5 and 7·8 per cent of purified acidic polysaccharide respectively. The substances dissolve slowly in water to give clear and viscous solutions, with $[\alpha]^{25}_{5461} = +\ 9{\cdot}4°$ and + 93·5° respectively.

The acidic capsular polysaccharide obtained from *E. coli RVC*2907(0141 *K*85(*B*) · *H*4) sediments in the ultracentrifuge as a homogeneous band. From the sedimentation constant of $s_{20} = 3{\cdot}5\ S$ and the diffusion constant of $D_{20} = 2{\cdot}6$ cm^{2}sec^{-1}, a molecular weight of approximately 100,000 is calculated for the polysaccharide. Its main constituents are mannose (33 per cent), rhamnose (18 per cent), glucuronic acid (19 per cent) and glucosamine (17 per cent). In addition the following radicals were found: *O*-acetyl (4 per cent), *N*-acetyl (4 per cent) and *N*-acyl (6 per cent, calculated as β-hydroxy-myristic acid). Hydrolysis with 0·25 N sulphuric acid at 100° C for 45 min liberates *N*-acetyl glucosamine and a hitherto unidentified *N*-acyl glucosamine. Chromatographic investigation of hydrolysates of the acidic polysaccharide revealed a fatty acid which could not be differentiated from β-hydroxy-myristic acid. But the isolated *N*-acyl glucosamine was chromatographically distinct from synthetic (D,L)-*N*-β-hydroxy-myristinoyl-D-glucosamine[11]. This indicates that the polysaccharide contains an *N*-acyl glucosamine not identical with, but very closely related to, *N*-β-hydroxy-myristinoyl-D-glucosamine.

The lipopolysaccharide (O antigen) isolated from *E. coli RVC*2907(0141 *K*85(*B*) · *H*4) is composed of glucosamine, heptose, galactose and glucose, thus belonging to chemotype I (ref. 1). It should be noted that in the lipopolysaccharide and in the acidic polysaccharide, glucosamine is the only common sugar constituent, whereas heptose, galactose and glucose are found in the lipopolysaccharide, and mannose, rhamnose and glucuronic acid are present in the acidic polysaccharide only. With the acidic polysaccharide all tests for galactose and/or glucose were practically negative, indicating that it contains, if any, less than 2 per cent of contaminating lipopolysaccharide. The *E. coli RVC*2907 strain, therefore, produces two polysaccharides of very different composition.

Hydrolysis of the acidic polysaccharide with N sulphuric acid at 100° C for 3 h gave rise to an aldotriuronic acid, $[\alpha]^{25}_{5461} = +\ 4°$, consisting of two mannose and one glucuronic acid residues, mannose being at the reducing end. The trisaccharide was isolated by preparative paper chromatography in 12 per cent yield (based on the acidic polysaccharide). Apart from the removal of *O*-acetyl groups, the acidic polysaccharide is stable in aqueous alkali. Periodate oxidation of the polysaccharide results in the uptake of 0·35 moles of oxidant and the liberation of 0·1 mole of formic acid per mole of anhydrohexose. The formation of formaldehyde is negligible. During periodate oxidation all the rhamnose, 55–60 per cent of the glucuronic acid and about 20 per cent of the glucosamine are destroyed. Mannose is resistant to oxidation.

An acidic polysaccharide with the same composition has been isolated from strain *D*295(0141 *K*85*ac H*4), which is a K antigen mutant isolated from *RVC*2907, and also from *CG*9, which is a wild-type strain serologically identical with *D*295 but of independent origin.

The acidic polysaccharide obtained from *E. coli A*295*b*(08 *K*42(*A*) —), after purification by the aforementioned procedure, still contained 3–5 per cent protein, which could be removed by incubation with papain or by treatment with 45 per cent phenol at 65° C for 15 min. Neither method alters the chemical composition of the acidic capsular polysaccharide, which contains galactose (37 per cent,) fucose (18 per cent), galacturonic acid (33 per cent), glucosamine (2·5 per cent), and in addition the following radicals: *O*-acetyl (6·4 per cent), *N*-acetyl (0·6 per cent) and *N*-acyl (0·8 per cent, calculated as β-hydroxy-myristic acid). Hydrolysis with N sulphuric acid at 100° C for 2 h gave rise to an aldobiuronic acid, $[\alpha]^{25}_{5461} = +\ 53{\cdot}3°$, consisting of galacturonic acid and fucose as the reducing end. The disaccharide was obtained by preparative paper chromatography in a yield of 26 per cent. The lipopolysaccharide of *E. coli A*295*b*(08 : *K*42(*A*) —) was found to be composed of glucosamine, heptose, galactose, glucose and mannose (chemotype III (see ref. 1)) with additional acetyl groups and long-chain fatty acids. Thus, the acidic polysaccharide can be chemically differentiated from the lipopolysaccharide of the same strain by its content of fucose and galacturonic acid; its purity with respect to possibly contaminating lipopolysaccharide can be judged by testing for the absence of heptose, glucose and/or mannose.

Serological tests were carried out in order to elucidate the relationship of these acidic polysaccharides to known *E. coli* antigens. In gel-diffusion tests, saline extracts of living organisms of the two closely related strains *RVC*2907 (0141 : *K*85*ab* → *ac* : *H*4) and *D*295(0141 *K*85*ac* . *H*4) were tested against homologous (and heterologous) antisera. In addition to common precipitation lines, each strain showed a characteristic band as an expression of the differences in specificity found also by agglutination techniques, that is, differences of the K antigens. These bands were identical with those obtained by precipitation of the purified acidic polysaccharides and the corresponding bacterial antisera. By exhaustive precipitation (absorption) using the acidic polysaccharide, it was possible to remove from an OK serum the precipitins responsible for the strain-specific K antigen line and all homologous K agglutinins. It should also be mentioned that in gel diffusion tests the lipopolysaccharides (O antigens) of these two strains are identical and unrelated to the K antigen bands. With and without Freund's adjuvant, the purified acidic polysaccharide of *RVC*2907 is non-antigenic in rabbits

In serological tests with the acidic polysaccharide from *E coli A*285*b*(08 . *K*42(*A*) —) we found that this substance will remove from a homologous OK serum almost all agglutinins for the living organisms, whereas O antibodies were not absorbed In gel-diffusion tests with bacterial extracts (saline) the absorbed serum did not give any precipitation line characteristic for the K antigen Clear-cut results were also obtained using the passive hæmagglutination technique[12,13] For this purpose the following sera were used an OK serum against 08 *K*42, a pure *K*42 serum produced by absorption of the OK serum with *E coli D*284(08 . *K*-), a K^--form of the strain 08 *K*42, and a pure O serum Human blood group B erythrocytes were sensitized with the lipopolysaccharide (O antigen) or with the acidic polysaccharide respectively It was found that the acidic polysaccharide modified erythrocytes directly, that is, without alkali or heat pre-treatment[13] In the hæmagglutinating systems the acidic polysaccharide was found to be highly reactive with OK and K sera (titres of 1 20480 in both systems), but not reactive at all with O serum (titre 1 10) On the other hand, the lipopolysaccharide was highly reactive with OK and O sera, but not reactive at all with K serum

Prof Michael Heidelberger, Institute of Microbiology, New Brunswick, New Jersey, kindly tested the two acidic capsular polysaccharides, described here, for cross-precipitation with *Pneumococcus* horse antisera He found the acidic polysaccharide of *E coli* 0141 *K*85(*ab*→*ac*) to cross-react strongly with type V antiserum and less markedly with type II antiserum The acidic polysaccharide from *E coli* 08 *K*42 showed cross-reactivity with type XXV antiserum These cross-reactions, which are based on common structures probably including identical uronic acid units, may be of value for the elaboration of specific structures in the respective acid polysaccharides

Since there are several similarities between *E coli* strains with K antigens of the A type and capsulated *Klebsiella* strains, it might be mentioned here that recently Barker *et al*[14] have described an acidic polysaccharide from a *Klebsiella* strain which is mainly composed of glucose, fucose and glucuronic acid

The acidic polysaccharides with serological K specificity are distinct from another type of acidic polysaccharides, which appear to be similar to or identical with those described by Goebel (colanic acid[15]), Anderson[16] and Wilkinson[17] and which are probably the *M* antigens common to many Enterobacteriaceae These rather ubiquitous acidic slime substances were separated from the acidic polysaccharides with K specificity by fractional 'Cetavlon' precipitation Separation is effected as follows the aqueous phase of phenol/water extracts (65° C (ref 6)) is dialysed, concentrated and ultracentrifuged at 105,000*g* (sedimentation of the lipopolysaccharide) and the supernatant is lyophilized The resulting material is dissolved in 0 25 molar sodium chloride and 'Cetavlon' is added to precipitate the nucleic acid fraction, which is removed by centrifugation The supernatant is stirred and slowly diluted with water The acidic slime substance precipitates as 'Cetavlon' salt after the addition of 0 5–1 volume of water, whereas the acidic capsular polysaccharide with K specificity is precipitated only after the addition of 1 5–2 volumes of water The precipitates are removed by centrifugation as they appear As an example from the mucoid phase of *E coli* 059 *K*(?) *H*19, grown at 18° C, the following polysaccharides could be extracted and separated 2 5 per cent lipopolysaccharide (O antigen), composed of glucosamine, heptose glucose and mannose (and traces of galactose), 9 per cent acidic slime substance (*M*), composed of glucuronic acid, galactose, glucose and fucose, 2 7 per cent acidic polysaccharide (*K*)—composed of galacturonic acid, glucosamine, galactose and mannose From the same strain, grown at 37° C, only lipopolysaccharide (3 2 per cent) and acidic polysaccharide (4 8 per cent) were isolated, but no acidic slime substance It is remarkable that a single bacterial species is able to synthesize these different complex polysaccharides

More detailed accounts of our work on acidic polysaccharides isolated from capsular antigens of *Escherichia* strains will be published later

We thank Prof O Westphal for his advice and criticism and for his continuous interest in this work We also thank Prof M Heidelberger for carrying out cross-precipitation tests of the acidic *E coli* capsular polysaccharides with *Pneumococcus* horse antisera

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REVERSAL OF PHOTOPERIODIC INDUCTION OF STRAWBERRIES WITH MALEIC HYDRAZIDE

By P A THOMPSON

Scottish Horticultural Research Institute, Mylnefield, Invergowrie, Dundee

IT has been shown[1] that treatment with maleic hydrazide, at levels which do not reduce the rate of leaf production, can prevent flower truss initiation in strawberry plants growing in inductive short-day cycles This substance has also been found[2] to inhibit flower initiation in Biloxi soybean, chrysanthemum and peppermint, but it was not certain that the effect was directly on the processes of flower initiation rather than a general inhibition of plant growth

Experiments involving the application of maleic hydrazide to strawberry plants over the past few years have occasionally resulted in the time of treatment being coincident with, or immediately successive to, the time of flower initiation, and this has resulted in a reversion of the flower shoot to a more or less vegetative condition in several strawberry varieties

In the first experiment a range of concentrations of maleic hydrazide was applied as a single dose to the leaves of plants of the variety Talisman growing in a growth cabinet at 15° C in a photoperiod of fourteen hours Three weeks after the treatment, the plants were transferred to a warm glasshouse where they were grown

Table 1. EFFECT OF A RANGE OF CONCENTRATIONS OF MALEIC HYDRAZIDE (MH) ON THE INCIDENCE OF PHYLLODY IN THE STRAWBERRY VARIETY TALISMAN

Concn. of MH (μg/plant)	No. of flower trusses (per 12 plants)	Phyllody (per cent)	Mean No. leaves to flower
0	10	0	5·4
100	7	0	5·4
500	6	0	5·8
1,000	5	20	5·7
1,500	7	43	5·2
2,000	6	50	5·1
2,500	8	75	5·1
3,000	7	71	6·1

from May 2 until harvested on June 10. Table 1 shows the range of concentrations applied, the numbers of flower trusses found, the percentage of flower trusses exhibiting phyllody, and the mean number of leaves emerging between the time of treatment and the emergence of the flower truss.

Table 1 shows that the incidence of phyllody in the flowers increased with increasing concentrations of maleic hydrazide, and the appearance of a fairly extreme example of flower truss reversion is shown in Fig. 1. In this experiment the mean number of leaves between treatment and flower emergence was about 5·5, and as this variety normally has between five and six leaf primordia within its bud, it may be inferred that the treatment was applied within one plastochron of the time of flower truss initiation.

In a second experiment maleic hydrazide was applied to plants of the variety Climax by spraying whole plants with solutions containing either 125 or 62·5 p.p.m. of active ingredient twice a week for a period of six weeks—July 29–September 19. There were twenty plants in each treatment. This variety initiates flowers freely over a long period and from the beginning of the course of treatment flowers at all stages of development were present in the bud; a number of abnormal flower forms resulted. These were broadly of two types; flowers in which varying degrees of inhibition of the development of the female parts occurred, which have been described in a previous paper[3]; and inflorescences showing phyllodious development of the flowers. The former type was found on inflorescences which were two or more plastochrons old at the first treatment; the latter was confined to those inflorescences which were initiated within one plastochron before or after the start of treatment. The degree of phyllody ranged from an almost complete reversion of the whole truss similar to the condition illustrated in Fig. 1 to minor effects on individual parts of the flower, particularly the carpels, similar to that shown in Fig. 3.

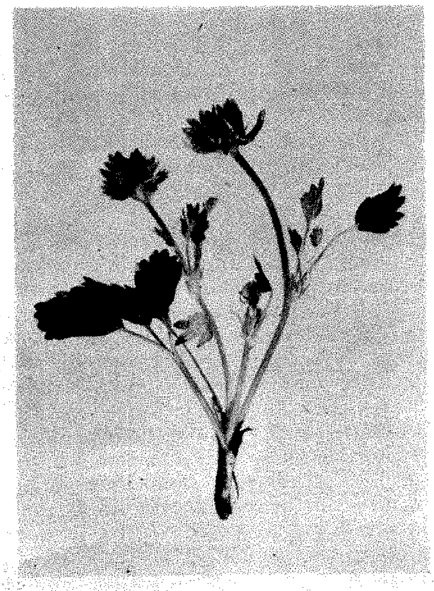

Fig. 1. Inflorescence of strawberry plant showing phyllody of the floral parts after treatment with maleic hydrazide

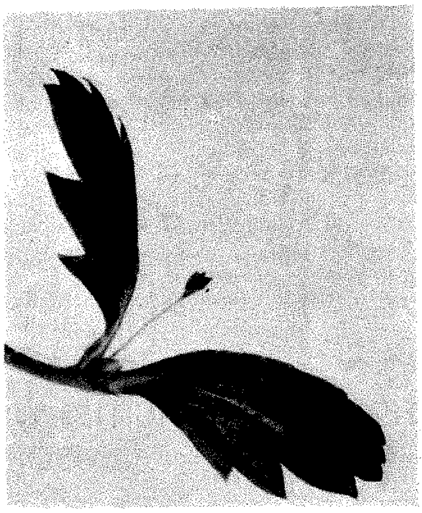

Fig. 2. Reversion of a primary flower of *Fragaria elatior* to a vegetative bud after treatment with maleic hydrazide

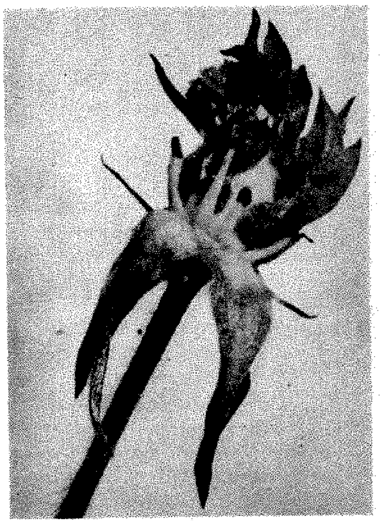

Fig. 3. Development of leafy bracts on the receptacle after treatment with maleic hydrazide

In the third experiment an aqueous solution of maleic hydrazide containing 75 p.p.m. of active constituent was sprayed twice weekly for four weeks—October 12–November 9—on to plants of the species *Fragaria elatior* (Ehr.) growing in a warm glasshouse; three plants out of the eight so treated produced flowers showing varying degrees of phyllody in positions which indicated that the flowers had just been initiated at the time of the first treatment. In one instance, illustrated in Fig. 2, the primary flower had reverted to a vegetative shoot bearing two bract-like leaves and a small bud in place of the flower, and other examples were found in which phyllody was confined to the development of leafy bracts on the receptacles in place of the carpels, illustrated in Fig. 3.

The results of these experiments show that not only can maleic hydrazide prevent the initiation of inflorescences in strawberries in response to favourable photoperiods, but also that it can cause a reversal of the processes following induction which normally lead to the development of a flower truss irrespective of the day-length.

The results also show that the timing of the spray was extremely critical, so that the effect was produced only if the treatment was applied within one plastochron of flower initiation. This is scarcely surprising, as the rate of development of strawberry flowers after initiation is rapid and the period during which they are subject to changes in their developmental processes is therefore short. It is not possible to indicate the nature of the changes which this treatment produced, since both the action of maleic hydrazide and the changes in the apical meristem accompanying the transition from the vegetative to the flowering condition are obscure. One may, however, conjecture that the changes which occur in the meristems are mediated through the RNA system and are produced or accompanied by alterations in the composition of this material.

In this connexion a recent paper by Butenko and Baskakov[4] is of particular interest, in which they demonstrated that maleic hydrazide-induced inhibition of callus tissue cultures could be overcome by the addition of uracil, and suggested that maleic hydrazide acts antagonistically to uracil by blocking its incorporation into the nucleic acid molecule. If the maleic hydrazide used in these experiments acted in a similar way on nucleic acid synthesis then this chemical may provide a useful tool for the investigation of the changes in the apical meristem occurring during photoperiodic induction.

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DIFFERENT SPECIFICITIES OF PROLINE-ACTIVATING ENZYMES FROM SOME PLANT SPECIES

By P. J. PETERSON* and L. FOWDEN

Department of Botany, University College, London

IN recent years many amino-acid analogues have been chemically synthesized and used as antimetabolites to natural compounds in the synthesis of proteins[1], while a large number of naturally occurring, newly characterized amino- and imino-acids[2,3] appear to have been neglected in such investigations[4], although often possessing structures closely akin to the protein amino-acids. Azetidine-2-carboxylic acid, a member of the latter group of compounds and the lower homologue of proline[5,6], has a restricted distribution in some members of the Liliaceae and the closely related Agavaceae[7]. It may occur in all parts of the plant as seen in *Convallaria majalis* and *Polygonatum multiflorum*[7,8], or its distribution may be restricted to certain plant organs, especially seeds[7]. The imino-acid, which is present in the aqueous ethanol-soluble nitrogenous fraction of these plants, is not known to occur naturally in plant proteins[9] and therefore has been loosely described as a non-protein imino-acid.

When azetidine-2-carboxylic acid was supplied to germinating seeds of different species in which it does not normally occur, for example, *Phaseolus aureus*, it produced marked growth inhibition and was lethal at high concentration[4]. The degree of inhibition was dependent on the concentration supplied and on the species investigated. The severity of growth inhibition produced by azetidine-2-carboxylic acid was greater than when the same concentrations of *p*-fluorophenylalanine were supplied to seedlings. Azetidine-2-carboxylic acid also inhibited the growth of carrot tissue cultures[10], *Escherichia coli*[11] and chick embryo[4]. This behaviour has been attributed to its action as a proline analogue, where a stoichiometric replacement of proline occurred with production of abnormal proteins having impaired biological activity[4,11]. The inhibitory action of azetidine-2-carboxylic acid was reversed by addition of proline, and therefore differences in endogenous proline concentrations could explain the differing degrees of growth inhibition observed with various plant species.

The absence of incorporation of azetidine-2-carboxylic acid into the proteins of liliaceous species producing this imino-acid (where the concentration may be as high as 50 times the lethal dose for *Phaseolus aureus*[8]) and its profound growth-inhibitory action on species not normally containing it suggest that some difference must exist in specific selection mechanisms for amino-acids in these two groups of plants. A differentiation between proline and azetidine-2-carboxylic acid could result from either of two causes[4]. The Liliaceae may have evolved a sub-cellular organization that excludes azetidine-2-carboxylic acid from the sites of protein synthesis, for example, vacuolar storage, or a more subtle enzymatic selection between proline and azetidine-2-carboxylic acid may occur at one (or more) points during the elaboration of protein molecules. The second, more interesting possibility has received our attention here.

The mechanism of protein biosynthesis is well established, the initial step being the enzymatic activation of amino-acids[12]. Investigations on more or less highly purified activating enzymes have shown that each amino-acid is activated by a specific enzyme and that these enzymes exhibit high specificity, often being able to discriminate between analogues and their normal amino-acid substrates[13]. In the majority of cases, if an analogue is activated, usually measured by the adenosine-5′-triphosphate-[^{32}P]pyrophosphate (ATP-[^{32}P]PP) exchange reaction, then it is incorporated into protein. However, discrimination at a subsequent intermediate reaction in protein synthesis has been reported[14], as has discrimination at the transfer step to *S*-RNA (transfer-ribonucleic acid)[15]. A selection at a later stage appears unlikely because a 'wrong' amino-acid becomes incorporated into protein if it is produced by chemical modification of the 'correct' amino-acid while the latter is attached to its specific *S*-RNA[16,17].

The varying abilities of the proline activating enzyme from several plant sources to differentiate between its normal substrate and azetidine-2-carboxylate, used as an analogue, are reported here. The results leave little doubt that the observed selective incorporation of azetidine-2-carboxylic acid into plant proteins aforementioned can be attributed to the differences of specificity observed for this enzyme when obtained from different sources. However, the reason why many liliaceous plants elaborate azetidine-2-carboxylic acid at all remains an intriguing, but unsolved, problem.

Enzyme Preparation and Assay Procedure. Four plant species were used in this investigation. Two did not contain azetidine-2-carboxylic acid, namely *Pisum sativum* L. var. Carter's little marvel (garden pea) and *Phaseolus aureus* (mung bean), while the other two, *Convallaria majalis* (lily-of-the-valley) and *Polygonatum multiflorum* (a Solomon's seal), contained azetidine-2-carboxylic acid in high concentration. The first two species were used for convenience as seeds, and ground to flour before use, while the latter species were collected as rhizomes in mid-March and stored at 1° C. The apical 3-cm long portion of the rhizomes was used for enzyme preparation. All manipulations were carried out in a cold room at 1° C.

Seed flour (45 g) was ground by pestle and mortar with washed sand (5 g) in a solution (135 ml.) of 0·1 M *tris* buffer (2-amino-2-hydroxymethylpropane-1,3-diol-HCl,

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* Holder of a New Zealand National Research Fellowship.

*p*H 7 5) containing 0 4 M sucrose. Rhizome tissue (60 g) was homogenized (0 5 min) at 12,000 r p.m with the same *tris* buffer-sucrose solution (150 ml) The slurries were pressed through cheesecloth and the liquid collected and centrifuged at 20,000*g* for 15 min (step *A*) The cell-free supernatant was diluted with water (45 ml) and then protein was precipitated by adding 0 1 M acetic acid to *p*H 4 2 and sedimented at 12,000*g* for 5 min The protein was re-suspended in *tris* buffer (95 ml) without sucrose, centrifuged to remove insoluble denatured material and fractionated over known *p*H ranges with centrifuging as before The precipitated protein was dissolved finally in 0 1 M *tris* buffer *p*H 7 9, 10 ml (step *B*) Further enzyme purification was achieved by ammonium sulphate precipitation of these fractions following standard procedure with additions of solid salt to give known saturations, precipitated protein was sedimented as before The precipitates were dissolved in *tris* buffer as above for the acid fractions and the solutions were dialysed for not less than 2 h against the same *tris* buffer (step *C*)

[^{32}P] Pyrophosphate was prepared from sodium [^{32}P] orthophosphate, by raising the *p*H to about 9, adding carrier Na_2HPO_4 and pyrolysing in a platinum crucible (500° C, 5 h)[18] Paper chromatography on 1 per cent (w/v) oxalic acid-washed Whatman No 2 paper with two solvent systems[19,20], followed by radioactivity scanning, showed the purity to be greater than 98 per cent pyrophosphate

The enzyme assay conditions are described in the footnote to Table 1 Reactions were stopped by cooling and adding cold 7 5 per cent (w/v) trichloroacetic acid (2 ml) Precipitated protein was sedimented (1,500*g*) and supernatant samples (1 ml) were added to 0 1 M sodium acetate (2 6 ml) ATP-[^{32}P]PP exchange was measured by a charcoal adsorption method[21,22] using acid-washed[23] 'Darco *G* 60' charcoal The two terminal phosphate groups of the charcoal-adsorbed ATP were hydrolysed (100° C, 15 min) with N hydrochloric acid (4 ml) and the radioactivity determined in 1 ml samples Inorganic phosphate determinations were also carried out on the hydrolysate[24] Protein content of the enzyme preparations was determined using bovine plasma albumin as standard[25] The results are expressed in terms of per cent exchange and rate of exchange[26] Each set of results presented was obtained from a different enzyme preparation

Results of Activation Investigations The soluble fractions obtained from different plant tissues after step *A* were tested for their ability to catalyse the ATP-[^{32}P]PP exchange The results shown in Table 1 (only two species are included to avoid repetition) indicate that added proline did not stimulate the exchange, although the addition of a 'complete mixture', that is, twenty protein amino-acids, did cause some stimulation of exchange Hence, in order to investigate proline activation a marked lowering of the endogenous exchange rate was essential

Table 1 EFFECT OF POTASSIUM FLUORIDE ON THE ACTIVATION OF AMINO-ACIDS BY HOMOGENATES OF *Pisum sativum* AND *Phaseolus aureus*, MEASURED BY ATP-[^{32}P]PP EXCHANGE

Species	Fluoride (μmole)	Amino-acid	Per cent exchange	*R* (μmole exchanged/h/mg protein)	*ΔR*
Pisum	20	Endogenous	16 58	0 010	—
	20	Complete mixture	25 76	0 016	0 006
	20	L-proline	16 60	0 010	N S
	0	Endogenous	32 38	0 021	—
	0	Complete mixture	36 60	0 025	0 005
	0	L-proline	32 40	0 021	N S
Phaseolus	20	Endogenous	38 32	0 055	—
	20	Complete mixture	40 74	0 060	0 005
	20	L-proline	38 39	0 055	N S
	0	Endogenous	40 81	0 061	—
	0	Complete mixture	44 33	0 067	0 006
	0	L-proline	40 71	0 060	N S

Incubation system ATP, 4 μmole, [^{32}P]PP, 4 μmole, about 10^5 c p m , magnesium sulphate, 10 μmole, *tris*-HCl buffer *p*H 7 6, 100 μmole, 0 6 ml enzyme solution L-proline, 5 μmole, 'Complete mixture', contained 20 protein L-amino-acids each at 2 5 μmole Final volume 1 ml Incubation at 37° C for 15 min All treatments duplicated

Table 2. ACTIVATION OF AMINO-ACIDS BY ACETIC ACID-PRECIPITATED PROTEIN FRACTIONS FROM HOMOGENATES OF FOUR PLANT SPECIES MEASURED BY ATP-[^{32}P]PP EXCHANGE

Species and acid fraction	Amino-acid	Per cent exchange	*R* (μmole exchanged/h/mg protein)	*ΔR*
Phaseolus *p*H 4 2–4 6	Endogenous	6 82	0 171	—
	Complete mixture	70 50	2 955	2 784
	L-proline	51 90	1 770	1 599
	L-A 2 C*	25 22	0 704	0 533
Pisum *p*H 4 2–4 6	Endogenous	2 33	0 055	—
	Complete mixture	38 31	1 131	1 076
	L-proline	9 30	0 228	0 173
	L-A 2 C*	3 79	0 091	0 036
Polygonatum *p*H 4 6–5 3	Endogenous	32 48	0 285	—
	Complete mixture	71 50	0 912	0 627
	L-proline	39 00	0 359	0 074
	L-A 2 C*	31 65	0 276	N S
Convallaria *p*H 4 6–5 0	Endogenous	16 09	0 478	—
	Complete mixture	90 40	6 380	5 902
	L-proline	37 80	1 293	0 815
	L-A 2 C*	16 29	0 484	N S

For incubation system, refer to legend Table 1 The fractionation *p*H ranges recorded were established on solutions which were first precipitated to *p*H 4 2 redissolved in 0 1 M *tris*-HCl buffer *p*H 7 6

*L-azetidine-2-carboxylic acid

An extract containing protein obtained from the original homogenate after precipitation at *p*H 5, that is, a '*p*H 5 fraction', showed some proline activating activity but the endogenous rate was still very high. Better results were obtained after step *B*, in which the protein was precipitated first down to *p*H 4 2, then redissolved to give an extract that was fractionally precipitated at several known *p*H values

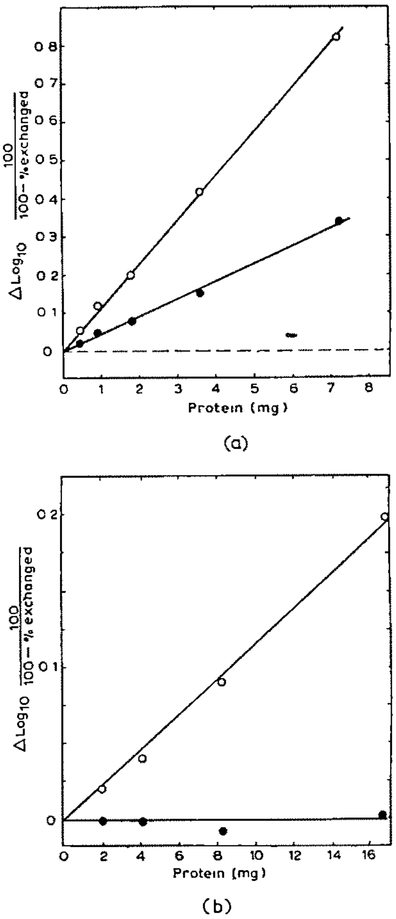

Fig 1 Effect of concentration of enzyme from protein fractions of (*a*) *Phaseolus aureus* and (*b*) *Polygonatum multiflorum* on L-proline (○) and L-azetidine-2-carboxylic acid (●) stimulated rates of ATP-[^{32}P]PP exchange Assayed under standard conditions described in Table 1

ATP-ase activity was present in the whole homogenate (step *A*) Potassium fluoride was added to inhibit this activity but also suppressed activation, especially in the pea seed extract, and was therefore omitted after subsequent purification steps where the ATP-ase was negligible This effect of fluoride on activation has been noted previously by other workers on higher plant extracts[27], but is absent in investigations on micro-organisms[22]

Table 2 shows the proline and azetidine-2-carboxylic acid activation values obtained for the four species together with results for the 'complete mixture' Data are given only for those *p*H values that correspond to the highest proline activation Protein precipitated over *p*H ranges above those shown exhibited markedly less activity, while measurements with protein precipitated below *p*H 4 2 were difficult owing to instability of the enzyme in these acid solutions The *p*H range over which the most active fraction precipitated was dependent on the origin and the purity of the protein and, in view of the differences in protein composition between dicotyledonous and monocotyledonous seeds, it is not surprising that species differences were encountered as is shown in Table 2

The most significant result seen in Table 2 is that azetidine-2-carboxylic acid is activated at about one-third of the rate of proline in pea and mung bean (species which do not normally contain azetidine-2-carboxylic acid), yet in the two liliaceous species, azetidine-2-carboxylic acid, which is normally present, is not activated The same relative activation values as those presented in Table 2 were obtained for the four species at all the other *p*H-precipitation ranges tested The possibility that the differences in the ability to activate azetidine-2-carboxylic acid were not a function of species but merely of the tissue chosen for enzyme preparation, that is, seed compared with rhizome, was eliminated by showing that similar preparations from seed of *Convallaria majalis* also failed to activate the analogue

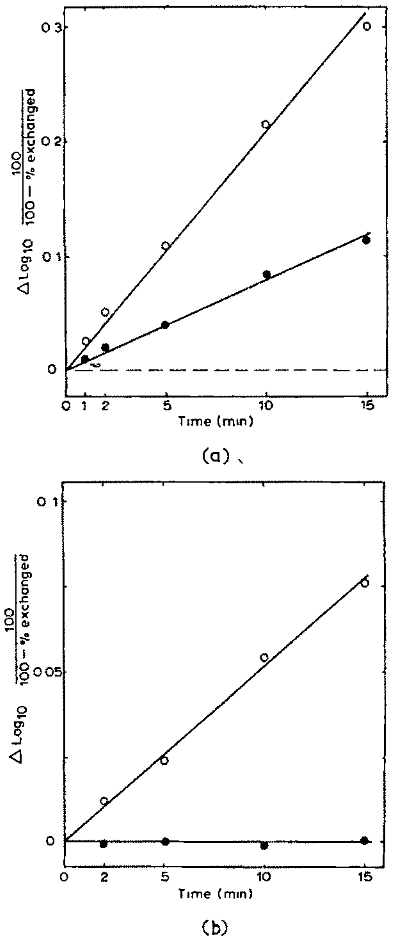

Fig 2 Effect of time of incubation of enzyme preparations from (*a*) *Phaseolus aureus* and (*b*) *Polygonatum multiflorum* on L-proline (○) and L-azetidine-2-carboxylic acid (●) stimulated rates of ATP-[^{32}P]PP exchange Assayed under standard conditions described in Table 1

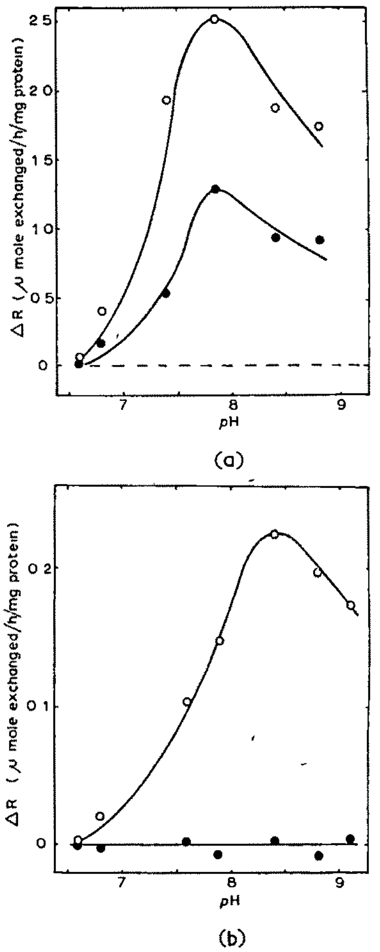

Fig 3 Variation of rate of L-proline (○) and L-azetidine-2-carboxylic acid (●) stimulated ATP-[^{32}P]PP exchange with *p*H, for enzyme preparations from (*a*) *Phaseolus aureus* and (*b*) *Polygonatum multiflorum* 0 1 M Citrate-NaOH buffer *p*H 6 6, or 0 1 M *tris*-HCl buffers *p*H 6 8–9 1 were used to dissolve final precipitated protein Assayed under standard conditions described in Table 1 except for the *p*H values of buffer used

A series of further experiments were performed to determine the relationships existing between the rates of activation of azetidine-2-carboxylic acid and proline and enzyme concentration (Fig 1), reaction time (Fig 2), and *p*H (Fig 3)

Throughout the fractionation procedure of mung bean or pea extracts, an observed ability to activate proline was always accompanied by a proportionately smaller activation of azetidine-2-carboxylic acid, indicating that only one enzyme was involved This conclusion was confirmed by adding both imino-acids together (5 μmole of each) when the rate of activation remained the same as when proline alone was added (Table 3) The same result was obtained when the imino-acids were added at 2 5 or 10 μmole concentration Had two enzymes been involved, the rate would have been close to the sum of the rates determined for the two imino-acids separately

The *p*H-precipitated protein fractions from mung bean and Solomon's seal rhizome extracts contained enzymes

Table 3 EFFECT OF ADDITION OF L-PROLINE AND,-OR, L-AZETIDINE-2-CARBOXYLIC ACID, ON THE RATE OF ATP-[^{32}P]PP EXCHANGE IN *p*H 4 2–4 6 ACETIC ACID PRECIPITATED PROTEIN FRACTION FROM *Phaseolus aureus*

Amino-acid (5 μmole)	Per cent exchange	*R* (μmole exchanged/h/mg protein)	*ΔR*
Endogenous	2 52	0 102	—
L-proline	40 55	2 090	1 988
L-proline + L-A 2 C*	40 20	2 070	1 968
L-A 2 C*	18 75	0 834	0 732

* L-Azetidine-2-carboxylic acid

capable of activating twelve and eleven other protein amino-acids respectively A protein fraction further purified by precipitation between 60 and 70 per cent saturation ammonium sulphate contained the highest proportion of the proline activating activity and also had the highest enzymatic specific activity This fraction also activated fewer amino-acids and their relative per cent activations compared with the value for proline were lower The ratios of proline to azetidine-2-carboxylic acid activation rates were the same for all the fractions precipitated from mung bean extracts by different concentrations of ammonium sulphate, none of the ammonium sulphate precipitates from Solomon's seal extracts showed any capacity to activate azetidine-2-carboxylic acid

Higher plants have rarely been used as experimental material for investigations of amino-acid activation and they have served even less often as starting material for enzyme purification Previously, only weak proline activating activity has been encountered in higher plant extracts, but we have found the mung bean especially to be excellent material for purification of this enzymatic activity and as a source of a number of other activating enzymes

Species differences observed in the properties of amino-acid activating enzymes do not appear to have been reported previously However, the following article describes a similar difference in the specificity of the methionine activating enzyme present in wild-type and in an ethionine-resistant mutant of *Coprinus* Presumably one may infer that species producing azetidine-2-carboxylate have evolved a proline activating enzyme differing slightly in protein conformational features (tertiary structure) from the enzyme present in other species, and possessing a subtly modified active centre that can reject azetidine-2-carboxylic acid The proposed structural modification may conceivably be attributed to the presence of different amino-acid sequences in the region of the active centres of the same enzyme from different plant species Structural variability of this type already has been established for certain proteins obtained from different animal species[28]

Further purification of the proline activating enzyme and the testing of other naturally occurring proline analogues and imino-acids have been carried out and will be reported shortly

We thank the New Zealand Council of Scientific and Industrial Research for a fellowship for one of us (P J P) and the Central Research Fund of the University of London for an apparatus grant.

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STRUCTURAL GENE FOR THE METHIONINE-ACTIVATING ENZYME AND ITS MUTATION AS A CAUSE OF RESISTANCE TO ETHIONINE

By Prof D. LEWIS, F R S

Department of Botany, University College, London

ON the present genetical theory of the synthesis of enzymes there should be, in all organisms, at least twenty genes for the postulated twenty enzymes which activate the twenty proper amino-acids in proteins Some of these enzymes, if they are hetero-polymers, would require two structural genes as in hæmoglobin None of these genes has been found in any organism The common method of obtaining biochemical mutants in micro-organisms by growing the cultures on supplemented media will only detect mutants of genes concerned with the synthesis of relatively simple molecules which can be supplied in the medium An enzyme which is primarily essential for protein synthesis would be irreplaceable under all conditions It is not surprising, therefore, that genes for the activating enzymes have not been detected A specific search for the gene of the histidine-activating enzyme in *Salmonella* has been made by Ames and Hartman[1] They tested for the histidine-activating enzyme in cultures with single-site and multi-site mutations in the cluster of the histidine biosynthetic genes No differences were found in the activating enzymes in these mutant stocks It is doubtful if this method, even if extended to the whole genome, would yield results, because most of these unselected mutations and deletions would cause complete inactivity or loss of the enzyme, and this would be lethal

The method I have used to detect the gene for the methionine-activating enzyme is to screen for small non-lethal changes in the active sites of the enzyme by using analogues of methionine

Analogues of five amino-acids, when applied to higher or micro-organisms, are known to become incorporated into protein, for reviews see refs 2 and 3 Some of these, such as azetidine-2-carboxylic acid, are highly toxic to many organisms[4,5] At the other extreme is selenomethionine, which not only replaces all the methionine in the protein of *Escherichia coli* without effect on growth but also can support linear growth in the complete absence of a source of sulphur[6]

Ethionine, the analogue of methionine, used in my experiments, is known to be incorporated into the protein of rat tissue[7], *Tetrahymena*[8], yeast[9,10] and *Bacillus subtilis*[10,11] It is toxic to yeast but not to *Bacillus subtilis* The toxicity of ethionine has been attributed to (1) its effect on the properties of proteins by incorporation, (2) by interfering with lipotrophic activity, and (3) by interfering with transmethylation[10]

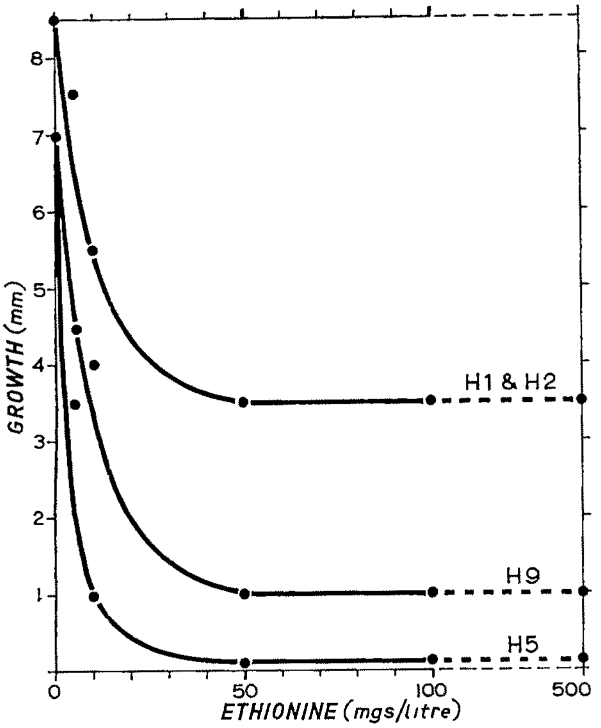

Fig 1 Radial growth of wild-type stocks on solid medium in 24 h, 100 mg/l = 0·61 × 10^{-3} M ethionine

Effect of Ethionine on Growth of Coprinus lagopus. The organism used in these investigations is the basidiomycete fungus, *Coprinus lagopus* (*sensu* Buller). Descriptions of its genetic system and method of culture are given by Lewis[12]. Methionine was the amino-acid chosen for investigation because seven structural genes for methionine synthesis and a system of suppressor genes have been mapped and investigated. The effects of different concentrations of ethionine are given in Fig 1, where it can be seen that all the wild-type stocks tested are inhibited by ethionine but to different degrees. The analogue norleucine, on the other hand, has no effect on growth even at saturation.

The inhibiting effect of ethionine was partially, but not completely, removed by methionine (Fig 2). A methionine-requiring mutant, *me*-1, reacts differently to the mixture, the methionine being much more effective on the *me*-1 mutant in removing the inhibitory effect of the analogue. This differential reaction to the mixture is under the control of a single gene, but the full significance of the differences is not known and will not be discussed in relation to the main subject of this report.

Mutation to Resistance to Ethionine. Mutations conferring resistance to ethionine were selected by sowing uninucleate oidia on solid minimal medium with the addition of 0·61 × 10^{-3} M L-ethionine. Four plates, with 3 × 10^{6} oidia each, were sown of *H*5 oidia. At this density of sowing there was an uncountable number of small irregular colonies with submerged hyphæ. Twenty of the largest of these colonies were isolated, but none were found to be resistant mutants. During the course of other experiments, where total oidial numbers were not known, two genuine resistant mutants have been obtained from untreated oidia. Treatment with ultra-violet light, which gave a kill of 84 per cent, produced three good resistant colonies from 2 plates sown with 5·4 × 10^{5} oidia per plate. One of these mutants, *H*5 *E*2, has been used extensively in these experiments; its growth on ethionine medium is given in Fig 3.

From the analysis of appropriate crosses the mutant *E* 2 was found to differ from its wild-type progenitor in a single gene which is recessive to the wild allele when tested in a dikaryon. This gene is linked to two methionine synthesizing genes, *me*-2 and *me*-6.

Mutants resistant to ethionine have also been selected from a stock *PR*2301 which requires methionine due to a mutation of the gene *me*-1 controlling the synthetic step between cystathionine and homocysteine[12]. When oidia of this stock are sown on minimal medium, 1 in 10^{5} grow as a result of recessive mutation of a suppressor gene[12]. If the oidia of the same *me*-1 stock are sown on minimal media with 0·61 × 10^{-3} M L-ethionine, 3 in 10^{7} oidia grow into colonies and have the double property of being both able to grow without methionine and are resistant to ethionine. These mutants are recessive. The growth on ethionine of one of these mutants, *Ms*13, is given in Fig 3. The significance of this double property of the mutant is the subject of another investigation, but this mutant is included here because, as will be described, it is of importance in the interpretation of the biochemical cause of the resistance to ethionine.

Incorporation of Ethionine into Protein. The resistance to ethionine in the mutants could be caused by a blockage in one or more steps in the entry into the cell or in its metabolism. It could be prevented from entry by the lack of or change in a permease system. It could enter and then be immediately metabolized to a harmless compound, or it may be accumulated in the cell and be prevented from being incorporated into the protein by a change in the specific activating enzyme or by a change in the specific transfer ribonucleic acid. Furthermore, it may be prevented from supplying ethyl groups to precursors of important methylated compounds.

To investigate the metabolic fate of ethionine, 0·14 × 10^{-3} M ^{35}S DL-ethionine, 13·7 mc./mM, obtained from the Radiochemical Centre, Amersham, was added to liquid minimal medium. This concentration of ethionine allowed enough growth in liquid medium of both sensitive and resistant strains for the analysis. Unshaken culture flasks inoculated with *H*5, *E*2 and *Ms*13 cultures were collected after 5 days' growth. The mycelium was macerated in sodium borate buffer *p*H 8·0 and the protein was precipi-

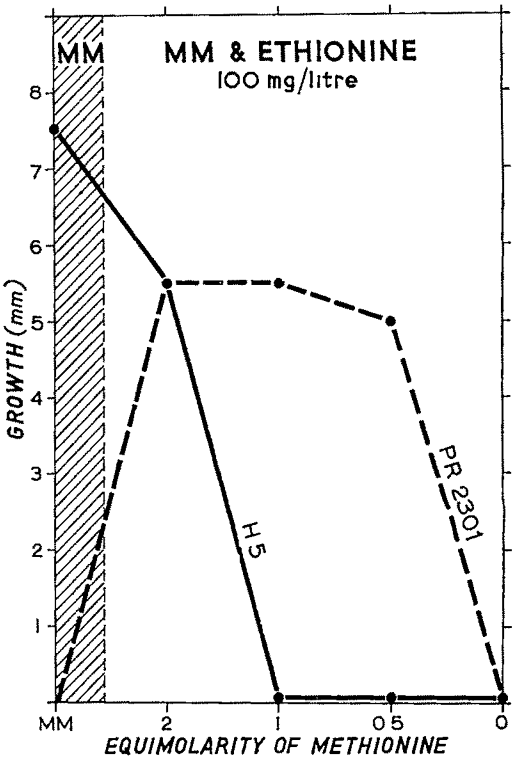

Fig 2 Radial growth of wild-type *H*5 and a methionine-requiring mutant *PR*2301 on minimal medium (*MM*) and on ethionine with and without methionine

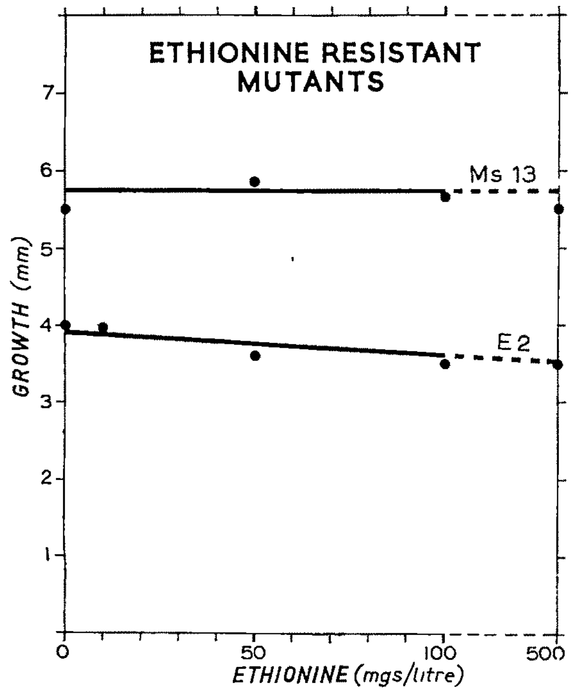

Fig. 3. Radial growth of resistant mutants on solid medium in 24 h

tated in 5 per cent trichloracetic acid. The washed TCA-insoluble fraction containing the protein was hydrolysed in hydrochloric acid. The amino-acids of both the TCA-soluble and insoluble fractions were separated by chromatography in a butanol–acetic acid–water mixture. The summary of the results is given in Tables 1 and 2.

From Table 1 it is clear that in all three cultures ethionine entered the cells and that radioactive sulphur amino-acids were present in the protein. The resistant mutants have less sulphur-35 compounds in both the free soluble pool and in the protein than the wild-type and mutant *E*2 has a lower amount than *Ms*13. From Table 2 it is apparent that ethionine is found in the TCA-soluble fraction of *H*5 and *E*2 but not in *Ms*13. The very large amount of the unidentified, ninhydrin positive spot *X* is characteristic of the soluble fraction but is absent from the protein. The results showed that in the protein of the mutant *E*2 ethionine is absent. Statistically it is not possible to exclude up to 3 per cent of the ethionine-level of the wild-type *H*5 or at about 0·3 per cent of the total activity of sulphur-35. In the wild *H*5 stock ethionine is 9 per cent and in the mutant *Ms*13 it is 17 per cent of the total activity of sulphur-35. The results with *E*2 in which ethionine is not incorporated would suggest that the inhibitory effect of ethionine is due to incorporation. Clearly from the fact that the resistant mutant *Ms*13 incorporates ethionine into its protein we must conclude, however, that the primary inhibitory effect of ethionine is not due to incorporation into protein.

Table 1. INCORPORATION OF ^{35}S FROM ^{35}S-ETHIONINE INTO THE TCA-SOLUBLE (POOL) FRACTION, PROTEIN FRACTION AND ACID HYDROLYSATE OF THE PROTEIN FRACTION

Culture	Counts/400 sec/g dry weight mycelium		Counts/400 sec/mg protein after hydrolysis
	TCA soluble fraction	Protein fraction*	Protein hydrolysate
Wild *H*5	20,291	25,261	10,503
Resistant *E*2	14,642	7,902	4,002
Resistant *Ms*13	21,690	13,810	7,109

* By difference between radioactivity in homogenate and TCA-soluble fraction.

Table 2. INCORPORATION OF ^{35}S FROM ^{35}S-ETHIONINE INTO AMINO-ACIDS OF THE TCA-SOLUBLE AND PROTEIN FRACTIONS

	Radioactivity (as % of total counts in the three *S*-containing labelled spots)					
Culture	'Pool' amino-acids			Protein hydrolysate		
	Spot X	Methionine	Ethionine	Cyst(e)ine	Methionine	Ethionine
Wild *H*5	96·7	0·0	3·3	54	37	9
Resistant *E*2	93·6	0·0	6·4	65	45	0
Resistant *Ms*13	91·0	9·0	0·0	47	36	17

Methionine-activating Enzyme in Wild-type and Mutant E2. Cultures of wild-type *H*5 and resistant mutant *E*2 were grown in 1·8 litres of liquid minimal medium. To the *E*2 culture flasks 0·01 × 10^{-3} M L-ethionine per litre was added to discourage growth of back mutations to wild-type. Collection and extraction of the protein were carried out at 4° C. The washed mycelium was homogenized in sucrose *tris* buffer, *p*H 7·6. The activities of the activating enzymes were determined by the pyrophosphate, adenosinetriphosphate exchange method as followed by Peterson and Fowden[4].

The whole crude homogenate of *H*5 possessed an endogenous activity that was slightly increased equally on addition of methionine or ethionine. The mutant *E*2 was similar except that the increase in activity with ethionine was about a quarter that obtained with *H*5. The levels above the endogenous rates were so low that these results were of no value without further purification of the enzyme.

Fractionation at three different *p*H's with acetic acid did not improve the activity. But ammonium sulphate fractionation resulted in a spectacular increase in the methionine activity in the 50–100 per cent saturation fraction. The bulk of the protein was in the 0–50 per cent saturation fraction but this had little or no activity, towards methionine.

Table 3. TOTAL COUNTS IN 400 SEC ON 4 SPECIMENS OF EACH ASSAY. THE AMOUNTS OF PROTEIN IN THE *H*5 AND *E*2 TESTS ARE NOT THE SAME, BUT A VALID COMPARISON CAN BE MADE AS A PERCENTAGE OF THE ACTIVITY WITH THE COMPLETE AMINO-ACIDS (LAST COLUMN)

*H*5	Actual counts	Counts above control	% above control
Control	3,346	—	—
Ethionine	4,760	1,414	7·9
Ethionine + methionine	6,329	2,983	16·0
Methionine	6,306	2,960	16·0
Complete amino-acids	21,897	18,551	100
*E*2			
Control	4,836	—	—
Ethionine	5,064	228	0·5
Ethionine + methionine	11,993	7,157	16·8
Methionine	12,500	7,664	18·0
Complete amino-acids	47,221	42,385	100

The results of counts of radioactive ATP from the 50–100 per cent fraction, given in Table 3, show that in the wild *H*5 ethionine is activated at a level of 48 per cent of the methionine activity. In the mutant *E*2, on the other hand, ethionine is not activated or at most to about 3 per cent of the methionine-level. A chromatographic test of the purity of the L-ethionine used for the activation test showed trace impurities of cysteine and methionine, which could account entirely for the slight activation observed with the mutant. The result from the mixture of ethionine and methionine shows that only one enzyme is concerned with the activation of both methionine and its analogue.

Resistance of Choline-less Mutants. In view of the hypothesis of the inhibitory mechanism of ethionine expounded in the next section it became important to test for the resistance to ethionine of certain choline-less mutants. Two mutants chol 1 and chol 2 (ref. 13) were tested on choline medium with 50 mg/l. of L-ethionine. Growth was at about 75 per cent of that occurring on medium containing choline alone, showing that they were resistant to ethionine.

Discussion and Conclusions The ethionine-resistant mutant $E2$ differs from wild-type in that the mutant does not incorporate ethionine into protein and the methionine-activating enzyme does not activate ethionine The gene for ethionine resistance is therefore the structural gene for the methionine-activating enzyme, and is designated *meth AE* The mutational change must have changed the active sites in a way that has altered the specificity so that the mutant sites can effectively fit methionine but not ethionine Presumably a single amino-acid substitution in the active site has occurred The difference discovered by Peterson and Fowden[4] between the proline-activating enzyme of different species of plants could have a similar mutational origin of long standing

The resistant mutant, $Ms13$, incorporated ethionine into the protein at a level that was nearly twice as high as ethionine incorporation into the sensitive wild-stock The inhibitory effect of ethionine is therefore not caused by incorporation It is also not caused by ethionine being an end-product repressor of methionine synthesizing enzymes since methionine is synthesized and incorporated in the wild-type in the presence of ethionine

The hypothesis proposed is that ethionine is inhibitory in the sensitive $H5$ because it transethylates the precursors of metabolically important methylated compounds such as choline

In support of this is that cholineless mutants which are unable to synthesize choline are resistant to ethionine If this hypothesis is accepted then the mutant gene *meth AE* of $E2$ must not only be a block to the incorporation of ethionine into the protein but also a block to transethylation From this it follows that the methionine-activating enzyme mediates not only the first step in amino-acid incorporation but also the first step in transmethylation In the $Ms13$ mutant the block is not at the first step of the activating enzyme but at a later step in the transmethylation system This mutant can transfer methyl, but not ethyl, groups There would, therefore, be an accumulation of an ethylated compound, and this in turn would relieve the demand for ethionine and increase its availability for incorporation into protein Hence the double amount of ethionine in the protein of $Ms13$

Cantoni[14] has shown that S-adenosylmethionine is the active form of methionine for transmethylation, and that this is formed by a reaction requiring ATP and releasing PPi and Pi catalysed by an activating enzyme Parks[10] has shown that S-adenosylethionine is synthesized in the presence of ethionine and that transethylation occurs with an *in vitro* system There are two possibilities to explain the results of the work recorded here (1) The methionine-activating enzyme for the synthesis of methionine-AMP-enzyme complex leading to incorporation, and the activating enzyme for the synthesis of S-adenosylmethionine leading to transmethylation, are one and the same enzyme, (2) two or more enzymes are involved and the S-adenosylmethionine is synthesized sequentially from the methionine-AMP-complex

The first possibility invokes one enzyme carrying out two parallel reactions, the second postulates a complicated dephosphorylation and rearrangement of the adenosine from the terminal carboxyl group to the sulphur atom Experiments to distinguish between these possibilities are planned

The significance, if any, of the close linkage of the *meth AE* gene with the complex of two structural genes concerned in methionine synthesis is not apparent Close linkage of genes with sequential functions suggests a common repressor system by the end product (Jacob and Monod[15]) It is unlikely that the *meth AE* enzyme is repressed by methionine, for this would stop protein synthesis Possibly the control of transmethylation may be a factor to consider if repression is established

At the beginning of the experiments it was hoped that genes controlling the synthesis of specific transfer RNA would also be found in the methionine system, but if the hypothesis of transmethylation is correct there is no selective technique available for obtaining viable mutants of the transfer RNA with the methionine system

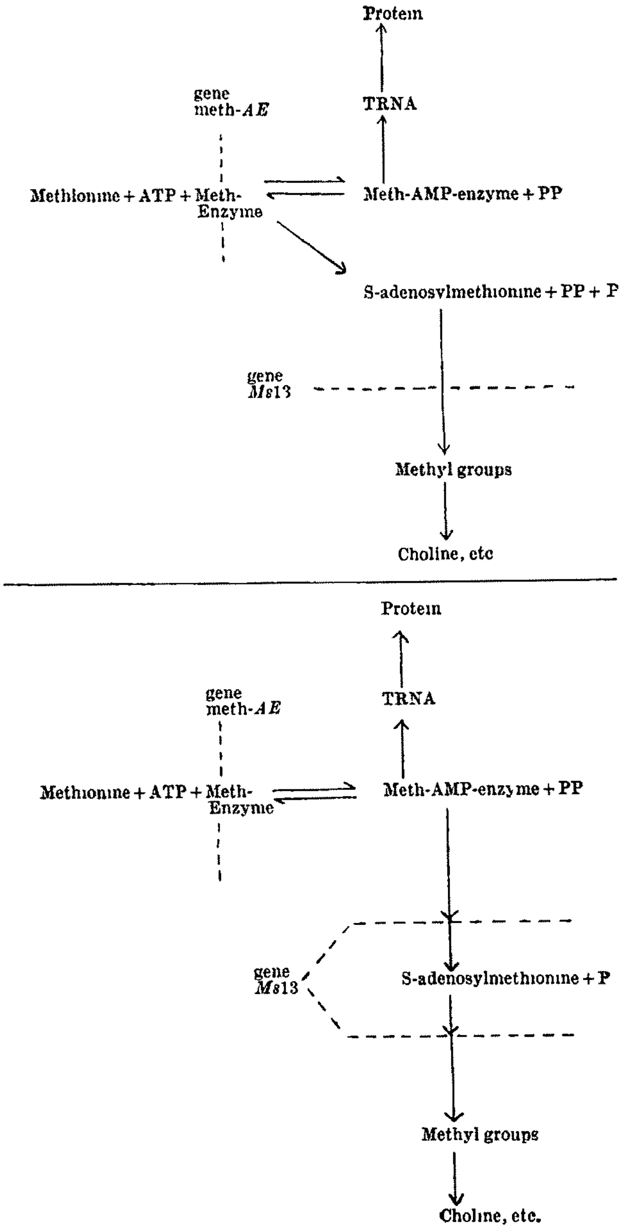

Fig 4 Alternative hypotheses to explain the action of the *meth AE* and the *Ms*13 genes

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LETTERS TO THE EDITOR

RADIOPHYSICS

Galactic Background Polarization at 610 Mc/s

AFTER the first successful attempt to measure the polarization of the galactic radio radiation at 408 Mc/s (refs 1 and 2), the observations were continued with improved receivers, resulting in a nearly complete survey of the polarization at 408 Mc/s of the part of the sky visible with the Dwingeloo radio telescope[3,4] The receivers used were all based on the correlation principle, correlating the signals of two crossed dipoles The results obtained were confirmed by observations made at Cambridge using the difference between the signals on two crossed dipoles[5,6] and by results obtained at Sydney, by rotating a feed consisting of two parallel dipoles[7]

Although the measurements at 408 Mc/s showed some interesting details, the presence of interstellar Faraday rotation made interpretation of these details in terms of magnetic field structure impossible Measurements at other frequencies would be needed to obtain this interstellar Faraday rotation, thereby at the same time gaining additional information about the magnetic field The only results which yielded a positive determination of the intensity and polarization angle at a frequency different from 408 Mc/s are measurements made with the 210-ft Parkes telescope, of a rather limited part of the sky[7] These measurements showed that a large variation over small distances in the sky existed in the apparent spectral index. This might be due to an effect of depolarization, by which different regions would be observed at different frequencies Some measurements were also made at 178 Mc/s at Cambridge These observations showed the probable existence of polarized radiation at this frequency, but no intensity or polarization angle could be obtained[8]

To obtain some measurements at a frequency different from 408 Mc/s in the northern sky, one of us (C A M) designed a receiver with a centre frequency of 610 Mc/s During June 1963 this receiver was used in connexion with the 25-m radio telescope of the Netherlands Foundation for Radio Astronomy at Dwingeloo By using a larger bandwidth (3 6 Mc/s) than at 408 Mc/s (1 8 Mc/s), and by using two-channel detection, which utilizes all available information, a signal-to-noise ratio equal to that at 408 Mc/s was obtained, even though the intensity of the polarized radiation was roughly a factor three lower As, moreover, the spurious effects turned out to be also less by about a factor three, the reduction process as used to obtain the 408 Mc/s results could be maintained[3,4] As a test of the reality of the observed radiation, the observed polarization angles were plotted against the Faraday rotation expected for each measurement on the basis of a parabolic F_2-layer, for those points in the sky which were observed frequently for calibration purposes The ionospheric data used in this comparison were kindly put at our disposal by Drs H J A Vesseur of the Dutch Meteorological Institute, K N M I , at De Bilt One of these comparison plots is shown in Fig 1 Although the ionospheric Faraday rotation at 610 Mc/s is rather small (ref 9), a correlation between the two parameters is obviously present This shows that the observed signals are indeed due to a source beyond the Earth's atmosphere, and not merely caused by unknown instrumental effects[9]

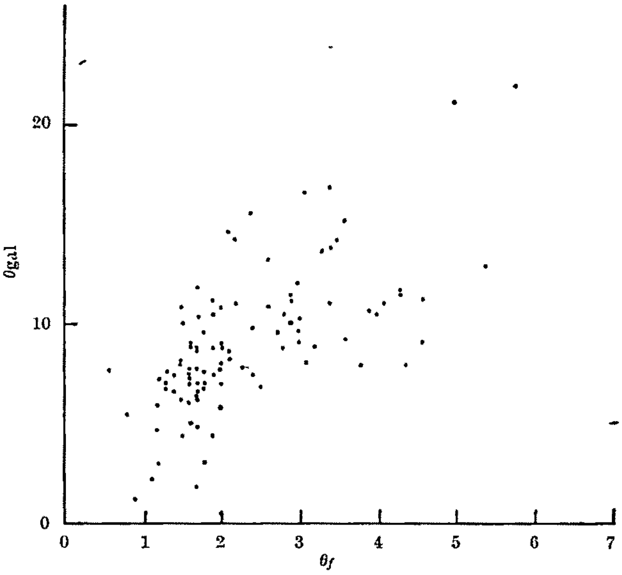

Fig 1 Comparison of the observed polarization angle (θ_{gal}) and the Faraday rotation expected on the basis of a parabolic F_2-layer (θ_f)

The results of the measurements are shown in Fig 2 The average internal mean error was 0 11° K for one measurement, but we suspect the presence of systematic errors in certain parts of the sky, which will not be eliminated by repeated measurements, of up to 0 3° K The observations were mainly restricted to those regions where a high polarization brightness temperature had been found at 408 Mc/s Some data about the points which were measured ten times or more are summarized in Table 1 The intensities and position angles for the same points at 408 Mc/s are included for comparison Combining the two sets of measurements, an apparent spectral index and an apparent Faraday rotation at 408 Mc/s can be deduced If we assume that the apparent Faraday rotation calculated in this manner represents the real interstellar Faraday rotation, the product $\overline{N H_{\parallel}} L$ can be calculated This product is shown in the last column of Table 1

The most pronounced feature on the map in Fig 2 seems to be a region in which the Faraday rotation is almost zero, and which extends from $l^{II} = 120°$, $b^{II} = -50°$ up to $l^{II} = 140°$, $b^{II} = +10°$ To the immediate left of this region the Faraday rotation is largely anticlockwise, while to the immediate right it is clockwise This would suggest that in this region the line of sight may be at

Table 1

				610 Mc/s				408 Mc/s					
b^{II}	l^{II}	α	δ	T_b^p	θ_{gal}	θ_{eq}	n	T_b^p	θ_{gal}	θ_{eq}	n	Apparent β	Apparent $\overline{N H_{\parallel}} L$
+ 8 0°	141 0°	57 0°	+ 64 0°	1 6° K	4°	43°	211	5 6° K	174°	33°	429	3 1	− 7 × 10⁻⁷ Gauss cm⁻³ parsec
+ 27 4	123 0	—	+ 90 0	0 2	15	—	78	0 9	18	—	244	3 8	+ 2
+ 59 3	120 0	195 0	+ 58 0	0 3	9	4	12	1 7	2	177	17	4 4	− 5
+ 63 4	8 0	217 0	+ 14 0	1 3	106	50	171	5 0	2	126	285	3 4	+53 −88

right angles to the direction of the magnetic field, while to the right of the region a component away from the observer and to the left a component towards the observer exist.

It may be noted that in the large region of high polarization between 120° and 155° longitude and 0° and +12° latitude the directions of polarization are much more parallel at 610 Mc/s than at 408 Mc/s As the Faraday rotation at 610 Mc/s is roughly equal to the differential rotation between 610 Mc/s and 408 Mc/s it must be expected that the original radiation over this entire field is practically parallel, and perpendicular to the galactic equator

Another remarkable fact is the spectral index, which for the majority of the observed points is about three, showing that, although the Faraday rotation is appreciable in some places, the observed intensities are not markedly influenced by it This indicates that probably the Faraday rotation does not exceed 180 degrees at 408 Mc/s, suggesting that either the magnetic field involved is fairly small, or that the radiation originates in the neighbourhood of the Sun

The region near the galactic north pole has a rather complicated structure, which may be due to the presence of the north polar spur It seems at the present moment to be impossible to disentangle the complicated structure of the galactic magnetic field by means of polarization observations at only two frequencies More measurements, and more accurate measurements, at other frequencies will be needed before any attempt can be made to prepare a map of the galactic magnetic field

We thank the staff of the Radio Observatory at Dwingeloo, who built and maintained the receiver and its digital equipment The reduction of the measurements was carried out on the electronic computer X1 of the Computing Centre of the University of Leyden The work was financially supported by the Netherlands Organization for the Advancement of Pure Research (Z W O)

C A Muller

Radio Observatory,
Dwingeloo

Elly M Berkhuijsen
W N Brouw
J Tinbergen

Observatory, Leyden

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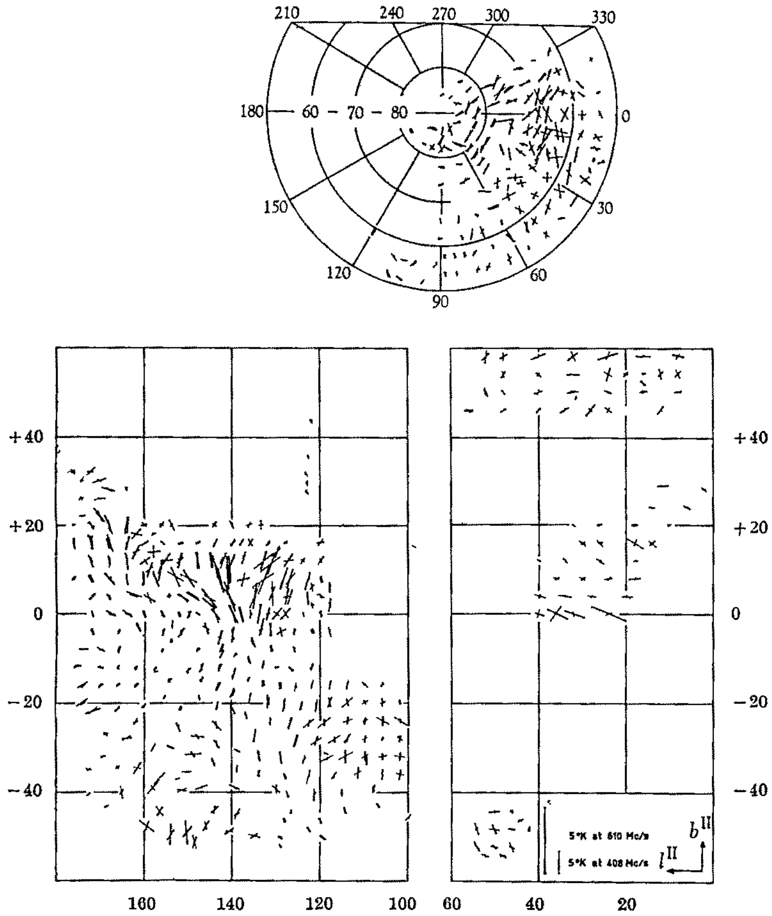

Fig 2 Intensities and position angles of the polarized radiation at both 610 Mc/s and 408 Mc/s

Radio and International Geophysical Research

In Dr R L Smith-Rose's excellent review of the achievements of radio studies in geophysical research[1] there appears a passage which might be misunderstood by those interested in practical ionospheric communications He says that research has shown it "to be desirable to use aerial systems which project the radiation at a greater angle of elevation than has hitherto been considered the most appropriate"

In fact, as a result of research, efforts are being made by communicators to radiate at lower angles than have been used in the past, so as to benefit from the secant law of ionospheric propagation Dr Smith-Rose's remark no doubt alluded to the hypothetical assumption of zero angle radiation which is adopted in drawing conventional frequency predictions for long routes

P A C Morris

Cable and Wireless, Ltd ,
Mercury House,
Theobald s Road,
London, W C 1

[1] *Nature*, **199**, 11 (1963)

I thank Mr Morris for pointing out that the passage in question may be somewhat ambiguous to those concerned with practical communications But the phrase quoted must not be read out of context with the rest of the paragraph I was, in fact, recording the conclusions reached in a study of electron density/height profiles and their application to high-frequency communication[1] I agree that some practical communication systems were designed to use higher angles of transmission and reception, and this means that improved results would probably be obtained by reducing the angle of elevation, having due regard to the frequency in use and the range of communication in question

R L Smith-Rose

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PHYSICS

Use of an Electron Beam for detecting Superconducting Domains of Lead in its Intermediate State

SUPERCONDUCTING domains have been detected in lead by means of an electron beam technique previously used to detect magnetic domains[1] A spectroscopically pure cylinder of lead, 1 1 cm long and 0 28 cm in diameter, was mounted with its axis vertical in a modified horizontal electron diffraction camera A 20-kV electron beam was focused about 15 cm from the cylinder and was deflected at 50 c/s in a vertical direction by means of suitable coils, so as to produce an electron shadow of the lead cylinder Fig 1c shows a photograph of a shadow taken with the lead in its normal state In this case the smooth edge is merely a geometrical projection of part of the curved surface of the cylinder The cylinder, which could be heated electrically by a non-inductively wound heater, was attached to the outside of a metal vessel containing liquid helium A uniform magnetic field could be applied to the lead by means of a pair of small Helmholtz coils These were mounted symmetrically on either side of the lead with their common axis horizontal and perpendicular to the mean direction of the undeflected electron beam

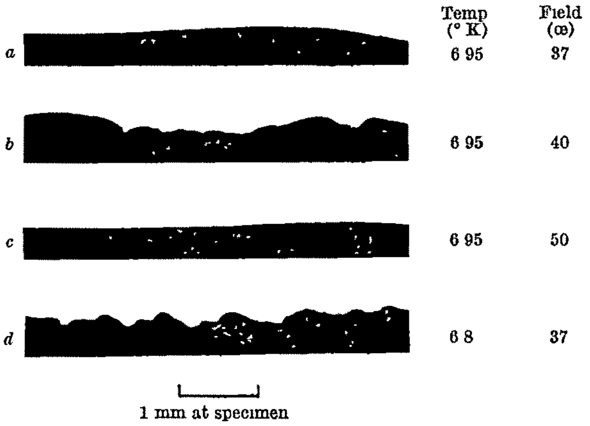

Fig 1 Electron shadows of the surface of a lead cylinder, showing lead in the following states (*a*) superconducting, (*b*) intermediate, (*c*) normal, (*d*) intermediate

The electron shadow shown in Fig 1*a* was taken after the applied field had been increased from zero to 37 œrsteds When the field was further increased to 40 œrsteds the shadow edge ceased to be smooth (Fig 1*b*) It became smooth again for fields in excess of about 50 œrsteds (Fig 1*c*)

These observations are consistent with the following explanation At and below 37 œrsteds the lead was superconducting At 40 œrsteds the lead transformed to the intermediate state[2] in which alternate lamellæ of superconducting and normal lead were perpendicular to the axis of, and approximately periodically distributed along, the cylinder Since the applied field was expelled from the superconducting parts, a concentration of magnetic flux occurred in the normal regions The resulting periodic distortion of the previously uniform field led to the 'corrugated' electron shadow shown in Fig 1*b* Above about 50 œrsteds the intermediate state transformed to the normal state Taking 50 œrsteds as the critical field this would indicate that the results so far described were obtained with the lead at about 6 95° K (ref 2)

Fig 1*d* was obtained by cooling the lead in a field of 37 œrsteds from a temperature at which the cylinder was in its normal state to 6 8° K The irregularities in the electron shadow may again be explained by assuming that the applied field was distorted because the lead was in its intermediate state The distance between adjacent minima on the shadow in Fig 1*d* corresponds to distances varying from 0 08 to 0 29 mm at the specimen It follows that these distances give the order of the periodicity of the superconducting domains in lead under the conditions of the experiment The variation of this periodicity as a function of applied field and temperature has not yet been studied in detail A full account of the work and details of the apparatus will be published in due course

We thank Dr B R Coles and Dr J G Park for useful discussions One of us (A T P) is also indebted to E M I. Ltd for a maintenance grant

M Blackman
A E Curzon
A T Pawlowicz

Department of Physics,
Imperial College of Science and Technology,
Prince Consort Road,
London, S W 7

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Gamma-ray Spectrometric Determination of Cæsium-137 in Sea-water by using Ammonium Molybdophosphate as Scavenger

A NUMBER of methods have been proposed for determining very low concentrations of cæsium-137 in sea-water by γ-ray spectrometry The sodium potassium cobaltinitrite method[1,2] and the nickel ferrocyanide method[3] were proposed by several workers for identifying traces of cæsium-137 Special considerations should be made on the purity and the size of the cæsium sample to be counted and the simplicity of the procedure to be suited for shipboard operations

The ammonium molybdophosphate method was used by Yamagata and Yamagata[4] originally for the radiochemical determination of cæsium-137 in biological materials, the precipitate of ammonium molybdophosphate being made *in situ* in a sample solution Meanwhile, the work of Smit[5] revealed the ion-exchange properties of ammonium molybdophosphate, and the use of solid ammonium molybdophosphate by adding to sample solution as a specific scavenger was made by Yamagata *et al* [6] in the neutron activation analysis of common cæsium and by Miyake *et al* in the determination of cæsium-137 in sea-water[7]

The best conditions for scavenging concerning temperature, acidity, the quantity of carrier cæsium and the quantity of ammonium molybdophosphate were checked by Yamagata *et al* [6] The experiments on a small scale showed about 0 1 per cent quantity of the ammonium molybdophosphate (wt /vol) was enough to recover more than 95 per cent of cæsium labelled with cæsium-137 in sea-water in acid conditions However, the large-scale operations on 100–200 l of sea-water by this method resulted in only 70–80 per cent of recoveries with the addition of 5 mg quantity of carrier cæsium per litre[8] The reduction in recoveries might have been caused by losses in the large-scale operations, and the addition of carrier cæsium as well because of the limited ion-exchange capacity of the ammonium molybdophosphate

To overcome the difficulties involved in the addition of appreciable amount of carrier cæsium for checking the recovery in full-scale operation, the radioactive cæsium-132 was examined for its use as a spike This radioisotope was produced by a (γ, *n*) reaction on the purified cæsium chloride using a linear accelerator at the Japan Atomic Energy Research Institute It had specific activities of about 1 mc /g cæsium when used as a spike and several hundred μμc were applied to 100 litres of sea-water sample This means the addition of only less than 1 μg of the stable cæsium to 50 μg of naturally occurring cæsium in the sea-water, as compared with 500 mg of carrier cæsium added by other workers[6]

The radiochemical purity of the spike cæsium-132 was checked by following up the decay over the period of ten half-lives and also by measuring γ-ray spectra Half-life was measured as 6 8 days and no characteristic photopeaks other than those attributed to cæsium-132 were observed

Surface waters were collected in the Sagami Bay, off Enoshima, at 35° 16′ N , 139° 28′ E on May 14 and transported to the laboratory To 100-l aliquot of the sample were added several hundred $\mu\mu$c of standard spike solution of cæsium-132 and the mixture agitated mechanically for half an hour after the addition of 1 l. of concentrated hydrochloric acid and 90 g of the solid ammonium molybdophosphate After settling overnight the supernatant liquid was siphoned off and the scavenger collected in a plastic assay cylinder, 3 in in diameter After the scavenger had been allowed to settle it was placed on top of a sodium iodide crystal (3 in × 3 in) and the γ-ray spectrum taken for 100 min by using a 256-channel pulse height analyser

Recovery of spike cæsium-132 was determined on the characteristic photo-peak of 0 67 MeV by comparing with the spectrum from adding known amounts of spike solution to the scavenger The contribution of cæsium-137 activity (0 66 MeV) in the sample was estimated as approximately 3 per cent and this was subtracted from the results after the measurement of cæsium-137 Duplicate independent scavengings showed 91 and 97 per cent of recovery

Two months were required for allowing the spike activity to decay, and then the γ-spectrum was taken for 1,000 min to determine the activity of cæsium-137 in the spectral region almost identical to that for the determination of cæsium-132 Comparison made with the spectrum from adding known amounts of standard cæsium-137 to the scavenger resulted in 20 ± 5 $\mu\mu$c per 100 litre seawater The spike activity in the sample was calculated as being less than 1 $\mu\mu$c , which was negligibly small compared with the errors involved in the activity measurement

One of the advantages of this spike method of determination would be the use of identical spectral region around 0 66–0 67 MeV for both recovery and activity measurements, making feasible the use of only a single-channel pulse height analyser Adsorption of potassium to the ammonium molybdophosphate was too small to affect the γ-ray background, and the difficulty involved in the sodium potassium cobaltinitrite method[2] would be overcome Contamination of the ammonium molybdophosphate with radionuclides other than cæsium-137 which may be present in sea-water was checked by tracer experiments Table 1 summarizes the results Only zirconium–niobium-95 would interfere with the determination by use of a single-channel analyser if appreciable amounts of these nuclides are present in sea-water

Table 1 CONTAMINATION OF THE AMMONIUM MOLYBDOPHOSPHATE WITH OTHER RADIONUCLIDES IN ACID MEDIA (0 1 G AMMONIUM MOLYBDOPHOSPHATE IN 100 ML SEA-WATER)

Tracer	Contamination (% of applied radioactivity)	Specific activity of the tracer (c /g)	Gamma-ray energy (MeV)
$^{51}CrO_4$ (II)	1 6	51 6	0 33
^{54}Mn (II)	< 0 14	1 0	0 84
^{60}Co (II)	< 0 40	5 0	1 17, 1 33
^{65}Zn (II)	< 0 10	1 2	1 12
^{89}Sr (II)	< 0 50	C F	
^{91}Y (III)	2 3	C F	
^{95}Zr–^{95}Nb	11 8	C F	0 72, 0 77
^{106}Ru–^{106}Rh	< 0 17	C F	0 52
^{144}Ce (III)	0 38	C F	0 13

Table 2 CONCENTRATION OF CÆSIUM-137 IN THE COASTAL WATERS OF JAPAN

Date collected	Position	Cæsium-137 ($\mu\mu$c /l)
May 14, 1963	Sagami Bay 35° 16′ N , 139° 28′ E	0 2 ± 0 05
July 30, 1963	Sasebo Harbour 33° 08′ N , 129° 43′ E	0 6 ± 0 1
August 1, 1963	Kure Harbour 34° 06′ N , 132° 37′ E.	0 6 ± 0 1
August 3, 1963	Tsuruga Bay 35° 44′ N , 136° 03′ E	0 7 ± 0 1

Finally, considering the high efficiencies with which the cæsium-137 in sea-water can be carried by the proposed method and the errors involved in activity measurements, neither addition of carrier cæsium or spike, nor correction of the results for recovery would be necessary

The simplicity of the method makes it convenient for processing samples of large volume If other fission activities or corrosion products, such as cobalt-60, are to be measured on the same sample, the manganese dioxide method proposed by Yamagata *et al* [9] may be successfully applied preceding the ammonium molybdophosphate scavenging Table 2 summarizes the results of determination of cæsium-137 by using the proposed method

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NOBORU YAMAGATA

Institute of Public Health,
Tokyo

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Stimulated Optical Emission of a Terbium Ion Chelate in a Vinylic Resin Matrix

RECENTLY, the observation of optical maser action of a europium chelate (europium *tris*[4,4,4-trifluoro-1-(2-thienyl)-1,3 butanedione]) in polymethylmethacrylate (Eu TTA in PMMA) was reported[1]

This communication describes the probable stimulated emission of the terbium-substituted counterpart of the material system described here, that is, Tb TTA in PMMA This compound was prepared by the method described by Crosby *et al* [2] The Tb^{3+} ion was excited by optically pumping into the absorption band of the chelating molecules (TTA), which is approximately in the wave-length interval 3200 Å–3700 Å (maximum excitation at 3350 Å) The absorption coefficient of this chelate's excitation band is comparable with that observed for Eu TTA excitation band[1] approximately 10^6 mole^{-1} cm^{-1} At 77° K, energy is efficiently transferred from the metastable triplet-state energy-levels of the TTA molecule to the resonance energy-level $^5D_4$ of the Tb^{3+} ion[2] I have observed that the fluorescence of Tb TTA in PMMA is quenched at 300° K

Strong fluorescence of the Tb^{3+} ion occurs at approximately 5450 Å due to a transition from the $^5D_4$ to the $^7F_5$ energy-level The terminal $^7F_5$ energy-level of this transition is known to be about 2100 cm^{-1} above the ground state The fluorescence spectrum of Tb TTA in PMMA is strongly correlated in wave-length with that of anhydrous terbium chloride, which has its strongest line $\simeq$ 5450 Å The normal fluorescence lifetime and total line-width for the 5450 Å line of Tb TTA in PMMA was measured to be $\simeq$ 0 7 m amp and 60 Å, respectively, at 77° K

In the experiments we carried out several short, straight, polymethyl-methacrylate fibres doped with a Tb TTA concentration of approximately 0 08 per cent of the weight of the PMMA The fibre for which results are presented by Figs 1–3 was approximately 0 75 mm in diameter by 60 mm in length, both ends of the fibre were polished and one end was silvered Other small fibre samples (terbium in TTA) showed similar behaviour

The experimental apparatus was assembled as follows the fibre was immersed in liquid nitrogen inside of an optically transparent, cylindrical 'Pyrex' Dewar A tubular absorption filter composed of 'Corning 7–54' glass was interposed between the fibre and the inside wall of the Dewar The 'Corning 7–54' filter permitted the desired pumping radiation to reach the fibre and, at the same time, reduced the pump radiation component at 5450 Å In addition, by rejecting the infra-red radiation of the flash-lamp, the filter reduced cooling problems and minimized thermal decomposition of the terbium TTA A helical, xenon flash lamp, which surrounded the Dewar, provided the pumping radiation The radiation from the fibre was detected with a 6,199 photomultiplier A monopass interference filter (maximum transmission at 5450 Å) was interposed between the photomultiplier cathode and the output end of the fibre An opaquely coated light pipe to guide the radiation from the end of the fibre to the photomultiplier, plus the action of the filters, reduced the amount of pump radiation (leakage) incident on the photodetector The light pipe also served the function of allowing the entire fibre to be immersed in liquid nitrogen To eliminate the possibility of spurious response in our apparatus, I substituted undoped fibres of the same dimensions in place of the doped fibres I observed only a moderate flash-lamp leakage in the output

The radiation output versus time curves observed for the fibre described here are shown for three different pumping-levels in Figs 1–3 Note that sufficient pumping leads to distortions of the normal fluorescence decay curve We interpret this to be evidence for stimulated emission Comparison of these photographs with those of europium TTA in PMMA fibres tested in our apparatus indicate very similar behaviour To corroborate that the distortion of the fluorescence decay curve represents stimulated emission, I am at present measuring the coherence properties on the output radiation

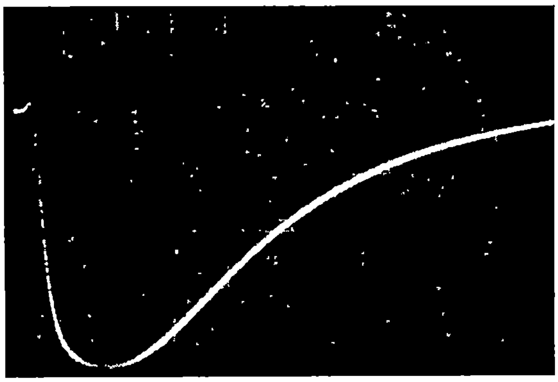

Fig 1 Normal fluorescence decay at 50-joules input Sweep 200 μs/cm

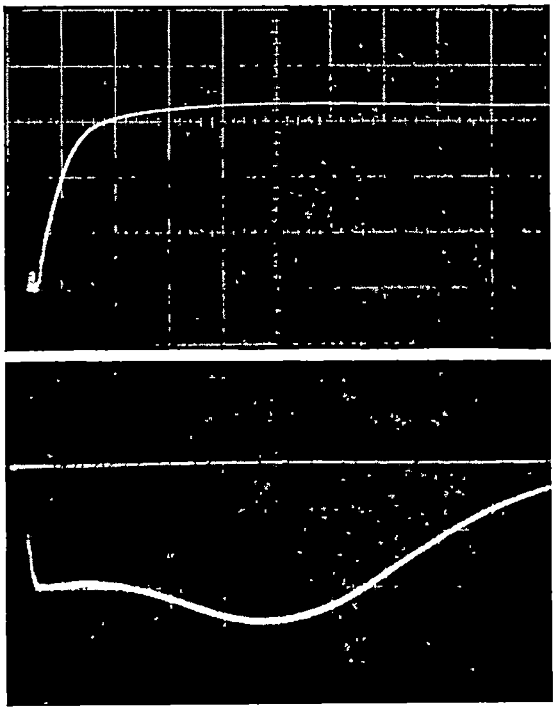

Fig 2 Upper, pump light at 225-joules input, lower, evidence of near threshold stimulated emission at 225 joule input Sweep 200 μs/cm Vertical amplifier gain is half that used in Fig 1

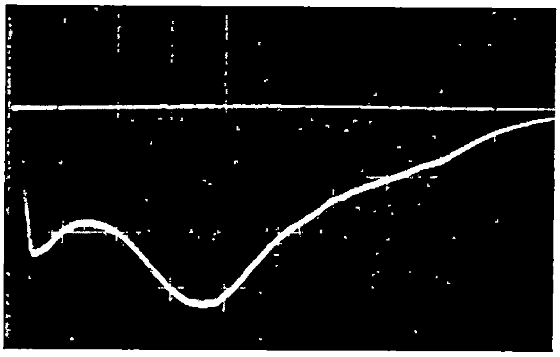

Fig 3 Evidence of stimulated emission at 400-joules input Sweep 200 μs/cm Vertical amplifier gain equal to half that used for Fig 1 The pump light outputs for Figs 1 and 3 (not shown) were similar in shape to the flash-lamp output of Fig 2

I am indebted to the late Dr B Herzog, of this laboratory, for invaluable discussions and advice, and for encouragement and support Furthermore, I thank William DeLaCruz and S L McCall for performing the experiments and in designing some of the experimental apparatus

E H Huffman

Philco Corporation,
A Subsidiary of Ford Motor Co ,
Western Development Laboratories,
Palo Alto, California

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Axial Migration of Particles in Poiseuille Flow

We have extended recent observations of the radial movements of single rigid[1,2] and deformable[3] spheres suspended in Newtonian liquids flowing through straight circular tubes to include other particle shapes and visco-elastic fluids

The experiments in Newtonian liquids were conducted as before[3,4] using single rigid spheres, rods and disks of the same density as the liquids but flowing at particle Reynolds numbers (Re_p) between 3×10^{-4} and 7×10^{-3} instead of less than 10^{-6} The tubular pinch effect previously observed with rigid spheres[1,2] at these Re_p was also exhibited by the rods and disks Particles placed initially near the tube-wall migrated inwards, while particles near the tube axis moved outwards until an equilibrium radial position (r) close to one-half the tube radius (R) was reached (Fig 1) For a given particle shape, the rate of radial migration increased with increasing flow rate, particle size and radial displacement from the equilibrium position Furthermore, rods and disks assumed limiting rotational orbits which were independent of the conditions of initial release The long axis of the rods and the faces of the disks became oriented in planes passing through the axis of the tube, corresponding to spherical elliptical orbit constants $C = \infty$ and 0 respectively[4], these are the orbits in which the particles make the maximum contribution to suspension viscosity[5] in Couette flow At low Re_p ($< 10^{-6}$) the radial positions and orbit constants of single rigid rods and disks remained

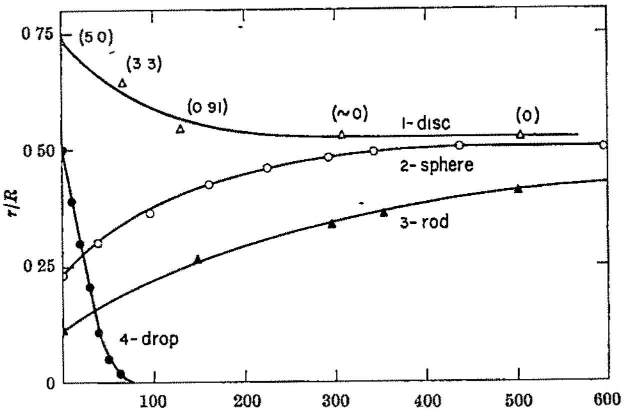

Fig. 1. Radial migration inwards (curve 1) and outwards (curves 2 and 3) to the equilibrium position $r/R = 1/2$ approximately, exhibited by spheres, rods and disks at high Re_p ($> 10^{-3}$) when suspended in polyglycol oils flowing through a tube $R = 0.2$ cm. The curves are: (1) polystyrene disk of radius 0.034 cm having an initial orbit constant $C = 5.0$, the numbers in parentheses indicating the decrease in C with radial distance and time; (2) polystyrene sphere of radius 0.050 cm; (3) nylon rod of length 0.11 cm. The steady inward migration to the tube centre of a glycerol drop in a polyglycol oil mixture, viscosity ratio suspended phase/suspending phase = 10, is shown by curve 4

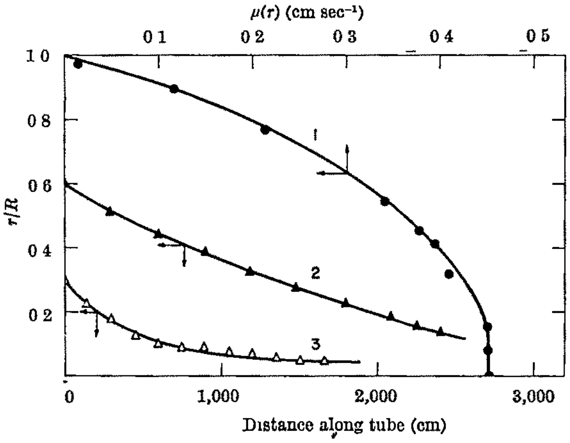

Fig. 2. Inward radial migration of 'Plexiglass' spheres in viscoelastic fluids flowing through a tube $R = 0.3$ cm at low Re_p ($< 10^{-6}$). Curve 1 shows the velocity profile, blunted near the tube centre, of a 6 per cent by weight solution of polyisobutylene in 'Decalin'. The inward migration to the central region of no shear of spheres of radii 0.016 and 0.021 cm suspended in the same solution is shown by curves 2 and 3 respectively

fixed at their initial values[4]. The drift in C and r at higher Re_p presumably are related effects due to inertia[6].

In striking contrast to rigid particles, liquid drops and elastomer filaments, which were deformed by the shear field in the tube, migrated inwards to $r = 0$ just as they did at low Re_p (refs. 3, 4). This behaviour was shown by liquid drops having a viscosity as high as 10 times that of the suspending medium (Fig. 1). When the ratio reached 50, and the drop deformation appeared negligible, the behaviour was as for rigid spheres, that is, no migration at low Re_p and migration to $r/R = 1/2$ at high Re_p. Thus the migration due to deformation[3,4] can dominate that due to inertia, and vice versa.

Inward migration of rigid particles occurred in the viscoelastic fluids at low Re_p ($< 10^{-6}$). Solutions of 3–6.3 weight per cent polyisobutylene ('Vistanex *L*-100', Enjay Chemical Co.) in decahydronaphthalene ('Decalin'), which have been well characterized rheologically[7], were used as the medium. As expected from the decrease in apparent viscosity with increasing rate of shear, the velocity profile $u(r)$ across the tube, instead of being parabolic as with the Newtonian liquids, was blunted in the central regions (Fig. 2). Rigid spheres, rods and disks placed near the wall rotated and migrated inwards to radial positions at which the velocity profile was nearly flat (Fig. 2). The rate of migration increased with r and particle size. During migration, rods and disks drifted into rotational orbits corresponding to $C = 0$ and ∞ respectively; these orbits correspond to *minimum* energy dissipation in Couette flow and are in agreement with Saffman's theoretical prediction and observations for rigid spheroids in a non-Newtonian fluid of the type used[8]. Particles in the flat portion of the profile, where the velocity gradient was zero, neither rotated nor moved radially. The particle migration observed in these experiments may arise from the combined action of normal stresses in the fluid and the variation in velocity gradient across the particle.

These experiments reveal three distinct mechanisms for radial migration during the flow of suspensions through tubes of which only one, that due to deformation at low Re_p, has been explained with any degree of completeness[4]. It is possible that there are additional mechanisms, especially at concentrations at which appreciable particle interaction can occur. These phenomena are of interest in connexion with the development of particle-free peripheral zones in the flow of various suspensions such as pulp fibre suspensions and blood through tubes.

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A. KARNIS
H. L. GOLDSMITH
S. G. MASON

Pulp and Paper Research Institute
of Canada,
and
McGill University, Montreal

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Secondary Electron Emission of Evaporated Titanium Films

IN our recent communication[1] we attributed the different secondary emission characteristics of oxidized titanium sheet and oxidized titanium films to the irregular surface on the sheet material influencing the incidence of the primary electron beam. However, we incorrectly stated that raising the incident angle of a primary electron beam should lower the value of the primary electron energy at which the secondary electron emission coefficient of a target reaches a maximum value. In fact the reverse is true, that is, raising the incident angle should increase the value of V_{max} (ref. 2). Our experimental results are not affected by this error, but the relation between the V_{max} for the oxidized titanium sheet and titanium films cannot be attributed to incidence.

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L. HOLLAND
L. LAURENSON

Surface Physics Division,
Edwards High Vacuum, Ltd.,
Manor Royal,
Crawley,
Sussex

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ENGINEERING

Cleavage Fracture of Polycrystalline Aggregates

Both Cottrell[1] and Petch[2] consider that the fracture process is divided into two parts (1) the formation of a crack nucleus by dislocation coalescence, (2) the growth of this crack until it reaches a critical size when cleavage fracture spreads catastrophically Using the well-established relationship between the lower yield stress and grain size[3] they show that fracture should just be able to occur at the yield point when

$$\sigma_y k_y d^{1/2} = \beta\mu\gamma \qquad (1)$$

where σ_y is the lower yield stress, k_y is a grain size parameter related to the stress required to unpin a dislocation, β is a mechanical restraint factor, μ is the shear modulus and γ is the effective surface energy of the fractured faces The effective surface energy, γ, required to produce cleavage fracture in a correctly orientated grain includes a plastic work term and will be larger than the true surface energy However, it will be smaller than the specific energy G_{1c} (called the plane strain fracture toughness by Irwin[4]) required to propagate the fracture through a large number of randomly orientated grains Thus the size of the microcracks predicted by the Cottrell model[1] (using the effective surface energy γ) is an order of magnitude smaller than those observed by Hahn *et al*[5] Microcracks can only grow larger than one grain under the yield stress given by equation (1) if there are adjacent grains the orientations of which are favourable Therefore only a small proportion of the microcracks formed will be able to grow large enough to enable the energy release to be sufficient for fracture propagation through a randomly orientated structure

If, in a plain tension specimen, the probability that any one grain is favourably orientated with respect to the applied stress is p and the critical crack-length for initiation of a fracture through randomly orientated grains is nd, then the probability for the growth of such a crack at the yield stress is p^n Thus the Cottrell model[1] will give the criterion for cleavage fracture at the yield stress in plain specimens provided that the number of grains in the specimens is greater than p^{-n}

In a notched specimen, the stress is non-uniform and only a limited number of grains will be subjected to the yield stress given by equation (1) Near the root of the notch the stresses will be of the order

$$\sigma = \frac{K}{\sqrt{2\pi r}} \qquad (2)$$

where K is the stress intensity[6] and r the radial distance from the root of the notch Only within a radius of

$$r = \frac{K^2}{2\pi\sigma_y^2} \qquad (3)$$

or

$$r = \left(\frac{Kk_y}{\beta\mu\gamma}\right)^2 \frac{d}{2\pi} \qquad (4)$$

will the stress be greater than σ_y The number of grains in this region is

$$N = \frac{1}{\pi^2}\left(\frac{Kk_y}{\beta\mu\gamma}\right)^4 \qquad (5)$$

If the mean of the probability of favourable grain orientated over the highly stressed region is p and the critical crack length is again nd, cleavage fracture at the lower yield stress will occur only if

$$N = \frac{1}{\pi^2}\left(\frac{Kk_y}{\beta\mu\gamma}\right)^4 > p^{-n} \qquad (6)$$

There has not been a great deal of experimental work on the effect of grain size in notched specimens The results that there are do indicate that cleavage fracture is dependent on grain size However, there is some doubt[7] that the effect is entirely due to grain size *per se* and not to changes in the internal structure brought about by the treatment that results in a finer structure Thus it is not certain that the inequality (6) is necessarily satisfied and in certain cases the fracture strength need not be dependent on grain size This may mean that the fracture strength of electro slag welds with their very large grain structure may not be as low as previously thought

B Cotterell

Department of Mechanical Engineering,
University of Sydney

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METALLURGY

Effects of High Pressure on the Strength and Structure of Martensites

Following the direct observation of fine-scale twinning ($\sim$100 Å spacing) in the martensitic structures formed by the rapid quenching of certain steels and other alloys[1], relationships between the incidence of twinning and the high mechanical strength of martensites have been postulated, explicitly in a qualitative strengthening model[2] and implicitly in a quantitative solution-hardening model[3] In view of the suggested importance of the twinning contribution to strength, an attempt has been made to test its validity by experiments on martensitic transformations in the iron–carbon and iron–chromium systems at high pressure and in the iron–nickel system at atmospheric pressure The experimental apparatus and techniques used have been described elsewhere[4]

In the Kelly–Nutting model[2], the rapid increase in martensite strength (beyond that attributed to solution hardening) and incidence of twinning with increasing carbon content are considered to arise from the effect of carbon in depressing M_s from above to below a critical temperature range for twinning (approximately 300°–220° C) which is essentially composition independent It has been demonstrated previously for the iron–carbon system that the temperature at which martensite formation begins on cooling (M_s) is depressed substantially at high pressure[4] Accordingly, if the twinning model were correct, the strength of martensite formed in all compositions which have their M_s depressed at a given pressure to below the critical temperature range should attain or approach that of fully twinned martensite formed at atmospheric pressure Microhardness measurements on a series of iron–carbon martensites formed at atmospheric pressure and at 42 kilobars are compared in Fig 1 At the high pressure, the lowest composition for which M_s is below the critical range is reduced from 0 75 down to 0 4 wt per cent carbon[4] However, the expected increase in hardness to high values characteristic of fully twinned martensites in this system (925 VPN) is not observed for the two alloys in this composition region Instead, the hardness of the 0 4 wt per cent carbon alloy increases to only 780 VPN and the higher carbon alloy to a similar value, that is, the hardness increase is smaller for the latter alloy Furthermore, the 0 08 wt per cent carbon alloy, even though it transforms above the critical range at 42 kilobars, exhibits an increase in hardness as large as that for the 0 4 wt per cent alloy The specimens of high-purity iron and the 0 75 and 1 2 wt per cent carbon alloys show only minor changes, this result suggests that any plastic deformation arising from quasi-hydrostatic conditions in the high-pressure cell does not contribute strongly to the hardness increases in the intermediate alloys

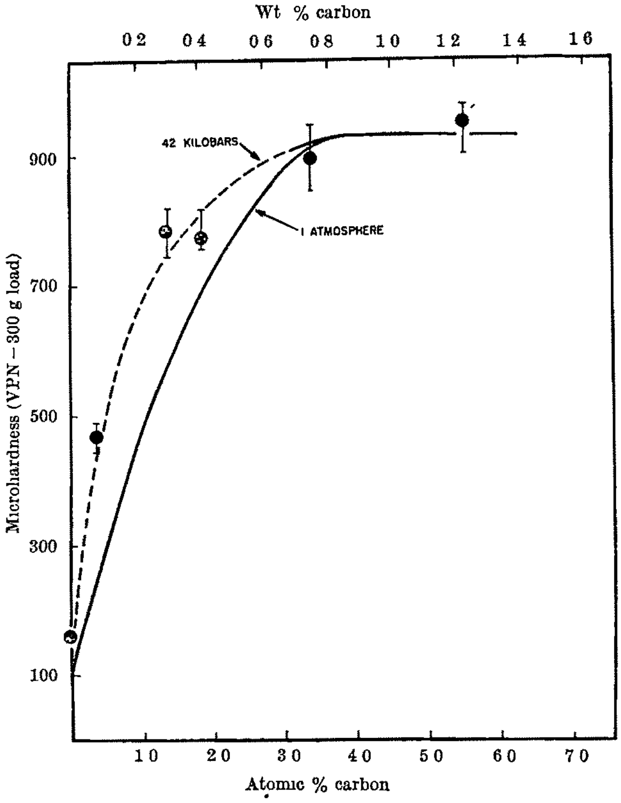

Fig. 1. Effects of pressure on hardness of martensites formed in iron–carbon alloys

The lack of hardness change for martensites containing more than 0.75 per cent carbon is consistent with the twinning model in that such martensites are normally fully twinned, so that further depression of M_s would not be expected to change the incidence of twinning. However, the hardness changes for the intermediate alloys are not in keeping with the model. Instead, it appears likely that the changes observed are attributable to a reduction in the loss of carbon from solution by self-tempering, that is, to an increase in solid-solution hardening. Self-tempering is not normally important in high carbon martensites, so that high pressure would not be expected to affect them, as is observed. Confirmation of this interpretation by transmission electron microscopy has not yet been possible because of difficulties encountered in preparing suitable thin-foils from the small specimens used in the high-pressure investigations.

In addition to the concept of a critical temperature range, the qualitative twinning model attributes a large twinning contribution to the strength of body-centred cubic martensites, in which the solution-hardening characteristic of tetragonal martensites containing carbon is absent. In the iron–nickel system, body-centred cubic martensites containing finely spaced twins can be formed only in alloys containing more than some 20 atomic per cent nickel. Alloys lower in nickel form the so-called 'massive' structures[6], which do not exhibit internal twinning. It is to be noted that over this composition range the M_s is indeed above the suggested critical range for twinning. In the iron–chromium system, the high-temperature face-centred cubic phase (γ) is restricted to a loop-shaped phase field with a maximum chromium content of 13.5 atomic per cent chromium, and all alloys quenched from within this field exhibit 'massive' structures. At high pressure, the extent of the γ-phase field is extended so that, in principle, it should be possible to quench alloys sufficiently high in chromium to have an M_s below the critical range. Such quenched alloys would be expected to show twinned martensitic structures. Thus, an examination of the iron–chromium system at high pressure should permit an additional test of the validity of the concept of a critical range for twinning. Furthermore, observation of the changes in hardness with solute content for quenched iron–chromium and iron–nickel alloys provides a test of the twinning contribution to strength, since a discontinuous increase in strength should be observed on reaching compositions at which twinned martensites are formed.

In Fig. 2, the increase in the extent of the γ-field in the iron–chromium system at 42 kilobars is shown, as measured from metallographic examination of equilibrated and quenched specimens and continuous electrical resistance measurements. At this pressure, the maximum chromium content of the loop is increased to ~21 atomic per cent chromium. These measurements confirm and extend the previous measurements shown of the depression of the γ-field in low-chromium alloys[5]. The composition dependence of the hardness of alloys quenched from within the loop region at 42 kilobars does not exhibit a discontinuity, although there is a possible indication of the beginning of one for the 20 atomic per cent chromium alloy. However, the measured M_s values indicate that even for the higher chromium alloys the transformation temperature is depressed only into and not below the critical range. The structures are 'massive' except for the 20 atomic per cent chromium alloy, which has an acicular structure. As for the iron–chromium alloys, replica electron microscopy proved inadequate to confirm the nature of the structure, and transmission microscopy has not yet been possible. It is clear that in the iron–chromium system a higher pressure is needed to depress adequately the transformation temperature, either directly or by the use of alloys still higher in chromium.

In Fig. 3, the composition dependence of hardness for a series of iron–nickel alloys quenched into liquid nitrogen

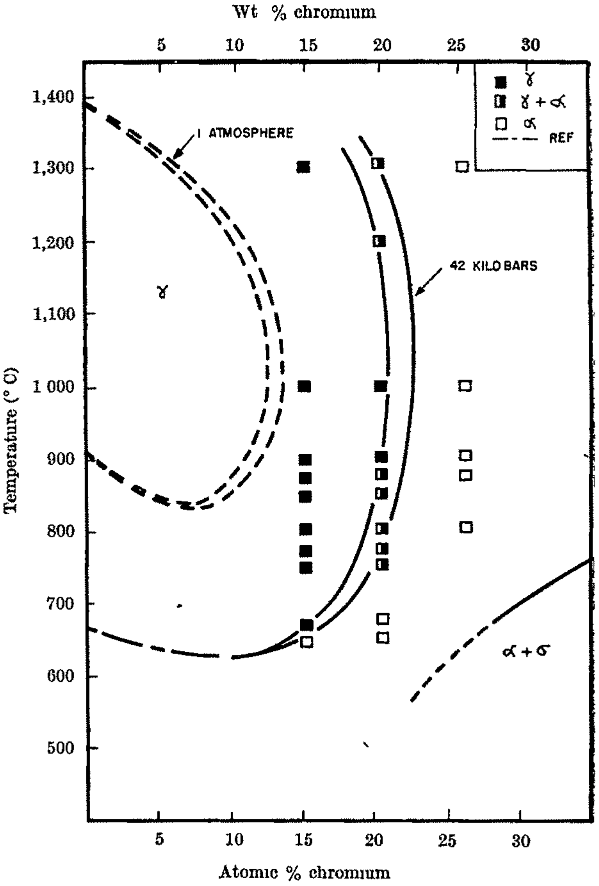

Fig. 2. Shift of γ-loop in iron–chromium system at 42 kilobars

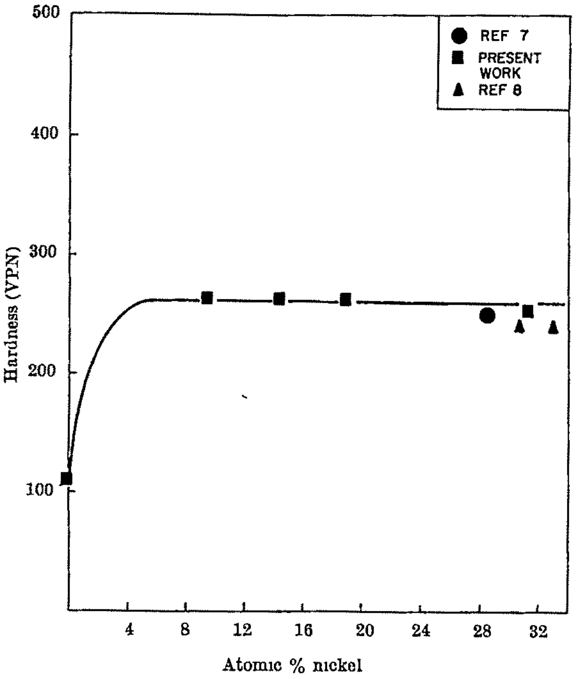

Fig 3 Effect of nickel content on hardness of iron–nickel alloys transformed by quenching at atmospheric pressure

(−196° C) to ensure completion of transformation is shown, together with reported data for high-nickel twinned martensites[7,8] The transition between massive and twinned martensitic structures with increasing nickel is well established[6], and the lack of any discontinuity in hardness is clear evidence of the lack of any singular contribution to strengthening from the presence of fine internal twinning It is interesting to note that the maximum hardness attained by twinned iron–nickel–carbon martensites[9] is only 630 VPN, compared with 260 VPN for twinned iron–nickel martensite and 925 VPN for iron–carbon martensites Thus, the presence of nickel strongly reduces the hardness of carbon martensites, even in the presence of internal twinning

In the Winchell–Cohen model of the strengthening of carbon martensite by solution hardening[3], the fine spacing of internal twins is considered to define the effective length of active dislocation line—an important parameter in the model In iron–carbon alloys and plain carbon steels, the proportion of internally twinned martensite plates is small up to some 0 34 weight per cent carbon, and complete twinning occurs only above 0 8 weight per cent carbon As the greater part of the hardness increase with increasing carbon occurs by 0 34 weight per cent carbon, the applicability of such a model to carbon martensites in general is doubtful Furthermore, since the present results show that structural twinning *per se* is not important to strength in iron–nickel martensites, the validity of using the twin spacing to define an effective length of dislocation in iron–nickel–carbon martensites is in question

It is concluded that the presence of fine structural twins does not make an important contribution, either directly or indirectly, to strength in tetragonal or body-centred cubic martensites in iron-alloys Structural twinning is an important part of the mechanism of martensite formation[1] and mechanical twinning is important in limiting the maximum strength which can be attained[10] However, the principal strengthening mechanisms operative during the initially rapid increase in strength with increasing carbon content must depend primarily on a solid-solution hardening mechanism, possibly one similar to that suggested by Winchell and Cohen, but in which the effective dislocation length is defined by a structural feature other than twinning

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S Victor Radcliffe
Department of Metallurgy,
Case Institute of Technology,
Cleveland, Ohio

M Schatz
Boston, Massachusetts

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CHEMISTRY

Role of Free Radicals in the Photo-oxidation of Fe^{+2} in Acidic Solutions of Ferrocyanide

Neutral solutions of $K_4Fe(CN)_6$ have been known to become alkaline when irradiated by ultra-violet light[1–3] It has been suggested that photolysis of $Fe(CN)_6^{-4}$ first takes place to give $Fe(CN)_6^{-3}$ and $(CN)^{-1}$, and the latter then hydrolyses, producing free alkali

$$Fe(CN)_6^{-4} \xrightarrow[h\nu]{} Fe(CN)_5^{-3} + (CN)^{-1} \quad (1)$$

$$(CN)^{-1} + H_2O \rightarrow (HO)^{-1} + HCN \quad (2)$$

$$Fe(CN)_5^{-3} + H_2O \rightarrow [Fe(CN)_5\ H_2O]^{-3} \quad (3)$$

When the light source is cut off, all the three reactions are reversed and the original *p*H is restored unless irradiation has been far too prolonged[2]

We have found quite a different effect of ultra-violet light on $Fe(CN)_6^{-4}$ under acidic conditions Percolation of dilute solutions (about 2 g/l) of $K_4Fe(CN)_6$ through beds of the strong acid ion-exchanger, '*IR*–120', gave solutions which had a *p*H of about 1 5 When freshly prepared, they had a straw colour which slowly changed to light blue in diffuse sunlight Irradiation with ultra-violet light from a Philips mercury discharge lamp very soon gave an intense blue colour The blue substance has been identified as ferri-ferrocyanide which came down as a precipitate on standing

The composition of the acidic solutions before irradiation is determined mainly by the following three equilibria

$$H_4Fe(CN)_6 \rightleftarrows 4H^+ + Fe(CN)_6^{-4} \quad (4)$$

$$Fe(CN)_6^{-4} \rightleftarrows Fe^{+2} + 6(CN)^{-1} \quad (5)$$

$$CN^{-1} + H^{+1} \rightleftarrows HCN \quad (6)$$

Calculations made from known values of the instability constant[3] of $Fe(CN)_6^{-4}$ and the dissociation constant of HCN (ref 4) showed that, at a *p*H of about 1 5, very little $Fe(CN)_6^{-4}$, as such, would be present, and the predominant species would be Fe^{+2} and HCN The freshly prepared acidic solution, however, titrated as a strong acid Its *p*H titration curve (Fig 1) indicated four strong H^+ ions per Fe^{+2} ion During the titration, a gradual shift of equilibria (5) and (6) occurs and the solution titrates as $H_4Fe(CN)_6$, which is a strong acid and gives back $K_4Fe(CN)_6$ on complete neutralization

On irradiation photo-oxidation of the Fe^{+2} ions takes place and this gives the ferri-ferrocyanide Jortner and Stein[5,6] have shown that Fe^{+2} ions in acidic solutions of

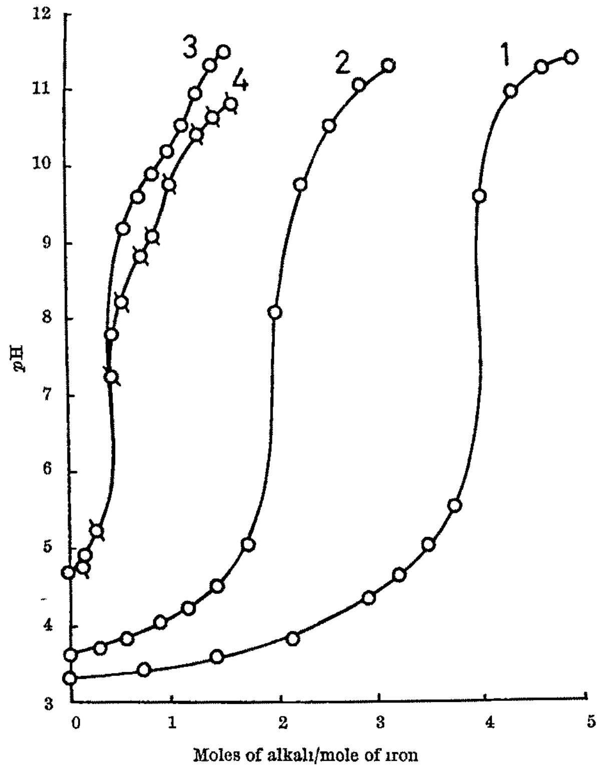

Fig 1 1, No irradiation, 2, 2-h irradiation, 3, 4-h irradiation, 4, 6-h irradiation

$FeSO_4$ are oxidized to Fe^{+3} under the influence of ultraviolet light We found, however, that the rate of oxidation of Fe^{+2} ions in an acidic solution of $FeSO_4$ was only about 5 0 per cent of the rate at which ferri-ferrocyanide was formed in an equimolar solution of $K_4Fe(CN)_6$ of the same *p*H Evidently, the mechanism of oxidation is not quite the same in the two cases We believe that, on irradiation, photolysis of HCN present in the acidic solutions of ferrocyanide first takes place to give H and CN radicals

$$\mathrm{HCN} \xrightarrow{h\nu} \mathrm{H}+\mathrm{CN} \qquad (7)$$

The CN radical then oxidizes Fe^{+2} to Fe^{+3}, either directly, or through a water molecule

$$\mathrm{CN}+\mathrm{Fe}^{+2}\rightarrow\mathrm{Fe}^{+3}+\mathrm{CN}^{-} \qquad (8)$$

$$\mathrm{CN}+\mathrm{H_2O}\rightarrow\mathrm{HCN}+\mathrm{OH} \qquad (9)$$

$$\mathrm{OH}+\mathrm{Fe}^{+2}\rightarrow\mathrm{Fe}^{+3}+\mathrm{OH}^{-} \qquad (10)$$

The acidic solutions contained dissolved oxygen, in the presence of which the H atom (equation 7) oxidizes three Fe^{+2} ions

$$\mathrm{H}+\mathrm{O_2}\rightarrow\mathrm{HO_2} \qquad (11)$$

$$\mathrm{HO_2} + \mathrm{H_2O} + 3\mathrm{Fe}^{+2} \rightarrow 3\mathrm{Fe}^{+3} + 3\mathrm{OH}^{-} \qquad (12)$$

Jortner and Stein also used equations (11) and (12) to explain photo-oxidation of Fe^{+2} However, while their only source of H was $[Fe^{+2}(H_2O)_n]$, we have an additional and, apparently, a more efficient source, namely, HCN

The Fe^{+3} ions produced by reactions 8, 10 and 12 react with $Fe(CN)_6^{-4}$ to produce Prussian blue The overall reaction is

$$8\mathrm{H_4Fe(CN)_6}+\mathrm{O_2}\rightarrow4[\mathrm{HFe(Fe(CN)_6}]+24\mathrm{HCN}+2\mathrm{H_2O} \qquad (13)$$

The titratable amount of the strong acid present in the solution decreases as irradiation proceeds and, ultimately, one-eighth of the strong acid remains (Fig 1) This is what one would expect from equation (13), if it is assumed that ferri-ferrocyanic acid is also a strong acid The presence of HCN, as a weak acid component, is clearly indicated by the titration curve obtained after complete irradiation However, the amount of HCN is smaller than what would be expected from equation (13) A large amount of HCN escapes during irradiation, the solution smelling strongly of the gas Some CN may also be lost by secondary reactions, for example, formation of $(CN)_2$

R P MITRA
D V S JAIN
A K BANNERJEE
K V RAGHAVACHARI

Physical Chemistry Laboratory,
University of Delhi

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Concentration Effects in Measurements of Characteristics of Deoxyribonucleic Acid by Transport Methods

A CLOSE qualitative similarity between the mode of variation of the reduced viscosity ($\eta sp/C$) and that of the reciprocal of sedimentation coefficient ($1/S$) with concentration of deoxyribonucleic acid (DNA), pointed out recently[1], is also apparent from the following two expressions

$\eta sp/C$ at a finite low concentration C (gm/ml) may be related to its zero concentration value $[\eta]$ (the intrinsic viscosity in ml /g) as[2–5].

$$\eta sp/C=[\eta](1+k[\eta]C) \qquad (1)$$

where k is a dimensionless factor having a magnitude characteristic of the system[2] and is a function of all significant interactions (solute–solute and solute–solvent interactions) at this concentration[4]

$1/S$ at the concentration C may be related to its zero concentration value $1/S_0$, as [5–7]

$$1/S=(1/S_0)(1+\alpha[\eta]C) \qquad (2)$$

where α is a dimensionless factor$=Ks/[\eta]$, obtained empirically (see ref 5) It is reasonable to suppose that α is also like k, a function of all interactions in the system which are significant at this concentration

Both α and k decrease generally with increase of added sodium chloride in DNA solution[1,8] and in many cases with increase of rotor speed[9] and shear rate[8], respectively The numerical values of α and k obtained for large varieties of DNA samples in solvents containing sodium chloride of the order of 10^{-1} M are also comparable $\alpha=0\ 8\pm0\ 1$ (refs 5,10) and $k=0\ 5\pm0\ 2$ (ref 5), 0 77 (γ-ray irradiated DNA solutions[11]) Some of these values of k are again close to the theoretical[3] value of 0 73 for rod-like molecules There are, however, instances where DNA solutions in these solvents show lower or even zero values for α and k

To explain the observed results, a consideration of the factors (solute–solute and solute–solvent interactions in the system) contributing to the values of α and k may be made Three types of interactions between pairs of single macromolecules may be the contributory factors[4], (*a*) Long-range hydrodynamic interaction has always a positive contribution, (*b*) intermolecular attraction and repulsion may have positive or negative contribution, (*c*) formation of double molecules (the existence of which even though may be fleeting may provide equilibrium concentration of aggregates) in good and poor solvents, respectively, have negative and positive contributions

It is evident that, depending on the nature of solute and solvent, a combination of these three factors may result in either positive, zero or negative values of α and k Additional negative contribution may arise when α and k are dependent on rotor speed and shear rate, respectively When charge interactions, aggregation and shear rate or rotor speed effects are absent, the values of α and k will be positive and will be determined by long-range hydrodynamic interactions in the system Thus, for effectively uncharged macromolecules both $1/S$ and $\eta sp/C$ should be concentration dependent It may be interesting to note[12] that for uncharged solid spherical molecules, $k \approx 2$ was predicted theoretically[13] and was obtained experimentally[14] for polystyrene latex Also one may obtain $\alpha \approx 2$ for polystyrene latex from the solvent density corrected sedimentation data[14], and also for spherical molecules from the theoretical relation[6] between Ks and $[\eta]$ (using channel constant = 1 8). Thus, it appears that for spherical uncharged macromolecules the values of α and k may be the same If there occur interactions between the charged polymers and their counter-ions, resulting in polymer–polymer interactions of the types (*b*) and (*c*) described here, the applied forces arising out of shear rate or rotor speed may interfere with such interactions A relevant discussion pertinent to the magnitude of k in DNA solutions has been made in ref 8 Thus, when a DNA solution is such that its equilibrium composition readily alters with handlings involved in different experiments, the magnitude of the factor dependent on interactions will also alter

The effect of interactions at concentration C expresses itself in equation (1) as an increase in the effective volume of the particle[4] . There will be corresponding increase in the viscosity of the medium, so that one may write $(\eta sp/C)/[\eta] = \eta_{eff}/\eta_0 = 1 + k[\eta]C$, where η_{eff} is the effective viscosity of the medium arising out of all interactions at concentration C and η_0 is the viscosity of the medium (solvent) in absence of all interactions Defining a frictional coefficient f^c at concentration C which varies with η_{eff}, one can describe both the equations (1) and (2) as variation of frictional coefficient with concentration Thus, in general, one can express the concentration dependence of frictional coefficient for all transport processes[12] involving migration of macromolecules through viscous medium under the influence of infinitesimally small applied forces as

$$f^c = f^0(1 + \lambda[\eta]C) \qquad (3)$$

where f^0 is the frictional coefficient at zero concentration and λ is the factor the magnitude of which will be characteristic of all the interactions in the system at finite low concentration C of the macromolecule The concentration dependence of $1/D$ and $1/\mu$ (D = diffusion coefficient, μ = electrophoretic mobility) may be expressed on the basis of this equation (3), assigning a distinctive symbol in place of λ for each type of experiment

Thus, a general approach towards correlation of concentration effects observed in measurements of characteristics of DNA (which should also be applicable for other macromolecules or macro ions) by various transport experiments has been outlined here A similar attempt[15] proposes, in effect, that (instead of η_{eff} proposed here) the macroscopic viscosity of the solution is the function of all interparticle interactions On the basis of considerations presented in this communication one may judge the reasons for observed agreement and disagreement of such theory with practice, and also evaluate other explanations of concentration dependence of sedimentation coefficient[16] Considerations on the observed similarity between concentration dependence of S and of DNA charge mentioned earlier[1] and other details pertinent to this communication will be presented elsewhere

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PRASANTA K GANGULI

Biophysics Division,
Saha Institute of Nuclear Physics,
Calcutta 9

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Quantitative Analysis of Ketosteroids by Means of Paper Chromatography

IN a previous paper[1] we described a method for the detection of small amounts of ketosteroids on chromatograms by means of DNPH The subsequent removal of the background colour enables the use of this method for quantitative analysis, as previously suggested A satisfactory solvent system, by which the hydrazones were eluted from strips and then determined by spectrophotometry, was found The procedure described here requires little time, and is suitable for routine estimations We have used this method in metabolic investigations *in vitro* as well as for measurement of the progesterone concentration in sheep's ovarian venous blood In both cases very satisfactory results were obtained

The procedure adopted for quantitative purposes was essentially like that previously described, except that the time of reaction with DNPH was shortened to 5 min. and the strips were neutralized in ammonia vapour instead of sodium carbonate solution Equally good results were obtained when washed in permanganate solution for 5 min without previous neutralization of the strips Whatman paper No 20 previously washed with methanol proved to be the most satisfactory one of all those tested The coloured hydrazone spots and respective blanks were cut out, then cut into small pieces and extracted four times at room temperature for 10 min each time, with a mixture of methanol–methyl acetate 1 4, the first portion of the eluent being 0 6 ml , the three subsequent ones 0 35 ml each It was necessary to use freshly dried strips, since storage caused an incomplete elution

The volume of the combined extracts was then adjusted to 1 5 ml immediately before measurement and the samples were read against appropriate blanks at 380 mμ The exact maximum absorption wave-length depended on the keto-group position of the particular steroid, for example, the peaks of pregnenolone, progesterone and testosterone lay at 365, 375 and 380 mμ respectively

The optical density was found to be 0 090–0 100 per μg progesterone, pregnenolone and testosterone have given respectively lower values

Glass cells of 1-cm light path and the Russian quartz spectrophotometer model *SF*-4 were used The calibration curves of pure steroids alone or with various lipid material added were usually linear in the range of 0 5–10 μg of diketones, or 1–20 μg monoketones, sometimes, however, they showed little curvature The minimum amount which can be determined is about 0 5 μg of progesterone The standard error expressed in extinction units was as high as 0 015 This value was found to be constant in the foregoing ranges of steroid quantities. This method was controlled radiometrically, using progesterone-4-^{14}C (The Radiochemical Centre, Amersham, England) and a current-flow gas counter, the loss of radioactivity during the procedure described did not exceed 5 per cent of the initial quantity added

R Stupnicki
Elżbieta Stupnicka

Institute of Animal Physiology
and Nutrition,
Jabłonna, nr Warsaw

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Stability of *N*-Glycuronyl Amino-acids

In assessing the stability of the various possible linkages joining carbohydrates to amino-acid residues and in order to avoid base-catalysed acyl migration, we have synthesized *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycine and its β-DL-phenylalanine analogue and examined their behaviour on hydrolysis The syntheses were effected by using as condensing agents either di*cyclo*hexylcarbodiimide[1], or tetraethylpyrophosphite[2]

1,2 3 4-Di-*O*-isopropylidene-D-galactose[3] was oxidized[4] with potassium permanganate and the crystalline potassium 1,2 3,4-di-*O*-isopropylidene-D-galacturonate obtained converted into the free acid by ethereal extraction of an acidified aqueous solution The acid (21 g) was added to a stirred solution of freshly prepared benzylglycinate (13 g) in diethyl phosphite (50 ml) Tetraethylpyrophosphite (22 g) was added and, after heating the stirred mixture at 100° for 10 min, it was poured into water The precipitate was washed with saturated aqueous sodium hydrogen carbonate and recrystallized from ethanol to yield benzyl *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycinate (20 g), m p 101°–102°, $[\alpha]_D$ −89 5° (*c*, 1 0 in chloroform) (found C, 60 1, H, 6 6, N, 3 3 $C_{21}H_{27}NO_8$, requires C, 59 9, H, 6 4, N, 3 3 per cent)

Alternatively, glycine benzyl ester (1 39 g) was suspended in dry ether (100 ml) and a mixture of di*cyclo*hexylcarbodiimide (3 1 g) and 1,2 3,4-di-*O*-iso-propylidene-D-galacturonic acid (3 g) added After stirring at room temperature for 12 h, glacial acetic acid (3 drops) was added and the di*cyclo*hexylurea removed by filtration The residue (4 99 g) after evaporation of the filtrate was crystallized from ether to yield benzyl *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycinate (1 21 g), m p 101°–102°, $[\alpha]_D$ −87 5° (*c*, 0 9 in chloroform), which was identical with the product described here Debenzylation could be effected with sodium and liquid ammonia to yield *N*-(1 2 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycine, m p 212° (from ethyl acetate), $[\alpha]_D$ − 97 1° (*c*, 0 8 in chloroform) (found C, 51 0, H, 6 5, N, 4 25 $C_{14}H_{21}NO_8$, requires C, 50 8; H, 6 3, N, 4 2 per cent)

In a similar way, β-DL-phenylalanine benzyl ester (2 8 g) in methylene chloride (100 ml) was condensed with 1,2 3,4-di-*O*-isopropylidene-D-galacturonic acid (3 1 g) in the presence of di*cyclo*hexylcarbodiimide (3 5 g) to yield benzyl *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-β-DL-phenylalanate (0 8 g), m p 115°–116° [from ethyl acetate–light petroleum (b p 60°–80°)], $[\alpha]_D$ −92 8° (*c* 0 88 in chloroform) (found C, 65 7, H, 6 2, N, 3 0 $C_{28}H_{33}NO_8$; requires C, 65 7, H. 6 5, N, 2 7 per cent) Debenzylation afforded *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-β-DL-phenylalanine with m p 211°–215° (from ethyl acetate–light petroleum (b p 60–80°)), and $[\alpha]_D$ − 130 5° (*c*, 1 0 in chloroform) (found C, 59 5, H, 6 6, N, 3 4 $C_{21}H_{27}NO_8$, requires C, 59 8, H, 6 45, N, 3 3 per cent)

The stability of the amide linkage in the two analogues was assessed by determination[5] of the hydrolytically released amino-acid with ninhydrin Aliquots (2 ml) containing *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycine (144 μg) in N hydrochloric acid were hydrolysed to extents of 24 5, 42 9, 53 6 and 59 8 per cent after 1, 3, 4 and 6 h at 100° The compound was more unstable in alkali and, after similar treatment with N sodium hydroxide at 100°, the percentage hydrolysis was 52 1 (0 5 h), 69 6 (2 5 h) and 83 7 per cent (3 5 h) By contrast, *N*-(1,2 3,4-di-*O*-isopropylidene-D-galacturonyl)-β-DL-phenylalanine was more unstable in acid than in alkali Thus, aliquots (2 ml) containing amino-acid derivative (1 mg) in 30 per cent aqueous alcoholic 0 5 N hydrochloric acid were hydrolysed to the following extents 10 (1 h), 13 (2 h), 18 (3 h) and 35 per cent (6 h) at 92° compared with 4 (1 h), 11 5 (2 h), 24 per cent (4 h) in 30 per cent aqueous alcoholic 0 5 N sodium hydroxide at the same temperature

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S M Amir
S A Barker
A B Foster
D C Lamb

Department of Chemistry,
University of Birmingham

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Influence of Hydrostatic Pressure on Orientation in Electrophilic Aromatic Substitutions

M G Gonikberg *et al* [1-3] have recently found that at high pressures the attack of phenyl radicals on tertiary butyl benzene yields an appreciably higher proportion of the sterically strained 2-phenyl isomer than it does at atmospheric pressure

We have observed a similar effect in the electrophilic nitration of 1,3-xylene by nitronium ions, NO_2^+ The nitrations were carried out under the same conditions and by the same methods that we had previously used in reaction rate measurements[4] The initial composition of the reaction mixtures, expressed in mole fractions, was nitric acid, 0 618, acetic acid, 0 369, 1,3-xylene, 0 013 We analysed the products by gas chromatography at several stages during the course of each reaction and found that the isomer ratios were independent of the extent of reaction Table 1 lists the results

Table 1 Mole Fractions of Isomers formed in the Nitration of 1,3-Xylene by Nitric Acid in Acetic Acid Solution at 0° C

	Nitro isomer		
Pressure (atm)	2-	4-	5-
1	0 107	0 880	0 013
800	0 150	0 833	0 017
1,200	0 164	0 816	0 020
2,000	0 171	0 829	

It will be seen that between 1 and 2,000 atmospheres the proportion of substitution at the sterically hindered 2-position increased by 60 per cent The change is larger than that observed by Gonikberg[1-3] presumably because in 1,3-xylene there are two adjacent groups tending to obstruct attack on the 2-position Our result supports Gonikberg's general thesis that an increase in pressure tends to favour the formation of sterically compressed isomers

However, some other experiments have shown that the steric factor is not the only important one in nitrations Under the same experimental conditions that applied in the nitration of 1,3-xylene, we found that toluene and chlorobenzene yielded decreasing proportions of the 2-nitro isomers as the pressure was increased The results are given in Table 2

Table 2 MOLE FRACTIONS OF ISOMERS FORMED IN THE NITRATION OF TOLUENE AND CHLOROBENZENE BY NITRIC ACID IN ACETIC ACID SOLUTION AT 0° C

Pressure (atm)	Toluene			Chlorobenzene		
	Nitro isomer			Nitro isomer		
	2-	3-	4-	2-	3-	4-
1	0 560	0 020	0 420	0 247	—	0 753
1,200	0 550	0 022	0 428			
2,000	0 543	0 024	0 433	0 204	—	0 796

Although the shift of isomer ratio in these cases was relatively small, there was a consistent trend with increasing pressure and its direction was the same whether the groups already present on the benzene ring had electron donating (activating) or electron accepting (de-activating) properties

Experiments at atmospheric pressure[5] have established that a change of solvent alters the ratios of isomers formed in the nitration of alkyl benzenes by nitronium salts The effect almost certainly arises from changes of dielectric constant and solvation Presumably similar factors operate when the dielectric properties of the nitrating solution are altered by an applied pressure, and they are supplementary to the simple steric effect Moreover, it is possible that compression may alter the distribution of electrical charge around the benzene rings We hope to distinguish these factors more clearly in future work

D W COILLET*
S D HAMANN

C S I R O
Division of Physical Chemistry,
Melbourne

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* Present address Department of Chemical Engineering, University of Sydney

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BIOCHEMISTRY

Amino-acid Sequences within the Ferrichrome Cyclic Hexapeptides

AN earlier communication[1] concerned with the structures of ferrichrome and ferrichrome *A* left open the particular arrangement of the 3 residues of neutral amino-acids and 3 residues of δ-*N*-hydroxyornithine[2] within the cyclic hexapeptide moiety of the naturally occurring ferric trihydroxamates The peptide portion of ferrichrome contains 3 residues each of glycine and δ-*N*-hydroxyornithine while, in ferrichrome *A*, 2 of the 3 glycine residues are replaced by serine residues In the present report we propose that the sequence for ferrichrome is *cyclo*-triglycyl-tri-δ-*N*-hydroxyornithyl while that for ferrichrome *A* is most probably *cyclo*-diseryl-glycyl-tri-δ-*N*-hydroxyornithyl

Ferrichrome was hydrogenated with Raney nickel in order to eliminate both the iron and the three oxygen atoms of the hydroxamate functions[3] The product was precipitated from methanol–acetone to yield colourless prisms with m p 262°–265° (uncorr , decomp) A synthetic specimen was obtained by the successive addition of three residues of α-*N*-carbo-benzoxy-δ-acetylornithine-*p*-nitrophenyl ester to triglycine ethyl ester, followed by de-blocking and cyclization of the linear hexapeptide This choice of sequence was predicted on the observation that diglycine appears during partial acid hydrolysis of ferrichrome and on the recent discovery by Czech workers[4] that triserine occurs in the related compound albomycin The natural and synthetic products displayed the same m p (decomp) and the same R_F in acidic, neutral and basic solvents Exposure of the two preparations to 12 N hydrochloric acid at 30° for 1 5 days provided an identical array of ninhydrin-positive spots after analysis by paper electrophoresis in 4 per cent formic acid

Ferrichrome *A* was degraded by a bacterium which had been isolated from soil through enrichment culture on the ferrichrome compounds as sole source of carbon and nitrogen The complex pattern of peptides present in the culture medium was separated by Moore-Stein[5] chromatography and selected members were subjected to sequence analysis by use of standard reagents such as fluorodinitrobenzene and hydrazine These investigations revealed the presence of the tripeptide seryl-glycyl-δ-*N*-acylhydroxyornithine A dipeptide, believed to be serylserine, was produced through partial acid hydrolysis of iron-free ferrichrome *A*

In summary, it appears that the cyclic hexapeptide core of the ferrichrome growth factors shares a common component with the albomycin antibiotics, namely, a tripeptide of δ-*N*-hydroxyornithine

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S J ROGERS
R A J WARREN
J B NEILANDS

Department of Biochemistry,
University of California,
Berkeley

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Hydrolysis of Some Antibiotics and of their Decomposition Products in the Presence of Cation Exchange Resins

THE conditions under which some antibiotics are hydrolysed may be important for the isolation and identification of the products of their decomposition since these are used in investigations of the structure of the antibiotics During hydrolysis with mineral acids some moieties, such as carbohydrates, may be destroyed, and much humin may be produced which makes it difficult to isolate the more stable products of decomposition in pure form

A new method of hydrolysis of some antibiotics with the aid of sulphocationic resins of the 'Dowex-50' type is described here By varying the conditions of the experiments (temperature, time, type of resin) different products of hydrolysis may be obtained without their complete degradation and without the production of humins At the same time basic substances can be separated from neutral and acid products The basic substances are adsorbed on the cationic resin while the neutral and acid products are retained in the mother liquor

By gradient elution with various eluants different basic products adsorbed on the ion-exchange resins can be separated The following sulphonic resins were used for hydrolysis 'Dowex-50 × 12' (50–100 and 100–200 mesh)

Table 1 DEGRADATION WITH THE AID OF ION-EXCHANGE RESINS OF VARIOUS ANTIBIOTICS AND OF THE PRODUCTS OF THEIR DECOMPOSITION

Antibiotic	Resin	Time of hydrolysis (h, 105°)	Products of hydrolysis observed	
			Eluate	Mother liquor
Monomycin	*KU*-2	1	Monamine, monomycin, second component	
,,	,,	2	the same	
,,	,,	4	monamine, second component	
,,	,,	48	glucosamine, diaminohexose, streptamine, traces of monamine	
,,	'Dowex 50 × 12' (100–200 mesh)	43	the same	
,,	'*KB*4*P*'2 (carboxylic)	43 43	monomycin, monamine, diaminohexose	
"Second component" after methanolysis of monomycin	'*KU*-2'	1 5	positive reaction with hydrophthalate	
,,		14	diaminohexose	ribose
Neomycin	'*KU*-2'	4 5	neamine, neobiosamine	
,,	,,	48	neamine, diaminohexose	
,,	'*KB*4*P*2'	43	neomycin, neamine, diaminohexose	
Kanamycin	'*KU*-2'	3	kanamycin, products of its partial hydrolysis	
,,	,,	24	two aminoglucoses, streptamine	
Amphomycin	'*KU*-2'	3	mixture of peptides, products of partial hydrolysis	
,,	,,	24	amino-acids, products of total hydrolysis	
Actinoidin	'Dowex 50 × 12' (50–100 mesh)	20		glucose, mannose, acid phenolic component

and '*KU*–2' in hydrogen form The following antibiotics were hydrolysed neomycin, kanamycin, amphomycin, as well as monomycin[1] and actinoidin[2] isolated in our laboratory The products of hydrolysis were identified on circular or descending chromatograms in appropriate systems of solvents[3] For each 100 mg of the substance to be hydrolysed 1·g of the resin and 50–100 volumes of 0 05 N hydrochloric acid were used Solutions of the substances under study were sealed in ampoules together with the resin and kept at 105° C with shaking for various periods of time After hydrolysis the resin was separated from the mother liquor, carefully washed and placed in a column for chromatography The mother liquor was analysed for the presence of neutral (sugars) and acid products

The elution of the basic products adsorbed on the cationic resin was carried out with increasing concentrations of hydrochloric acid (1, 2, 4 N) or ammonia The solvents were evaporated and the dry residues were dissolved in 50 per cent methanol The antibiotics neomycin, kanamycin and monomycin, as well as their nitrogenous decompositon products, are completely adsorbed on the resin and cannot be detected in the mother liquor On the other hand, actinoidin is not adsorbed by this type of resin and remains in the solution, but in the course of hydrolysis the nitrogenous products of decomposition are adsorbed by the resin In the latter case the course of hydrolysis was followed by measuring the decrease with time of the optical density at 280 mμ of the liquid filtered from the resin

The 'second component' of monomycin, consisting of diaminosugar and ribose, was originally obtained by methanolysis of the antibiotic in absolute methanol saturated with 0 5 N hydrochloric acid The hydrolysis of this component was followed by measuring the Bial reaction in the mother liquor and in the eluate from the resin Humins were not observed in the mother liquor or in the eluates

Observations on the hydrolysis of some antibiotics are presented in Table 1 The products of degradation of neomycin and monomycin after hydrolysis on the resin for 4 h are closely related to the products of methanolysis of these antibiotics The only difference is that the 'second component' derived from monomycin and the neobiosamine derived from neomycin possess free glucosidic hydroxyl groups after hydrolysis, whereas these groups are easily methylated during methanolysis When the 'second component' obtained by methanolysis of monomycin is hydrolysed with the aid of a cationic resin, the removal of the methyl group occurs in 1 5 h The complete splitting of this product into ribose and diaminohexose takes place in 12 h When actinoidin is hydrolysed under similar conditions, the mannose and glucose residues in this antibiotic can be detected in the mother liquor This mother liquor also contains an acidic phenolic component absorbing at 280 mμ, which had not hitherto been observed in acid hydrolysates of actinoidin

M G BRAZHNIKOVA
M K KUDINOVA

Institute of Antibiotics,
Academy of Medical Sciences,
Moscow, U S S R

---

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Purification and Analysis of Human Saliva Lysozyme

JOLLÈS[1,2] has previously demonstrated that lysozymes isolated from different organs of the same animal (dog spleen and kidney; hen egg-white and lung) have qualitatively the same biological activity but differ by their amino-acid compositions and their chromatographic behaviour

This work is part of a comparative study devoted to lysozymes of human origin The purification of human milk lysozyme[3] and the purification and analysis of human placenta lysozyme[4] have already been reported We describe here the purification and the analysis of human female saliva lysozyme

Lysozyme activity was determined by its bacteriolytic action on *Micrococcus lysodeikticus* and expressed as hen's egg-white lysozyme The proteins were titrated with the Folin–Ciocalteu reagent and also expressed as hen's egg white lysozyme after comparison with a standard solution containing lysozyme from Armour (Kankakee, Illinois) The amino-acids have been determined after total hydrolysis (6 N hydrochloric acid, 110°, 24 or 48 h, sealed tube) with a Technicon autoanalyser according to the method of Piez and Morris[5] The cystine and methionine contents have been determined on a performic acid oxidized sample Tryptophan has been determined according to Spies and Chambers[6]

Saliva was collected from women during the day and diluted each evening with water (1 4, v/v) The solution is adjusted to *p*H 4 5 with acetic acid and heated during 2 min at 100° C As with all previously studied lysozymes, saliva lysozyme resists to this treatment, contrary to a great number of other enzymes, and remains in solution Many impurities precipitate and can be eliminated by centrifugation The saliva is then stored at 4° until needed

The purification includes three steps, according to the procedure described by Jollès *et al* [7] (1) Adsorption of the lysozyme on 'Amberlite *CG*–50' buffered with a 0 2 M phosphate buffer of *p*H 6 5 20 ml of 'Amberlite' are added to 1,000 ml of diluted saliva The mixture is stirred overnight The resin is then put into a column and washed with water The lysozyme is eluted with a

0 8 M phosphate buffer of *p*H 6 5 After dialysis, against water at 2° C, a 'primary material' is obtained, its specific activity is 0·3 It should be kept in mind that the specific activity of hen's egg-white lysozyme is 1.

(2) Chromatography of the 'primary material' on carboxymethyl-cellulose (CM-cellulose) The 'primary material' corresponding to 200 ml of undiluted saliva is charged at *p*H 4 5 on a CM-cellulose column (70 × 1 6 cm) equilibrated with a 0 03 M phosphate buffer of *p*H 5 5 Gradient elution is begun after 100 ml by allowing successively a 0 03 M (600 ml), a 0 1 M (600 ml) and a 0 2 M phosphate buffer at *p*H 7 2 to flow into the buffer at *p*H 5 5 in a mixing chamber with a capacity of 250 ml The lysozyme is eluted between the 80th and 140th 10-ml fractions of the third gradient, the active fractions have a specific activity of around 2 5 The purified lysozyme is concentrated by adsorption on a few ml of 'Amberlite *CG*–50' and elution, as described here, it is then dialysed against a 0 2 M phosphate buffer of *p*H 6 98

(3) Ion-exchange chromatography on 'Amberlite *CG*–50' This chromatography is carried out on 16 × 1 1 cm columns of 'Amberlite *CG*–50', Type II, equilibrated with a 0 2 M phosphate buffer of *p*H 6 98

Lysozyme content of saliva In order to avoid some errors which might arise from the presence of activators or inhibitors in the saliva, this content has been determined after obtaining the 'primary material' Human saliva (from women) contains 150–200 μg lysozyme/ml (quantity expressed in hen's egg-white lysozyme)

Chromatography on CM-cellulose The lysozyme was obtained with a yield of 60 per cent and has undergone approximately a 10-fold purification

Ion-exchange chromatography It was carried out as indicated in Fig 1 without any change in the *p*H, the molarity or the temperature of the buffer Saliva lysozyme was eluted after 48 ml with a yield of 90 per cent, the specific activity inside the peak remaining constant (3 ± 0 5) Just before this main peak a very small one is eluted, its specific activity is lower It has not been submitted to amino-acid analyses

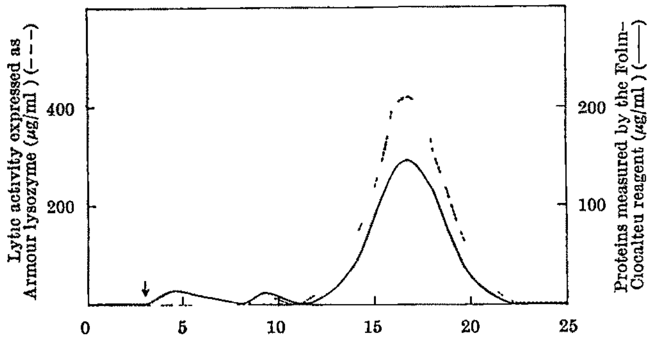

Fig 1 Ion-exchange chromatography on 'Amberlite *CG*-50' of human saliva lysozyme, 0-2 M phosphate buffer of *p*H 6 98

Amino-acid composition Table 1 indicates the amino-acid composition the preferred integer is derived from analyses carried out after 24 or 48 h hydrolysis on samples submitted or not to a performic acid oxidation With three different lots we have obtained the same results

Table 1 AMINO-ACID COMPOSITION OF HUMAN SALIVA LYSOZYME

Amino-acids	*r*	Amino-acids	*r*
Gly	13 ± 1	Ileu	4
Ala	12 ± 1	Phe	2
Ser	6 ± 1	Tyr	5
(Cys-)$_2$	6	Try	5 ± 1
Met	1	Asp	18 ± 1
Thr	6 ± 1	Glu	9 ± 1
Pro	3	Lys	4–5
Val	7	Arg	11 ± 1
Leu	8	His	1

r, Residues/mol

Molecular weight From the amino-acid composition it was possible to calculate a minimum molecular weight of around 14,000 Some experiments made with calibrated 'Cellophane' membranes established that this is the real molecular weight

Conclusion The new lytic enzyme is a lysozyme, as it possesses the principal properties of this group[2,8] it is a basic protein, with a low molecular weight (around 15,000), stable at acid *p*H, unstable at alkaline *p*H, active against suspensions of *M lysodeikticus* It has been obtained in a chromatographically pure state, after an ion-exchange chromatography The specific activity and the amino-acid analyses are constant from one lot to another

From our comparative studies, we can conclude that human placenta lysozyme[4] has the same chromatographic behaviour, the same specific activity and a quite comparable amino-acid composition (established after 18 and 24 h hydrolysis only) as human saliva lysozyme A comparison of the tryptic units will allow us to establish if the two lysozymes are identical or not Preliminary experiments have already shown that human milk contains in fact two lysozymes, one of which seems to be different from the two mentioned here[9]

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J F PETIT
P JOLLÈS

Laboratory of Biochemistry,
Faculty of Science,
96 Bd Raspail,
Paris

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Citrate as an Intermediary in the Inhibition of Phosphofructokinase in Rat Heart Muscle by Fatty Acids, Ketone Bodies, Pyruvate, Diabetes and Starvation

IN perfused rat heart, glycolysis is accelerated by anoxia and uncouplers of respiratory chain phosphorylation and inhibited by the respiration of fatty acids, ketone bodies and pyruvate[1–4] Measurements of the concentrations of hexose phosphates have led to the suggestion that these agents change the rate of glycolysis by altering the rate of the phosphofructokinase step[1–3] The rates of glycolysis and of the phosphofructokinase reaction are also diminished in hearts from alloxan diabetic or starved rats in which the release of fatty acids from glycerides for oxidation is enhanced[4,5] In extracts of guinea pig heart[6] and of rabbit skeletal muscle[7] the activity of phosphofructokinase is inhibited by increasing concentrations of ATP and stimulated by increasing concentrations of 5′ AMP, cyclic 3′,5′ AMP, ADP and inorganic phosphate These observations suggest that anoxia and uncouplers of respiratory chain phosphorylation increase the rate of the phosphofructokinase reaction in muscle (and hence of glycolysis) by increasing the concentrations of 5′ AMP, ADP and inorganic phosphate and diminishing that of ATP

On the other hand, when phosphofructokinase in rat heart is inhibited by the respiration of fatty acids, ketone bodies and pyruvate or by diabetes or starvation there is no increase in the concentration of ATP and no consistent decrease in that of 5′ AMP, ADP or phosphate This has led to the suggestion[4] that some intermediate in the respiration of fatty acids, ketone bodies and pyruvate might inhibit phosphofructokinase The metabolites which we have considered in this connexion are long-chain fatty acyl coenzyme A, acetyl coenzyme A and the acids of the citrate cycle

The concentration of fatty acyl coenzyme A in perfused rat heart has been assayed as coenzyme A (ref 8) after extraction and separation from the frozen and powdered muscle[9] In diabetes or starvation or after perfusion with 0 75 mM palmitate complexed with 2 per cent bovine plasma albumin the concentration of fatty acyl coenzyme A was increased 40–70 per cent (from control values of 2 4 to 5 0 μmoles/100 g) In hearts perfused with pyruvate (60 mg/100 ml) or DL β hydroxybutyrate (70 mg/100 ml) the concentration of fatty acyl coenzyme A was not increased It seemed unlikely, therefore, that these factors inhibited phosphofructokinase by raising the intracellular concentration of fatty acyl coenzyme A The concentration of acetyl coenzyme A in perfused rat heart was found to be too low for enzymatic assay[10], but acetyl coenzyme A at concentrations between 0 08 and 1 07 mM did not inhibit phosphofructokinase in extracts of rat heart Citrate, on the other hand, in the range of concentrations found in rat heart inhibited phosphofructokinase in extracts of the muscle Moreover, its concentration in perfused heart was increased by all the factors which are thought on the basis of measurements of hexose phosphate concentrations to inhibit the enzyme in the perfused heart These observations, which suggest that the inhibitory effects of fatty acids, ketone bodies, pyruvate, diabetes and starvation on phosphofructokinase in the intact muscle are mediated through an increase in the intracellular concentration of citrate, are briefly summarized

Rat hearts were perfused, frozen and powdered as described elsewhere[1,3] For assay of citrate the frozen powder was extracted with 0 5 M perchloric acid (3 ml /g), neutralized with 60 per cent potassium hydroxide, treated with '*IR* 120' (*H* form) to remove glutathione and neutralized again with 20 per cent potassium hydroxide Citrate was assayed enzymatically by the change in extinction at 340 mμ in a Beckman spectrophotometer on adding 10–20 units[11] of aconitase[12] (purified to the first ethanol precipitation) to a mixture of 0 5 ml muscle extract and 0 7 ml assay medium (70 mM, *tris* hydrochloric acid, 0 7 mM, EDTA, 2 mM, MnSO$_4$, 0 3 mM, NADP, pH 7 4) after removing isocitrate and glucose and fructose 6-phosphates with isocitrate dehydrogenase, glucose 6-phosphate dehydrogenase and phosphoglucoisomerase Citrate was re-assayed in some extracts with citrate lyase[12] (*EC* 4 1 3 6), with essentially similar results The concentration of citrate was assayed in hearts from fed rats perfused for 15 min with medium containing glucose (1 mg/ml) and insulin (0 1 unit/ml) and calculated as μmoles citrate/ml intracellular water The concentration of citrate was increased by starvation and alloxan diabetes from 0 47 ± 0 03 to 0 69 ± 0 08 and 2 38 ± 0 11 respectively, by 0 75 mM palmitate carried by 2 per cent bovine plasma albumin from 0 48 ± 0 08 to 1 64 ± 0 15, by pyruvate (60 mg/100 ml) from 0 67 ± 0 07 to 2 38 ± 0 24, by β-hydroxybutyrate (70 mg/100 ml) from 0 67 ± 0 07 to 1 40 ± 0 16 (mean ± *S E*) for six hearts in each group

For assay of phosphofructokinase in extracts hearts from fed normal rats were perfused anaerobically with glucose-free medium for 15 min (to remove glycogen) and extracted in an all-glass hand-operated Potter–Elvehjem homogenizer with 5 ml assay medium (see later) containing 2 5 mM ATP Phosphofructokinase was assayed in a recording fluorimeter by the disappearance of NADH, the fructose 1 6-diphosphate formed being converted to glycerol 1-phosphate The assay medium was that described by Passonneau and Lowry[7] with 2 5 mM, ATP, 0 05 mM, NADH, 30 μg/ml aldolase and 30 μg/ml glycerol phosphate dehydrogenase (if ammonium sulphate suspensions of enzymes are used they should be limited in volume to a total of 0 6 ml /100 ml as ammonium sulphate can block citrate inhibition) Rotenone (0 7 μg/ml) was added to inhibit NADH oxidase activity After steady-state conditions were attained with 10–20 μl muscle extract and 3 ml assay medium, the reaction was started by addition of 20 μl of 0 15 mM (glucose + fructose 6-phosphates) containing 10 μg/ml phosphoglucose isomerase The effect of various substances on the activity of phosphofructokinase was ascertained by the change in rate on their addition to the complete reaction mixture Control experiments showed that substances which altered the rate of conversion of fructose 6-phosphate to glycerolphosphate did not change the rate of conversion of fructose 1 6 diphosphate (7 μM), that is, that their influence was on phosphofructokinase

The activity of phosphofructokinase in extracts of rat heart was 140 μmoles fructose 1 6 diphosphate formed/g of wet heart/h (23 observations) In each of 10 experiments citrate (mM) reduced the rate to an average of 23 per cent of the control Definite inhibition (80 per cent control) was seen with as little as 0 2 mM citrate and the effect increased between 0 2 and 1 mM Citrate inhibition was not influenced by an equimolar amount of Mg^{2+} No inhibition was seen with (mM) *cis*-aconitate, L_s isocitrate, α-oxoglutarate, succinate, fumarate L-malate, tricarballylic acid, acetyl coenzyme A or coenzyme A The activity of phosphofructokinase was enhanced by 5′ AMP (0 17 mM), cyclic 3′,5′ AMP (0 17 mM), ADP (0 5 mM), inorganic phosphate (4 or 8 mM) and arsenate (8 mM), both in the presence and absence of mM citrate Inorganic phosphate, for example, increased the rate ten-fold at 8 mM Rat heart muscle phosphofructokinase is thus activated by those substances which activate phosphofructokinase from guinea pig heart or rabbit skeletal muscle[6,7] It is also inhibited by citrate Inhibition of brain phosphofructokinase by citrate has been observed by Passonneau and Lowry (personal communication)

These observations suggest that respiratory substrates and diabetes or starvation inhibit phosphofructokinase through an increase in the intracellular concentration of citrate The latter may be due to increased synthesis of citrate through the provision of additional acetyl CoA Other possibilities are alterations in mitochondrial permeability to citrate or in the utilization of citrate either in mitochondria or cell sap

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P B GARLAND
P J RANDLE
E A NEWSHOLME*

Department of Biochemistry,
University of Cambridge

* Present address Department of Biochemistry, University of Oxford

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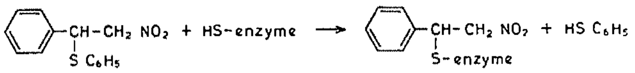
Antifungal Activity of Some 2-Nitro-1-phenyl-1-phenylthioalkanes

In view of the publication by Wain *et al* [1] on the fungicidal properties of some thiocarbamoylthionitroparaffins, we wish to describe the antifungal properties of some 2-nitro-1-phenyl-1-phenylthioalkanes and related compounds

The antifungal activity of αβ-unsaturated carbonyl compounds[2,3] is believed to be due to their ability to combine chemically with the thiol group of many enzymes and coenzymes, thereby causing their deactivation, and it seems likely that the fungitoxicity of β-nitrostyrenes[4–6] arises for similar reasons This is supported by the fact that those compounds possessing the double-bond most reactive towards nucleophilic reagents (for example, thiols) are the most powerful antifungal agents, namely, the β-nitrostyrenes[3,7,8] (Table 1) The compounds obtained by adding benzenethiols to αβ-unsaturated carbonyl compounds and β-nitrostyrenes might therefore be expected to have markedly diminished biological activity, Table 1 reveals that this is not necessarily so Substantially inactive thiol-addition products (type *B*) are apparently obtained only from compounds of type *A* possessing high antifungal activity and highly reactive double bonds (for example, compounds 2, 4, 7 and 9)

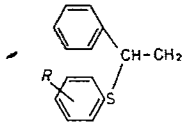
In view of this, and the fact that the parent sulphide, 1-phenyl-1-phenylthioethane, is inactive against all four test fungi at 1,000 p p m , it is tempting to suggest that the fungitoxicity of the 2-nitro-1-phenyl-1-phenylthioalkanes may be due to their reacting with the thiol groups of enzymes through exchange reactions



Such exchanges are less likely to occur with the compounds of type B in which the benzenethiol has been added to a highly reactive double-bond (compounds 2, 4, 7 and 9) In these cases, low fungitoxicity would be expected

The present hypothesis also suggests that 2-nitro-1-phenyl-1-phenyl-thioalkanes containing electron-attracting groups attached to the phenylthio moiety should exhibit the greatest fungitoxicity, as the foregoing exchange reaction will be facilitated This is supported by the biological results in Table 2, where compound 15 contains a 4-nitro-group The reverse effect is demonstrated by compound 17, which contains the electron-repelling methoxyl group

Table 2 EFFECT OF SUBSTITUENTS ON THE ANTIFUNGAL ACTIVITY OF SOME 2-NITRO-1-PHENYL-1-PHENYLTHIOETHANES



Compound No	*R*	Spore germination test (approx *LD*$_{50}$ in p p m)			
		B C	*S F*	*C F*	*V I*
5	H	500	20	10	20
11	4-F	35	20	20	10
12	4-Cl	10	35	35	10
13	4-Br	35	35	10	5
14	4-I	500	200	10	20
15	4-NO_2	10	10	10	3
16	4-CH_3	500	500	10	35
17	4-CH_3O	500	500	500	500
18	2,5-diCl	150	35	10	20
19	2,4,6-triCl	150	20	10	20

Anomalies in the biological results make it clear, however, that many more factors[9] must be considered in attempting to formulate any antifungal mechanisms

N G CLARK
A F HAMS
B E LEGGETTER

Research Department,
Boots Pure Drug Co , Ltd
Nottingham

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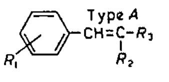
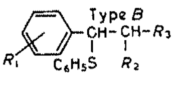
Table 1 ANTIFUNGAL ACTIVITY OF SOME ETHYLENIC COMPOUNDS AND THEIR BENZENETHIOL ADDUCTS

Compound* No	Substituents			Spore germination test (approx *LD*$_{50}$ in p p m)							
											
	R$_1$	*R*$_2$	*R*$_3$	*B C*	*S F*	*C F*	*V I*	*B C*	*S F*	*C F*	*V I*
1	H	—CO CH_3	—COOEt	6	20	3	50	500	15	10	150
2	H	—CO CH_3	—CO CH_3	50	20	6	12	500	500	500	500
3	3-NO_2	—CO CH_3	—CO CH_3	300	10	10	30	500	10	20	35
4	3-NO_2	—COOEt	—COOEt	250	10	10	2	500	500	500	500
5	H	H	NO_2	150	250	250	50	500	20	10	20
6	H	CH_3	NO_2	300	300	12	50	500	350	150	200
7	H	C_6H_5	NO_2	3	1	3	3	500	350	150	350
8	H	Cl	NO_2	150	35	350	150	150	20	20	10
9	4-NO_2	H	NO_2	10	35	150	10	500	500	500	250
10	4-NO_2	—COOEt	NO_2	50	50	300	50	500	50	50	50

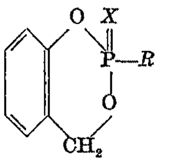
* Satisfactory analytical figures have been obtained for all new compounds
B C , *Botrytis cinerea* , *C F* , *Cladosporium fulvum* , *S F* , *Sclerotinia fructigena* , *V I* , *Venturia inaequalis*

Saligenin Cyclic Methyl Phosphate and its Thiono Analogue: New Insecticides related to the Active Metabolite of Tri-o-cresyl Phosphate

SALIGENIN cyclic phosphate was discovered as the active metabolite of tri-*o*-cresyl phosphate (TOCP) (ref 1 and 2) Analogous cyclic phosphorus esters were synthetically prepared for examination of their chemical properties[3,4] and biological activities[5,6] The biological activity is not always coincidental with the chemical reactivity and appears to be influenced by the size of a substituent on phosphorus being outside the hetero-ring of the cyclic esters The TOCP-metabolite causes ataxia in hens but has no insecticidal activity, while its analogous cyclic phosphates carrying a small alkyl group appear to have insecticidal activity. The following two esters are new promising insecticides in this unique series.

2-Methoxy-4*H*-1,3,2-benzodioxaphosphoran-2-one (salioxon) was prepared according to a described procedure[3] from saligenin and methyl phosphorodichloridate by the action of tertiary amine. Pyridine was better than triethylamine as the base. The product was a colourless oil, b.p. 110°–112° C (0.05 mm mercury), $n_D^{25°} = 1.5155$. Its thiono analogue (salithion) was not obtained in good yield by a similar procedure, but was by more drastic condition such as heating (80°–90°) the mixture of saligenin and methyl phosphorodichloridothionate in toluene for 15–20 h in the presence of potassium carbonate and copper powder. The thiono analogue consists of colourless needles (from methanol), m.p. 51°–53° C. Cyclic structure of these compounds was supported by infra-red-spectra, aminoantipyrine test[3] and determination of molecular weight.

Table 1. APPROXIMATE LD_{50} VALUES TO MOUSE AND HOUSEFLY OF NEW INSECTICIDES COMPARED WITH THEIR ANALOGOUS COMPOUNDS AND KNOWN INSECTICIDES



Compounds	*R*	*X*	LD_{50} Mouse*	LD_{50} Housefly†
Salioxon	OMe	O	52	0.035
Salithion	OMe	S	88	0.05
Ethyl salioxon	OEt	O	76	0.33
Propyl salioxon	OPr	O	—	7.1
Butyl salioxon	OBu	O	—	> 10 (40%)
Phenyl salioxon	OPh	O	—	> 10 (3%)
TOCP-metabolite *M*-1	OPh-2Me	O	140 (ref. 6)	> 10 (0%)
Phenylphosphonate analogue	Ph	O	—	> 10 (0%)
Parathion			5 (ref. 7)	0.04
Malathion			347 (ref. 7)	0.6

* Oral administration with addition of emulsifier, mg/kg, 24 h.
† Topical application of acetone solution, γ/female, 24 h.

These methyl esters have strong insecticidal activity comparable with parathion and less toxicity to mammals than the latter as shown in Table 1. They are also effective with some other insects such as the American cockroach, rice stem-borer and green rice leafhopper[7]. Another outstanding character of salioxon is its extremely rapid insecticidal action. It is interesting to note that these insecticidal cyclic phosphorus esters do not have any strong acidic group such as parathion, etc., have.

Analogous esters having a bulky substituent such as aryl group or big alkyl group are not insecticidal. This appears to be due to the differences in affinity for insect enzymes and also in penetration. Non-insecticidal phenyl derivative (R = OPh, X = O) is a strong inhibitor for housefly trunk aliesterase, but not for fly head cholinesterase. Another non-insecticidal phenylphosphonate (R = Ph, X = O) is a strong inhibitor of both enzymes *in vitro* but not *in vivo*. Salioxon inhibited both enzymes *in vivo* as well as *in vitro*.

MORIFUSA ETO
YOSHIRO KINOSHITA
TAKESHI KATO*
YASUYOSHI OSHIMA

Laboratory of Pesticide Chemistry,
Department of Agricultural Chemistry,
Kyushu University, Fukuoka, Japan.

* Research Fellow from Mikasa Chem. Co., Amagi.

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Relationship between Consistency of Wheat Grain and Flour Diastatic Activity

THERE are annually harvested in Australia more than 100 varieties of wheat, one of the chief reasons for this large number lying in the complexity of soil-type and climate encountered throughout the wheat-belt. It will be appreciated that a considerable diversity of quality types are represented by these varieties and in 1956 a phenomenon referred to as 'over-stability', characteristic of certain extremely vitreous material, received much attention. Flours obtained from these varieties were found to contain a high percentage of damaged starch, arising out of the increased roll-pressure it was necessary to exert on the grain in order to obtain a satisfactory flour yield. Consequently the diastatic activity of these flours was considerably augmented, and the increased dextrinization associated with this high diastatic activity imparted stickiness to the fermenting dough, the resultant loaves being dense, of low volume and poor, waxy texture. In order to ascertain which of the varieties being grown possessed this characteristic and were thus unsuitable for cultivation, a great number of varieties were tested for grain consistency and diastatic activity, and the relationship revealed between these two characteristics forms the subject of this communication.

Flour of each variety was obtained by milling 1 kg of tempered wheat in a Buhler automatic laboratory mill. Flour diastatic activity was measured by a modification of the Blish and Sandstedt procedure[1]. Grain consistency was measured by a particle size index test perfected by Symes[2] and since improved by the introduction of a *Ro*-tap sieve shaker. Ten grammes of grain are ground in a Labconco mill set at its finest setting, and the meal sieved

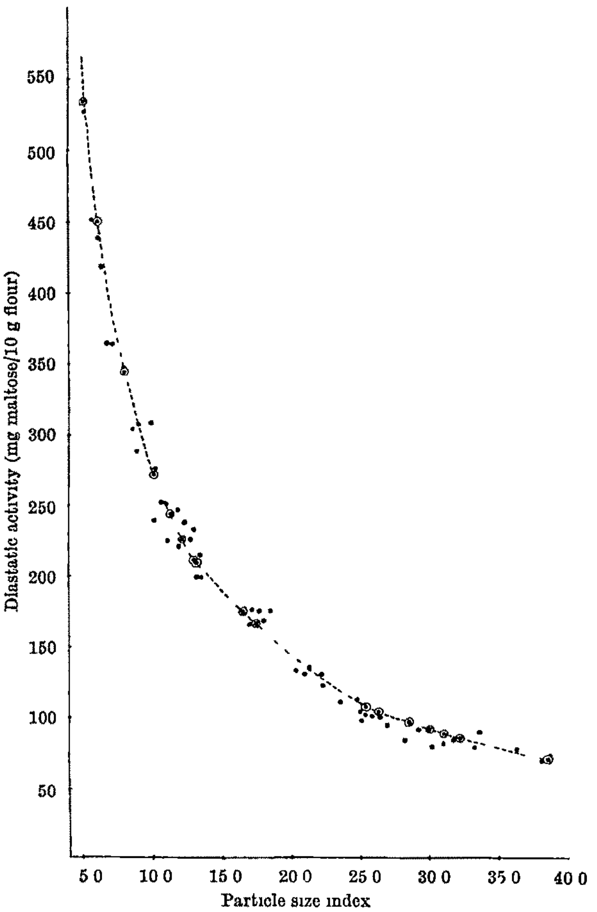

Fig. 1. Relationship between particle size index and flour diastatic activity. γ = 0.992, log Y = 3.441 − 1.004 log X. ○, Predicted flour diastatic activity; ●, observed flour diastatic activity.

Table 1 RELATIONSHIP BETWEEN OBSERVED FLOUR DIASTATIC ACTIVITY AND FLOUR DIASTATIC ACTIVITY PREDICTED FROM A KNOWLEDGE OF GRAIN CONSISTENCY, AS MEASURED BY PARTICLE SIZE INDEX SYSTEM

Variety	Particle size index	Observed diastatic activity (mg maltose/ 10 g flour)	Predicted diastatic activity (mg maltose/ 10 g flour)
Dural (*T durum*)	5 1	526	534
KBFB × *P_4* × Baringa (*T vulgare*)	6 1	440	450
Baringa	8 0	348	344
Curlew	10 1	276	272
Falcon	11 3	244	244
Festival	12 1	228	226
*M*993 (Bungulla × Gabo)	13 0	233	211
Gabo	13 1	214	209
Spica	16 6	175	175
Javelin 48	25 4	104	108
Bordan	26 3	101	104
Heron	28 5	89	96
Olympic	30 0	88	91
Quadrat	31 0	81	88
Sherpa	32 2	88	85
Sherpa	38 5	71	71

for 10 min on a 200-mesh wire sieve, the percentage of throughs being recorded as the particle size index *Durum* wheats have an index of 5, while a figure of 40 has been recorded for certain soft biscuit-type varieties

A close logarithmic relationship was found to exist between flour diastatic activity and grain consistency as measured by the particle size index system (Fig 1) By means of this relationship it was possible to predict the flour diastatic activity with some degree of accuracy from a knowledge of the particle size index of the grain (Table 1) From the range of varieties which were classified in this way, 12 varieties, chosen to cover the full range of diastatic activity encountered, were grown for 2 seasons in a series of 10 replicated trials throughout the Australian wheat-belt, in order to test the effect of environmental fluctuations on the classification Preliminary results from these trials have indicated that the logarithmic relationship referred to here is maintained under a variety of edaphic and climatic conditions

P. C WILLIAMS

Department of Agriculture,
Agricultural Research Institute,
Wagga Wagga,
New South Wales

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Effect of Vitamin B_6 and its Analogues on the Swelling of Rat Liver Mitochondria

IT has been observed[1,2] that substances like thyroxine, Ca^{++}, orthophosphate, induce swelling in rat liver mitochondria when suspended in a medium containing 0 3 M sucrose, and 0 02 M *tris*(hydroxymethyl)aminomethane, at *p*H 7 4 That the swelling of mitochondria is dependent on the respiratory chain is shown by the fact that 2,4-dinitrophenol, which is an uncoupler of oxidative phosphorylation, is a strong inhibitor of mitochondrial swelling[1]. The swelling of mitochondria is associated with the active water uptake, which was first pointed out by Raaflaub[3] The active water uptake is also inhibited by adenine triphosphate[4] Other substances like spermine, spermidine, and related polyamine also inhibit swelling[5] It has also been observed that many antihistamine drugs inhibit mitochondrial swelling[6] where permeability change has been suggested It is known that pyridoxal phosphate intensifies the uptake of amino-acids by Ehrlich ascites tumour cells[7] with the concomitant uptake of water A study of the effect of pyridoxal phosphate and other analogues of vitamin B_6 on the active water uptake by rat liver mitochondria will be of some interest

In the present investigation the rat liver mitochondria was isolated by usual methods, and swelling was studied in a medium containing 0 3 M sucrose and 0 02 M *tris*-(hydroxymethyl)aminomethane at *p*H 7 4 by observing the optical density at 520 mμ according to the method[8] published previously The results are shown in Table 1 Of all the various analogues of vitamin B_6 tested, pyridoxal phosphate and 4-deoxypyridoxine inhibit the swelling to the extent of 32 per cent and 75 per cent respectively when present in 2 mM-levels The rest of the compounds are inactive When 4-deoxypyridoxine and pyridoxal phosphate are present together at 1 mM-level, they prevent swelling completely These two compounds together cannot prevent the swelling of rat liver mitochondria caused by thyroxine When other analogues of vitamin B_6 are present along with 4-deoxypyridoxine they do not show any synergistic effect The inhibition of mitochondrial swelling by 4-deoxypyridoxine has similarity to the action of 2,4-dinitrophenol in that it releases the adenosine triphosphatase activity of the mitochondria to about four-fold that of the blank when present in equimolecular level, but on the contrary when 4-deoxypyridoxine and pyridoxal phosphate are present together it is inactive in releasing the adenosine triphosphatase activity *in vitro* of the mitochondria The other compounds are inactive in releasing the adenosine triphosphatase from rat liver mitochondria

Table 1 SWELLING OF RAT LIVER MITOCHONDRIA *in vitro* IN PRESENCE OF VITAMIN B_6 ANALOGUES THE SWELLING OF MITOCHONDRIA IN 0 3 M SUCROSE 0 02 M *tris* MEDIUM AT 15 MIN IS TAKEN AS 100

Compound	Concentration	Relative swelling
Pyridoxal	1 mM	103
Pyridoxine	1 mM	98
Pyridoxamine	2 mM	100
PMP*	1 mM	92
PLP†	1 mM	80
PLP	2 mM	68
DP‡	1 mM	53
DP	2 mM	25
DP + Pyridoxine	1 mM + 1 mM	80
DP + Pyridoxal	1 mM + 1 mM	88
DP + Pyridoxamine	1 mM + 1 mM	56
DP + PMP	1 mM + 1 mM	80
DP + PLP	1 mM + 1 mM	0

* Pyridoxamine phosphate † Pyridoxal phosphate, ‡ 4-Deoxypyridoxine

PRABHAT RANJAN PAL

Department of Biochemistry,
Indian Statistical Institute,
Calcutta, 35

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PHYSIOLOGY

Relationship between Venous Pressure and Cortical Blood Flow

IN man, acute occlusion of the superior vena cava results in complete obstruction to cerebral venous drainage, with gross changes in the electroencephalogram In the dog, however, complete constriction of the superior vena cava results in a rise in cerebral venous pressure, but no significant changes in the EEG[1] (Fig 1) Venous pressure is compounded of a number of variables[2] and a number of pressure-flow relations have been demonstrated in distensible vascular beds[3]

A veni-vasomotor reflex has been postulated for the finger[4] A fall in flow was observed following distension of the venous bed A similar mechanism may result in cerebral ischæmia following increases in intracranial venous pressure with coughing and straining It is, however, more probable that an alternative mechanism is present tending to stabilize cerebral blood flow despite fluctuations in venous pressure This communication supports this possibility

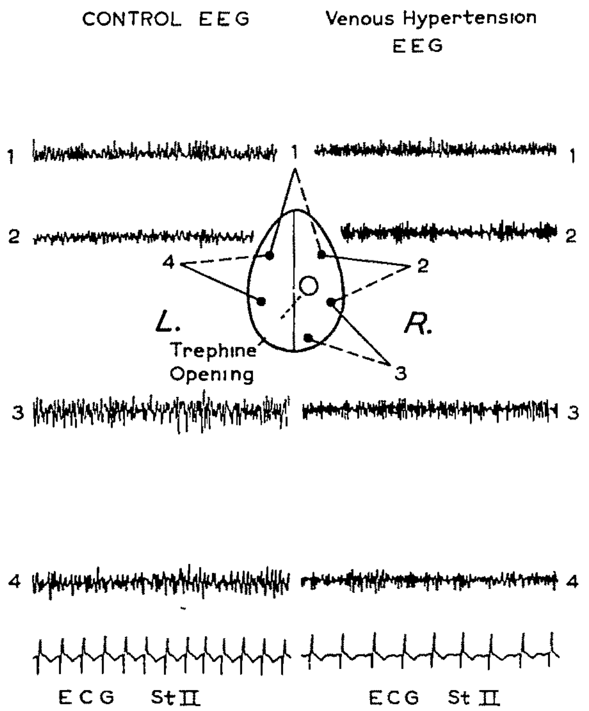

Fig. 1. The EEG before and after venous obstruction. There is no significant difference in the recordings. There was a slowing of the heart rate in this instance after constriction of the superior vena cava.

Table 1

Exp	Remarks	B P	V P	Art pCO_2	C B F	C B F″	Time
1		125	5	31.5	0.63	0.88	0
	S V C +	125	20	32.0	0.70	0.93	30
	S V C +	115	38	31.5	0.86	1.04	55
	S V C +	100	32	28.5	0.90	1.12	88
	S V C +	130	—	34.5	0.73	0.80	115
2		165	16.5	41.0	0.76	0.75	0
	S V C +	150	24	39.0	0.78	0.79	10
	S V C +	115	48	34.0	0.92	1.03	20
	S V C +	105	52	33.0	0.85	0.97	30
3		135	7	36.0	1.14	1.28	0
		145	6	36.0	1.00	1.12	30
	S V C +	145	23.5	34.0	1.57	1.76	55
	S V C +	145	17.5	36.5	1.71	1.74	75
	S V C +	140	12	37.0	1.11	1.13	95
		150	5	38.0	1.26	1.29	110
4		170	8	33.0	0.69	0.78	0
		175	7	34.0	0.68	0.76	10
	S V C +	170	15	31.5	0.96	1.11	60
		185	5	36.0	0.92	0.99	67
	S V C +	175	17	30.0	0.86	1.03	98
	Acrylic	180	7	35.5	0.52	0.56	113
	Acrylic	185	7	36.5	0.65	0.69	143
	Acrylic	190	8	33.5	0.71	0.79	158
	S V C +	180	18	34.0	1.08	1.21	173

Table 2

Exp	Remarks	B P	V P	Art pCO_2	C B F.	C B F″	Time
5		170	10	34.0	0.76	0.85	0
		170	10	33.0	0.86	1.00	35
		160	9	31.5	0.72	0.83	95
	S V C +	165	14	31.0	0.79	0.93	105
	S V C +	165	15	34.5	0.86	0.94	125
	S V C +	160	14	32.0	0.85	0.99	147
		165	7	32.5	0.69	0.79	182
		165	8	34.5	0.78	0.86	190
6		165	10	37.5	0.63	0.65	0
		160	10	41.0	0.70	0.69	15
	S V C + V A.	150	26	42.0	0.85	0.81	40
	S V C + V A	140	21	46.0	0.79	0.70	85
	S V C + V A	120	20	46.0	0.73	0.62	135
7		140	7	40.0	0.63	0.63	0
		155	8	39.0	0.57	0.58	40
	S V C +	155	17	45.0	0.71	0.64	75
	S V C +	150	16	38.0	0.67	0.69	95
	S V C +	155	17	36.0	0.70	0.75	115
	S V C + V A	135	32	36.0	0.47	0.51	135

Tables 1 and 2 show the overall experimental results. S.V.C. + indicates constriction of the superior vena cava; B.P., blood pressure mm mercury; V.P., sagittal sinus venous pressure cm water; Art. pCO_2, arterial carbon dioxide tension, mm mercury; C.B.F., cortical blood flow, ml./gm/min.; C.B.F.″, cortical blood flow normalized to a pCO_2 of 40 mm mercury.

Cortical blood flow (ml./g/min.) was measured in seven dogs with the krypton-85 clearance method of Lassen and Ingvar[5]. Anæsthesia was induced with thiopentone and maintained with nitrous oxide. Intermittent suxamethonium was administered to allow controlled ventilation. Arterial blood pressure was measured (mm mercury) and samples were obtained from a femoral artery cannula. Venous pressure (cm water) was measured from a cannula mounted in the sagittal sinus. Changes in venous pressure were induced by constriction of the superior vena cava exposed by thoracotomy. Arterial pCO_2 was determined using a micro-Astrup apparatus[6] and maintained within narrow limits in each dog by adjusting the stroke volume of the Starling respiratory pump. EEG activity was recorded on an Offner 8-channel *T.D.* electroencephalogram using stainless steel electrodes in bifrontal, fronto-temporal and fronto-occipital positions.

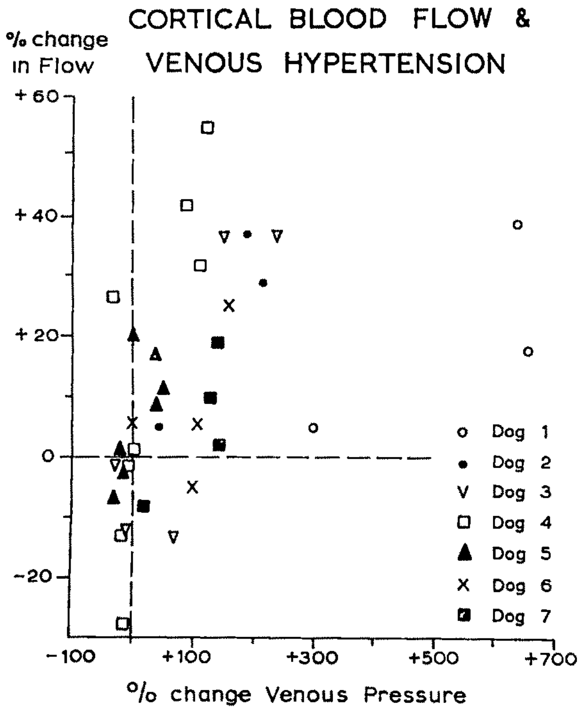

Fig. 2. Illustrates the relationship between the change in flow as a percentage of the initial controls in each dog and the change in venous pressure as a percentage of the initial controls.

After control measurements in three animals, progressive partial constriction of the superior vena cava was applied (dogs 1, 2 and 3). In one animal measurements were made during complete occlusion of the superior vena cava for 42 min and also following release of the constriction (dog 5). A further reduction in cerebral venous return was effected in two dogs by an additional ligation of the vena azygos (dogs 6 and 7). In one animal a closed intracranial system was simulated by sealing the trephine hole and plastic membrane[7] with methyl-methacrylate (dog 4).

The results are expressed in Tables 1 and 2. The control cortical blood flow values show a considerable variation between animals. This is probably attributable to the range in the control arterial pCO_2 levels and the considerable operative trauma required for the preparation. However, in each animal there was an increase in cortical blood flow after venous obstruction. In dog 7, after vena azygos ligation a fall in cortical blood flow occurred, together with a fall in systemic blood pressure and a much greater increment in venous pressure. A smaller increment in flow occurred in dog 6, with combined superior vena cava and vena azygos occlusion compared with the increase in flow in the other animals.

A graphic representation of the relationship between the percentage change in cortical blood flow—adjusted to an arterial pCO of 40 mm mercury—and the percentage change in venous pressure is shown in Fig 2 No simple relationship is evident However, the majority of flow results are within ± 15 per cent of the control values A coefficient of variation of 17 per cent in the mean estimations of several dogs in cortical blood flow has been reported using the krypton-85 technique at a $p\mathrm{CO}_2$ of 40 mm mercury[7] The change in cortical flow persists if venous hypertension is maintained for long periods, but there may be an upper limit of venous pressure after which a fall in flow occurs (dog 7) If a closed intracranial system is reconstituted the response to venous hypertension is unchanged (dog 4) In this animal, however, some loss of cerebro-spinal fluid had occurred prior to the fashioning of the acrylic window The consistent increase in cortical flow in every animal following constriction of the superior vena cava is at variance with reports on finger plethysmography and alterations in venous pressure[4] In this latter investigation, changes in venous pressure were induced by altering tissue tension The influence of tissue tension—cerebro-spinal fluid pressure in this instance—cannot be assessed in those animals using the standard krypton-85 technique[3,7-9] Excision of the dura and replacement with a plastic membrane allow a commensurate loss of cerebro-spinal fluid following alterations in blood flow and venous pressure The preliminary findings after closing the dural window in dog 6 suggest that moderate changes in cerebro-spinal fluid pressure may not alter the response to venous obstruction A provisional conclusion that there is a homeostatic mechanism stabilizing cerebral blood flow despite large fluctuations in venous pressure seems justifiable at this stage

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I JACOBSON
A. M. HARPER
D G McDOWALL

Departments of Surgery,
Royal Infirmary and Western Infirmary,
University of Glasgow

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Active Transport and Transfer of Energy

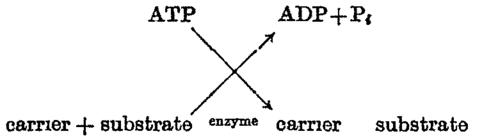
THE transport of ions and molecules across the cell membrane against a concentration gradient is usually defined by the term 'active transport' The word 'active' conveys the idea of active participation of the cells in these transport processes, the energy required being supplied by the metabolic processes within the cells

Certain important features of these processes are: (1) the rate of transport is not a linear function of the difference of concentrations on the two sides of the cell membrane, (2) competition among substances having similar configurations; (3) inhibition by enzyme inhibitors; (4) the temperature coefficients are often of the same order as those of enzyme reactions; (5) the rate of transport may be different even for optical isomers

The mechanisms which have been suggested to explain the active transport processes can be classified into three groups (1) Fixed carrier transport[1]. in this mechanism the carriers present on the cell membrane combine with the molecules or ions on one side of the membrane, then move to the other side and release them there (2) Mobile carrier transport[2,3] in this process the molecules or ions combine with mobile carriers on one side of the membrane, move to the other side and get released there (3) (*a*) Complex formation[4] with macromolecules such as proteins by chelation or electrostatic forces, (*b*) adsorption on colloidal particles

Since active transport processes include the movement of a wide variety of substances under different conditions, it is not unlikely that all these three mechanisms either separately or together are operating in different cases

The active transport processes usually require expenditure of energy The way this energy is utilized depends on the nature of the mechanism involved in any particular process The energy transfers in the two carrier transports are basically the same and are similar to those involved in other synthetic reactions within the cell.



The carriers in the mobile transport processes may be the common metabolites of the cell Due to the anisotropic distribution of enzymes and other reacting constituents on the two sides of the cell membrane, the combination with a metabolite (mobile carrier) on one side and its release on the other side may be more accelerated than the reverse process

The utilization of energy in the active transport processes operated by the mechanism of complex formation with a protein molecule or any other macromolecule may take place in the following ways

(1) Since the anabolism and catabolism of the protein molecules are under the dynamic control of the metabolic processes, the deficiency in the energy-producing processes will affect the steady state of the process

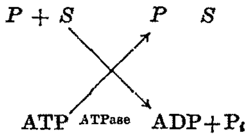
$P + S \rightleftharpoons P\quad S$, where P is a protein molecule and S is a substrate and hence will affect the active transport of S

(2) The enzyme ATPase is necessary for the breakdown of ATP to ADP:

$$\mathrm{ATP}\xrightarrow{\overbrace{[\mathrm{ATPase}]}^{\mathrm{Mg}^{++},\ \mathrm{Na}^{+},\ \mathrm{K}^{+}}}\mathrm{ADP}+\mathrm{P}_i$$

The rate of this reaction depends on the concentration of the complex of ATPase with Mg^{++}, Na^{+} and K^{+} which in turn depends on the concentration of these ions If they are below certain optimum concentrations, variations in their concentrations will greatly affect the rate of utilization of ATP.

(3) The energy may also be utilized by the following coupling processes



Two other phenomena are associated with active transport processes One is the resting potential of the cell and the other is transmission by nerves In the resting state the ions are partly in the bound form and partly in the free state Due to these complex formations there are unequal distributions of the ions on the two sides of the cell membrane The protein and other macromolecules are continuously undergoing repeated catabolism and anabolism At the time of catabolism of the macromolecules, the ions are liberated in the free state and these free ions tend to move to the other side of the cell membrane to attain equilibrium state. However, before the ions can attain the equilibrium state, the macromolecules are again synthesized and start forming complexes with the ions, which, therefore, move across the cell membrane in the reverse direction against the concentration gradient. Thus there is always an unequal distribution of certain ions and hence a potential difference between the two sides of the cell membrane.

Acetylcholine is, in the resting condition, in an inactive and bound form. Its release from the bound state and hydrolysis to choline are intimately connected with the transmission along nerves. The initiator in this process is either a configurational change in the protein molecule binding acetylcholine or liberation of free potassium ions from the bound state. The free potassium ions then accelerate the following process:

$$\text{acetylcholine}\quad\text{protein} \xrightleftharpoons{K^+} \text{acetylcholine} + \text{protein}$$

Acetylcholine esterase is present in conductive tissues throughout the whole animal kingdom. The most important feature of acetylcholine esterase is the high speed at which this enzyme can hydrolyse acetylcholine. The choline molecules formed cross the cell membrane and displace some of the sodium ions from the bound state. The free sodium ions enter the cell and cause liberation of some of the potassium ions from the bound state. The free potassium ions from inside the cell start moving to the outside medium. Also the increased concentration of free potassium ions accelerates the resynthesis of acetylcholine:

$$\text{choline} + \text{acetate} \xrightarrow[\substack{\text{choline acetylase}\\ + \text{ATP}}]{K^+} \text{acetylcholine}$$

Then the reverse processes start, choline coming in, sodium ions going out and potassium ions coming back inside the cell. The incoming and outgoing processes of sodium and potassium ions ultimately lead to the transport of bioelectric potential. There are two different types of movement of sodium and potassium ions, one associated with normal metabolic processes and the other with transmission by nerves. The rapid hydrolysis of acetylcholine by acetylcholine esterase in transmission by nerves makes the movement of sodium and potassium ions associated with this process much faster than that associated with normal metabolic processes. This enables the two processes to become distinct from each other and leads to the propagation of bioelectric potential associated with transmission by nerves.

NIKHIL RANJAN GHOSH

Physical Chemistry Division,
National Chemical Laboratory,
Poona, India.

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Anorexia produced by Direct-current Blockade of the Vagus Nerve

ACCORDING to a hypothesis presented in a previous communication[1] a tonic information from hepatic glucoreceptors might be stimulating the lateral hypothalamic 'feeding centre'. The reduction of this excitatory input by an increase of intracellular glucose (or some related metabolite) in the hepatic cells would inhibit intake of food.

As the vagus nerves are responsible for substantial innervation to the liver[2] it was thought that these nerves might be the main pathways for the afferent discharges of these hepatic glucoreceptors. Therefore, the blockade of one or both vagus nerves would represent an important reduction in the excitatory input to the 'feeding centre' and this would induce inhibition of intake of food. This would correspond to an increase of intracellular glucose in the liver such as is produced by intraperitoneal glucose injections or by injections of adrenaline[1,3].

A total of 45 experiments were performed on 8 adult cats with chronically implanted electrodes in one or both vagus nerves. In one of the cats, electrodes were also implanted in the glossopharyngeal. The animals were conditioned to reach for food when four 1/sec flashes were applied. In the experimental trials, direct current of 0·6–1·5 m.amp. was applied through the implanted electrodes after the second flash. The current intensities were previously confirmed to block nerve conduction in the vagus in acute experiments. The animals were tested after a 24-h fasting period.

In several control experiments it was observed that the cats ate eagerly all the meat portions (twenty) given to them. When direct current was applied to either or both vagus nerves after the second flash, the cats did not reach for the food but remained in the resting position. After the cessation of the current, the animals did not eat for a period of 5–10 min even if the flashes were repeated. When direct current was applied to the glossopharyngeal instead of the vagus, the animals' feeding behaviour was not modified.

The foregoing results establish that a direct current of sufficient intensity to block nerve impulses is capable of producing immediate anorexia when applied to the vagus but not when applied to the glossopharyngeal. This does not provide any direct evidence of the existence of hepatic glucoreceptors, but does indicate that some nervous information related to food-intake regulation is coursing the vagus, which agrees with the aforementioned hypothesis.

It was shown by one of us in another communication[4] that, if the direct current applied to the vagus is prolonged sufficiently, the animals enter into natural electroencephalographic sleep, indicating the close interrelationship existing between the regulatory-mechanisms of appetite and sleep, as pointed out by Soulairac[5].

J. H. PEÑALOZA-ROJAS
M. RUSSEK

Departamento de Fisiología,
Escuela Nacional de Medicina,
Escuela Nacional de
Ciencias Biológicas
and
Laboratorio de Fisiología Comparada,
Escuela Nacional de Ciencias Biológicas,
Instituto Politecnico Nacionale,
Mexico, D.F.

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PHARMACOLOGY

Anti-anaphylactic Action of Acetylsalicylate in Guinea Pig Lung

HISTAMINE, plasma kinins and slow-reacting substance (SRS-A) are released or produced in anaphylaxis[1-3]. In the guinea pig, intravenous injection of each of these substances causes bronchoconstriction[4,5]. The bronchoconstriction induced by kinins or SRS-A is readily prevented by acetylsalicylic acid or its salts (acetylsalicylate), but not by mepyramine, whereas that induced by histamine is prevented by mepyramine, but not by acetylsalicylate[4-7]. These facts suggest that both drugs together might pro-

Table 1 REDUCTION OF ANAPHYLACTIC BRONCHOCONSTRICTION IN THE GUINEA PIG BY ACETYLSALICYLATE AND MEPYRAMINE

Exp No	Group	No of animals	Treatment	Geometric mean maximal response (mm)	95 per cent fiducial limits	Significance of difference
1	*a*	35	*E* (2)	44	29–65	*a* versus *b* *P* < 0 01
	b	73	*M* (5) + *E* (2)	21	16–28	
2	*a*	18	*E* (2)	75	61–93	*a* versus *b*, *P* < 0 1
	b	18	*A* (1) + *E* (2)	44	26–73	*b* versus *c*, *P* > 0 1
	c	18	*A* (4) + *E* (2)	26	16–44	*a* versus *c*, *P* < 0 001
	d	18	*A* (16) + *E* (2)	28	17–46	*a* versus *d* *P* < 0 002
3	*a*	25	*E* (2)	50	34–73	*a* versus *b* *P* < 0 1
	b	20	*M* (2) + *E* (2)	28	16–48	*b* versus *d* *P* < 0 001
	c	20	*A* (8) + *E* (2)	20	9 6–41	*a* versus *c* *P* < 0 05
	d	20	*A* (8) + *M* (2) + *E* (2)	5 8	3 8–8 7	*c* versus *d* *P* < 0 01
4	*a*	16	*M* (2) + *E* (2)	14	8 2–24	*a* versus *c*, *P* < 0 005
	b	17	*A* (1) + *M* (2) + *E* (2)	12	8 0–17	*a* versus *b* *P* > 0 1
	c	16	*A* (8) + *M* (2) + *E* (2)	4 5	2 9–7 0	*b* versus *c* *P* < 0 005

A, Sodium acetylsalicylate, *E*, egg albumen, *M*, mepyramine maleate, in parentheses, doses in mg/kg intravenously, acetylsalicylate being expressed as weight of acid and mepyramine, of base Acetylsalicylate was given 15 min and mepyramine 10 min before egg albumen

tect guinea pigs against anaphylactic bronchoconstriction and that each drug separately might have a partial protective action Although mepyramine has been shown to exert some protection[6,8], previous attempts have failed to show that acetylsalicylate[6] or salicylate[9,10] were effective Stresemann[11] has recently reported that amidopyrine, phenazone and phenylbutazone partially protected guinea pigs against an aerosol of antigen; but large doses of drug were needed and he considered the effect non-specific The present communication describes a fuller investigation of the action of acetylsalicylate against anaphylactic bronchoconstriction in the guinea pig

Konzett-Rossler[12] preparations of guinea pigs sensitized to egg albumen were set up as previously described[4], to record pulmonary resistance to inflation, except that the side vent of the recording apparatus was automatically clamped for 7–9 sec during each ½ min to inflate the lungs more forcibly All treatments were intravenous and all animals received egg albumen, 2 mg/kg Some animals were also given mepyramine maleate, 2 or 5 mg base/kg, 10 min before egg albumen, some animals were treated with sodium acetylsalicylate 1 or 8 mg acid/kg, 15 min before egg albumen, some animals received both drugs From the record of resistance to pulmonary inflation obtained from each animal, the maximal excursion within 2 min of injecting antigen was measured

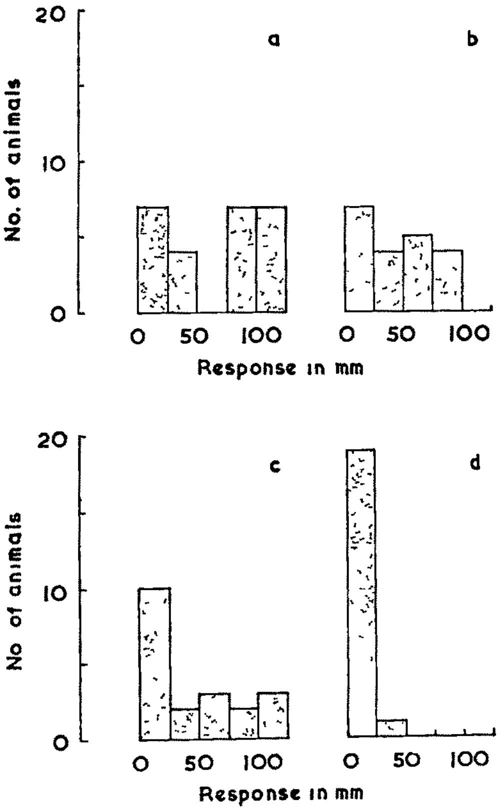

Fig 1 Frequency distribution of the size of the bronchoconstrictor response to antigen in the guinea pig after (*a*) no drug, (*b*) mepyramine, 2 mg/kg, (*c*) acetylsalicylate, 8 mg/kg, (*d*) acetylsalicylate, 8 mg/kg + mepyramine, 2 mg/kg Results from Exp 3 of Table 1

Table 1 summarizes the results of four experiments in a total of 314 guinea pigs The first experiment confirmed that mepyramine reduces anaphylactic bronchoconstriction The second experiment showed that acetylsalicylate also was active and the third that both drugs together were significantly more effective than either drug alone In the fourth experiment, in mepyramine-treated animals, a relationship was shown between the dose of acetylsalicylate and the extent to which bronchoconstriction was reduced

Fig 1 expresses the frequency-distribution of the size of response to antigen among the guinea pigs in experiment 3 of Table 1 It shows that, after either mepyramine or acetylsalicylate alone, anaphylactic bronchoconstriction was slight in some animals and intense in others, whereas after both drugs together it was always slight This finding suggests that those animals which were not protected by mepyramine alone would have been protected by acetylsalicylate and vice versa The previous attempt[6] to show the protective action of acetylsalicylate in anaphylaxis may therefore have failed because the guinea pigs used happened to have been almost completely protected by mepyramine In the present experiments, many more animals were used, among which an appreciable number were resistant to mepyramine

In the foregoing experiments, the possibility that acetylsalicylate may have acted by inhibiting release of histamine can be dismissed, because this requires a relatively high concentration of drug[13] and because it would not explain the synergism of acetylsalicylate with mepyramine, which is a much more potent inhibitor of histamine liberation in guinea pig lung[14] The possibility that acetylsalicylate may have acted by inhibiting formation of kinins and/or SRS-A cannot be dismissed, but, at the doses effective against anaphylactic bronchoconstriction, such an action has not been demonstrated in the guinea pig *in vivo* Acetylsalicylate itself constricts rather than relaxes guinea pig bronchioles It does not antagonize the bronchoconstrictor action of histamine, acetylcholine or 5-hydroxytryptamine, but it does antagonize that of kinins[4,6,7] and of SRS-A[5], probably competing with kinins for receptor sites on bronchioles[15] In view of these facts, the present findings indicate that kinins and/or SRS-A contribute to anaphylactic bronchoconstriction in the guinea pig and that acetylsalicylate lessens this effect by antagonizing one or both of these substances at their receptors in bronchial muscle If the anti-asthmatic action of acetylsalicylic acid in man, recently demonstrated by Stresemann[11], resembles in mechanism its anti-anaphylactic action in guinea pig lung, this drug might be expected to be more effective in bronchial asthma if given together with an anti-histamine

H O J COLLIER
A R HAMMOND
BARBARA WHITELEY

Department of Pharmacological Research,
Parke, Davis and Co,
Hounslow, Middlesex

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Effects of α-Methyl DOPA and DOPA on the Amphetamine Excitatory Response in Reserpinized Rats

IN the course of investigations into the actions of certain anti-depressant compounds on the excitatory response to amphetamine in rats[1], we have found that L-α-methyl-3,4-dihydroxyphenylalanine (α-Me DOPA) antagonizes the reserpine-induced modifications to the amphetamine response, but that the observed effects of this interaction are completely different if the α-Me DOPA is given after, rather than before, the reserpine

The excitatory response of male albino rats (Tuck Wistar strain), 160–240 g, to *dl*-amphetamine sulphate 5 mg/kg (intraperitoneal) was scored on the following arbitrary scale: 0, rat asleep, 1, alert but not moving, 2, moving around the cage, 3, stereotyped sniffing at bars, 4, licking bars, 5, 'mock' biting (momentarily touching bars with teeth), 6, gnawing bars Half values were scored for appropriate responses The individual scores for each of four rats in a group were noted and summed every half-hour (maximum score = 24) for 5–6 h after the amphetamine injection The scoring was performed by an operator who did not know what treatment the animals had received

Amphetamine in the dose used caused a moderate excitatory response, with all rats sniffing, rarely licking and never biting, the effect lasted 4–5 h (Fig 1) Treatment of the rats with α-Me DOPA 400 mg/kg (intraperitoneal) 2–3 h before the amphetamine reduced its response slightly, if given 18 h before amphetamine, α-Me DOPA did not modify the response at all Reserpine 2 5 mg/kg, injected intraperitoneally 18 h previously, markedly accentuated the amphetamine response both in degree and rate of onset, but reduced it in duration When the two treatments were given to the same rats, however, two quite different responses to amphetamine could be obtained, depending on the order of injection of the reserpine and α-Me DOPA

(1) If α-Me DOPA was given just before reserpine (that is, at 18 5 and 18 h respectively before amphetamine), the amphetamine response resembled that in untreated animals—that is, the modifications to the response induced by reserpine were abolished by α-Me DOPA

(2) If α-Me DOPA was injected 2 h before amphetamine into rats which had received reserpine 16 h earlier, all signs of excitation were abolished The rats sat immobile except for occasional stretching motions, and made no stereotyped sniffing or biting movements This blocking effect of reserpine and α-Me DOPA was not seen in rats treated with the mono-amine oxidase inhibitors nialamide 100 mg/kg or tranylcypromine sulphate 5 mg/kg 1 5–9 h after reserpine

The blockade of the amphetamine response by reserpine and α-Me DOPA could be 'reversed' by a subsequent subcutaneous injection of DL-DOPA 20–50 mg/kg given 0 5–1 5 h after amphetamine (Fig 2) The consequent excitatory response was indeed often greater than that to amphetamine after reserpine; the rats would spar and fight, or bite the bars of the cage strongly and incessantly This 'reactivation' effect lasted about 2 5 h, ending abruptly 5-Hydroxytryptophan 50 mg/kg or dopamine 100 mg/kg did not induce this effect Injection of DOPA to rats given reserpine and α-Me DOPA only, without amphetamine, caused some increased alertness and sensitivity to noise, but little motility and no signs of stereotyped sniffing movements (Fig 2) Similarly DOPA did not significantly increase the excitatory response to amphetamine alone in normal or in reserpinized rats which had not been injected with α-Me DOPA

The observation that, after prior treatment with α-Me DOPA, reserpine no longer modifies the pattern of amphetamine excitation in rats offers further evidence to that presented by Necina[2] and Day and Rand[3] that α-Me DOPA can in some way antagonize certain actions of reserpine, possibly by its metabolites taking over some of the physiological roles of their non-methylated analogues.

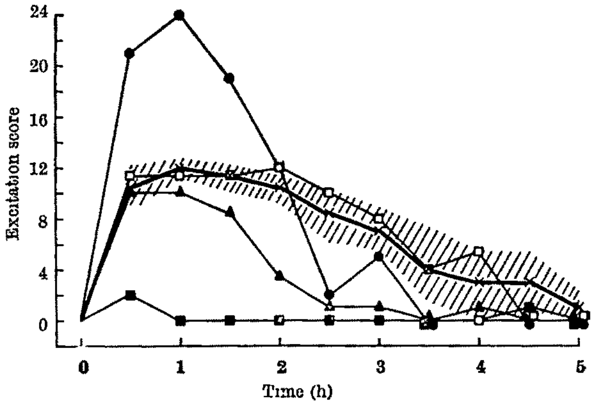

Fig 1 Time-course of the excitatory response to amphetamine in groups of 4 rats For scale, see text (maximum = 24) ×, Amphetamine only (shaded area = 95 per cent limits of error), ●, reserpine 18 h before amphetamine, ▲, α-Me DOPA 2 h before amphetamine; ■, reserpine 18 h, plus α-Me DOPA 2 h before amphetamine, □, α-Me DOPA 18 5 h plus reserpine 18 h before amphetamine

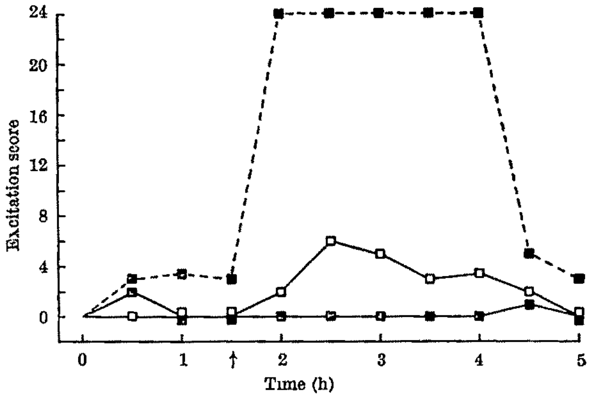

Fig 2 As Fig 1 ■——■, Reserpine 18 h plus α-Me DOPA 2 h before amphetamine, ■---■, reserpine 18 h plus α-Me DOPA 2 h before amphetamine plus DOPA injected at arrow, □——□, reserpine 18 h plus α-Me DOPA 2 h before saline plus DOPA at arrow

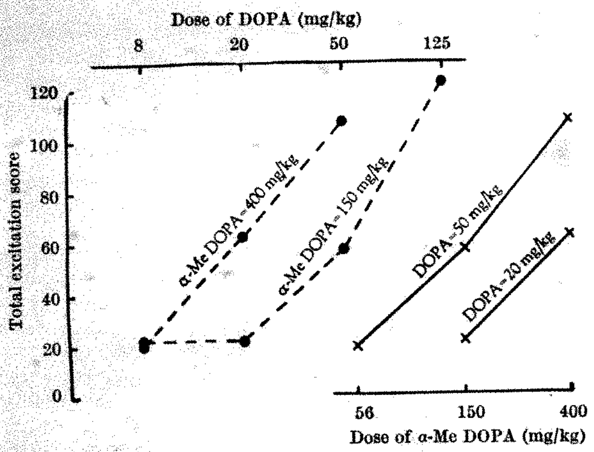

Fig. 3. Ordinate, total score for excitatory response to amphetamine in groups of 4 rats measured every 0·5 h from 2 to 5 h after amphetamine (maximum = 168); rats given reserpine 18 h and α-Me DOPA 2 h before and DOPA 1·5 h after amphetamine. Abscissæ, lower: dose of α-Me DOPA (solid lines); upper: dose of DOPA (broken lines)

(We have noted also that injection of α-Me DOPA before reserpine prevents the bradycardia normally induced by reserpine in rats, and in such animals a subsequent injection of imipramine no longer produces a marked tachycardia as it does in rats given reserpine only[1].) When α-Me DOPA is injected after reserpine and only 1–3 h before amphetamine, however, the combined treatment completely abolishes the excitant action of amphetamine. Under these conditions reserpine therefore appears to synergize the inhibitory action of α-Me DOPA, which is only slight by itself (although it can be increased somewhat by the use of higher doses than those reported here).

We have obtained certain indirect evidence which suggests that, in this test, amphetamine may act at least partly indirectly, via mechanisms involving catecholamines and/or 5-hydroxytryptamine. (For example, its action is markedly accentuated by imipramine-like drugs, and prolonged by MAO inhibitors; it is reduced by 5-HT antagonists, lysergic acid and brom-lysergic acid diethylamide, and abolished by tetrabenazine.) It seems reasonable, therefore, to suggest that the inhibition of amphetamine excitation by reserpine and α-Me DOPA may arise from a summation of their effects in depleting the brain of active amines. Possibly α-Me DOPA interferes with amine synthesis, binding or uptake at certain target sites of amphetamine action which are not entirely depleted of amines by reserpine (although tetrabenazine may deplete them fully). It appears unlikely that this action of α-Me DOPA is mediated purely by inhibition of amine synthesis by DOPA decarboxylase block (although such an action may contribute to its effect) since the potent decarboxylase inhibitor, 'Ro 4–4602' (ref. 4), does not mimic the action of α-Me DOPA in reserpinized rats.

The further phenomenon, of the restoration of the amphetamine response by DOPA after its inhibition by the combined action of reserpine and α-Me DOPA, presents greater difficulties of interpretation. No competitive antagonism between DOPA and α-Me DOPA seems evident, since the degree of excitation produced by DOPA in reserpinized rats in which the amphetamine response has been blocked by α-Me DOPA is directly proportional not only to the dose of DOPA injected but also to the dose of α-Me DOPA previously given (Fig. 3). If DOPA acts by overcoming an α-Me DOPA effect, the dose-response lines for these two substances would be expected to show opposite slopes. Furthermore, DOPA causes excitation in tetrabenazine-treated rats (in which the amphetamine response is blocked) only if α-Me DOPA has been given previously.

Perhaps this is a case where, under special conditions, a metabolite of α-Me DOPA (possibly α-Me dopamine, which may be considered as 3,4-dihydroxy-amphetamine) can be shown to exert a stimulant action; but the mode of action is obviously complex and demands further investigation.

We wish to thank Merck, Sharp and Dohme (Dr. J. Merry) for generous supplies of α-Me DOPA and Dr. H. Blaschko for helpful comments.

R. M. Quinton
G. Halliwell

Department of Pharmacology,
Pfizer Ltd., Sandwich,
Kent.

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Neuropathological Changes in Chronic Cyanide Intoxication

The presence of a small metabolically active pool of cyanide in the body is now well established. The conversion of cyanide to thiocyanate was first demonstrated in 1894[1]. Although thiocyanate is the main excretory product of cyanide, it has been shown that it can also be oxidized to carbon dioxide and formate, and therefore cyanide-carbon can enter into the metabolism of one-carbon compounds. Using $Na^{14}CN$, it has also been shown[2] that the radiocarbon appears in the methyl groups of choline and methionine and in the ureide carbons of allantoin, and some of it also finds its way into the cyano groups of cyanocobalamin.

The enzymatic conversion of cyanide to thiocyanate was first investigated in 1933[3], nearly 40 years after the observation[1] that cyanide was excreted by animals as thiocyanate. The enzyme system was named rhodanese and it has been found to be specific for free cyanide without action on organically bound cyano groups. This property indicates that the excretion of thiocyanates after the administration of organic nitriles results not from a direct action on the nitrile itself, but from HCN which had first been removed from the nitrile molecule by another mechanism. More recent studies of the distribution of rhodanese in tissues[4] show it to be widely distributed in the body, activity being greatest usually in the liver. The demonstration of very large amounts of enzyme available as compared with the relatively small size of the fatal dose of cyanide raises the question of what limits cyanide detoxification *in vivo*. The suggestion has been made that this limitation may be set by the availability of sulphur, although it seems more likely that the rhodanese system is not geared to combat non-physiological doses of cyanide.

Although rhodanese is an efficient antidote against the toxic effects of cyanide, there exist other pathways by which this may be mediated or assisted. It has been shown that hydroxocobalamin takes up cyanide avidly to form the harmless cyanocobalamin and can protect the experimental animal against lethal doses[5]. Furthermore, a dietary deficiency of vitamin B_{12} leads to increased thiocyanate excretion and it has been suggested that competition exists between hydroxocobalamin and rhodanese[6]. Cyanide can combine with cystine *in vitro* to form 2-iminothiazolidine-4-carboxylic acid[7]. The formation of this acid also occurs *in vivo*, for on injection of potassium cyanide (1·5 μg daily) subcutaneously into rats, the acid can be isolated from the urine in quantities corresponding to 15 per cent of the dose, although the main excretory product (80 per cent of the dose) is thiocyanate.

As well as a mechanism for converting cyanide to thiocyanate, there exists a reverse pathway which has been called thiocyanate oxidase[8]. This enzyme, which is found only in the erythrocyte, has been detected in man, dog,

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rabbit and rats. The presence of thiocyanate oxidase, which ensures a persistent trace of cyanide in the blood, indicates that in minute quantities the latter is an essential metabolite.

From an experimental point of view, the role of cyanide has been invoked as of great importance in the possible ætiology of demyelination[9,10]. This work may be criticized on the grounds that the doses used were compatible with the production of lesions secondary to anoxia, due to interference with enzyme mechanisms of which cytochrome oxidase is possibly the most important. It was also shown that sodium azide in small doses could produce demyelination[9], and this work has been quoted[11] as being the only successful attempt at producing a demyelinating disease in animals at all comparable with those observed in man. In this connexion it is interesting to note that biochemically sodium azide has an action similar to that of potassium cyanide. However, azides do not occur naturally, and it is difficult to implicate them directly in human demyelination.

It seemed to us that the effect of much smaller doses of cyanide, with a greater time-interval between each dose, should be utilized in an attempt to produce central nervous system damage. Accordingly, two groups of three adult Wistar albino rats were taken, and the three experimental animals injected with 0·5 mg of potassium cyanide subcutaneously once weekly for 22 weeks. The other three were kept as normal controls. After this period of time the six animals were killed by lightly anæsthetizing with ether and severing the great vessels in the neck. Blood and livers were collected for vitamin B_{12} and folate activity and the brains extracted for histological examination.

The brains were processed in paraffin and stained with hæmatoxylin and eosin, luxol fast blue, and by the methods of Nissl, van Gieson, the phosphotungstic acid–hæmatoxylin stain of Mallory and with a silver stain for axons, Weil's myelin stain and sudan black. Frozen sections were stained with sudan IV.

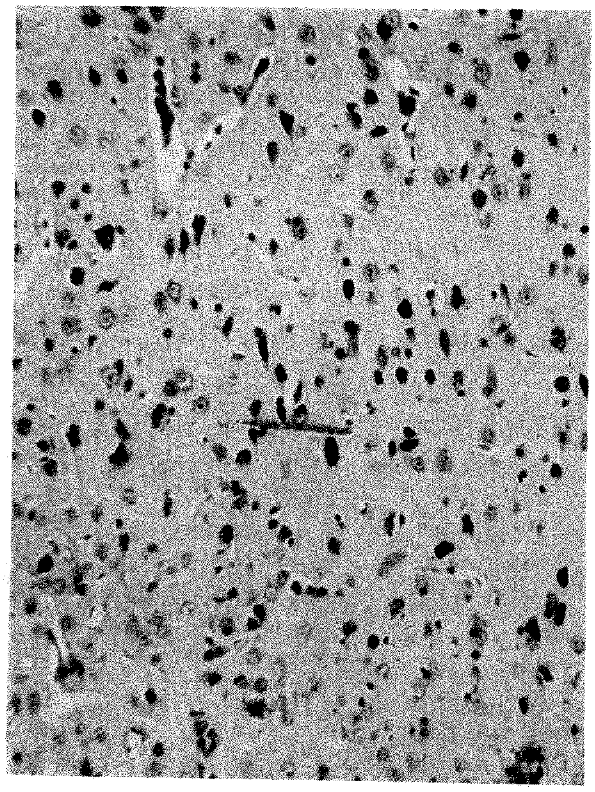

Fig. 1. Degenerated pyramidal cells in the cortex of a cyanide rat. (Hæmatoxylin and eosin × 400)

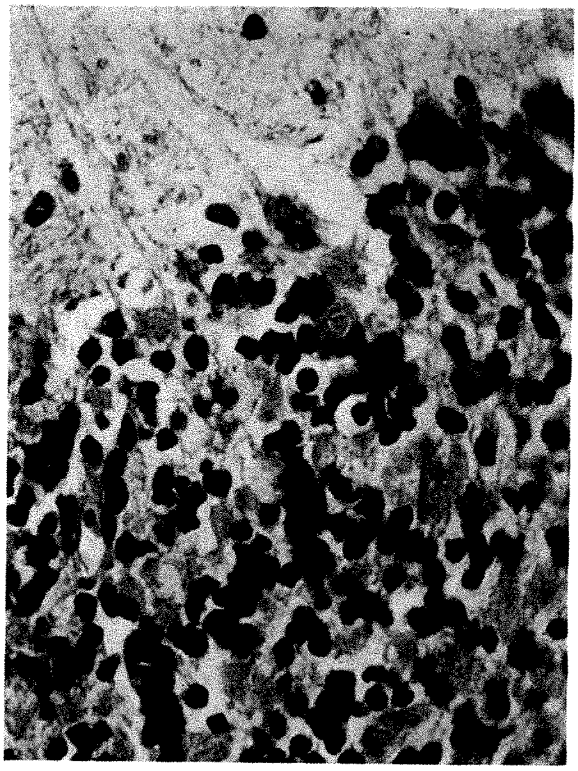

Fig. 2. Degenerated Purkinje cells in the cerebellum of a cyanide rat. (Hæmatoxylin and eosin, × 1,000)

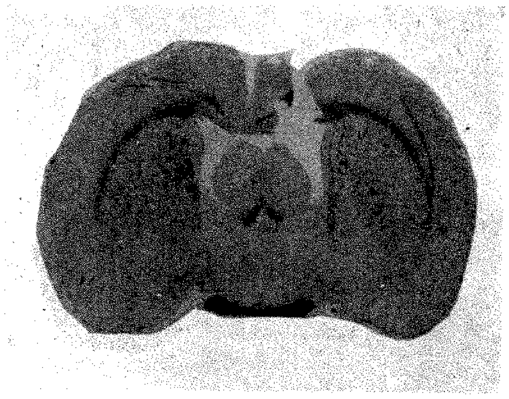

Fig. 3. Pallor of myelin in the corpus callosum of a cyanide rat. Compare the intact myelin in the optic chiasma. (Weil stain. × c. 3·3)

There are noticeable cellular changes in the cortex (Fig. 1), hippocampus and in the cerebellum (Fig. 2), with neuronal degeneration and cell loss in the brains of the animals receiving cyanide. There are minimal cellular changes in the brains of the normal controls, much less important than in the former group, and probably due to the anæsthesia. There is some pallor of myelin in the brains of the cyanide rats (Fig. 3), particularly in one of them, but whether there is demyelination is difficult to estimate.

The results of this small series indicate that cyanide in a comparatively small dose is capable of producing lesions in the central nervous system of the rat as described. In contradistinction to previous work along these lines, the animals tolerated the doses given without ill-effect or demonstrating manifest evidence of anoxia. It remains to be seen whether support has been given to the suggestion that a disorder of cyanide metabolism may be implicated in those diseases in which demyelination occurs within a picture of vitamin B_{12} deficiency[12]. We have been

Table 1

		Folate activity		B_{12} activity	
		Serum (μμg/ml.)	Liver (μg/g)	Serum (μμg/ml.)	Liver (μg/g)
(1)	Control	35·5	5·7	185	0·105
(2)	Control	27	5·3	110	0·182
(3)	Control	31	5·7	115	0·194
(4)	Cyanide	19	4·8	95	0·188
(5)	Cyanide	37·5	5·7	130	0·073
(6)	Cyanide	21·7	4·8	125	0·143

unable to confirm conclusively the observation[13] that cyanide intoxication probably produces depletion of liver hydroxocobalamin, although with the extremely small numbers used in this experiment it is impossible to make a valid comparison. Nevertheless, the average B_{12} activity of the livers in the cyanide treated rats is lower than that of the control animals (Table 1).

Further work requires the experiment to be repeated with the addition of cyanocobalamin and hydroxocobalamin to two groups subjected to a similar cyanide intake. If the latter confers protection to the central nervous system as opposed to the former then even greater questions of fundamental interest will be revealed.

A. D. M. Smith

Westminster Hospital.

S. Duckett

Maida Vale Hospital.

A. H. Waters

Postgraduate Medical School,
University of London, W.12.

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HÆMATOLOGY

Use of Ion Exchange Resins to alter the Concentrations of Individual Inorganic Ions in Blood *in vivo*

As yet no simple method is available to alter selectively the concentration of any ion in blood perfusing an isolated organ. Such a method would be of considerable value, and we report on the development of a suitable procedure.

The method depends on the fact that an ion-exchange resin equilibrated with a known electrolyte solution will approximately reproduce the original ionic concentrations in a small volume of a second solution replacing the first. The technique described here has been developed to study the effect of varying calcium and magnesium concentrations in the arterial blood supplying the parathyroid glands.

The cation exchange resin 'Dowex-50' (a cross-linked polystyrene sulphonic acid resin containing 8 per cent divinyl benzene, 20–50 mesh size) is purified by repeatedly washing with 5 N hydrochloric acid and 5 N sodium carbonate alternately. The final sodium form is then washed with distilled water and air-dried at room temperature. A column containing 15 g (wet weight) of this resin is washed with about 10 l. of a solution containing 140 m.equiv. sodium/l. (as sodium chloride and bicarbonate), 3·8 m.equiv. potassium/l. (as potassium chloride), varying amounts of calcium chloride and magnesium chloride and bubbled with 5 per cent carbon dioxide in oxygen so as to give a *p*H of 7·4. The resin is then poured into a conical flask and swirled with the same fluid. It is finally transferred to a thick polyvinyl chloride tubing column (15 cm × 1·6 cm) with inlet and outlet tubes (0·4 cm diam.) guarded by fine stainless steel mesh. The column is rid of all air bubbles by running in the equilibrating fluid from below; it is used once only.

A suitable artery in a heparinized (4,000 units/kg) animal is tied off distally and secured proximally to the afferent tube of the column. The effluent blood is pumped directly into the desired vascular bed. By adjusting the calcium and magnesium concentrations in the equilibrating fluid it is possible to obtain with reasonable accuracy any concentrations of these cations in the blood coming off the resin column. The concentrations of sodium, potassium and hydrogen in the blood plasma are not affected significantly. Other workers have noted absence of changes in various chemical and cellular components of blood following its passage through the resin[1–3].

Table 1. CATION CONCENTRATIONS IN RESIN EQUILIBRATING FLUID, SYSTEMIC BLOOD AND EFFLUENT BLOOD IN DOGS

(the last-named values are representative of levels obtained with perfusion for more than 3 h)

Exp. No.	Specimen	Ca	Mg	Na	K	*p*H
		(m. equiv./l.)				
1	Equilibrating fluid	3·2	0·4	145	3·8	—
	Systemic plasma	5·8	1·2	151	3·7	—
	Effluent plasma	5·9	0·7	150	3·7	—
2	Equilibrating fluid	3·6	2·7	141	3·7	7·40
	Systemic plasma	5·9	1·5	151	4·1	7·39
	Effluent plasma	6·2	3·7	146	3·9	7·41
3	Equilibrating fluid	2·0	1·3	140	3·8	—
	Systemic plasma	5·5	1·9	—	—	—
	Effluent plasma	4·1	1·9	—	—	—
4	Equilibrating fluid	4·6	1·4	140	3·8	—
	Systemic plasma	5·6	1·9	—	—	—
	Effluent plasma	7·0	1·9	—	—	—
5	Equilibrating fluid	3·0	1·2	137	3·9	7·40
	Systemic plasma	4·6	1·4	147	4·4	7·38
	Effluent plasma	4·6	1·7	143	4·0	7·36

Cation concentrations were estimated by flame spectrophotometer (Zeiss *PMM* 12) and *p*H was measured anaerobically in a blood *p*H apparatus (radiometer *pHM*4 with macro-Astrup assembly electrode). Table 1 shows the concentrations of cations in the equilibrating fluid, systemic blood plasma and resin-treated blood plasma in dogs. It will be noted that the levels of calcium and magnesium in the equilibrating fluid produce different plasma-levels in the effluent blood because these elements are partly protein-bound. With the resin column described, it is possible to produce almost constant cation concentrations for more than 5 h with flow rates up to 5 ml. blood/min. Larger columns may be used for greater flow rates or longer perfusion periods.

It appears that similar methods could be used to alter the levels of other inorganic ions in blood using suitably pre-treated cation or anion exchange resins. Besides the perfusion of organs and glands, this selective addition or removal of various electrolytes in blood may have therapeutic possibilities.

We are indebted to Mr. W. J. Dempster and the staff of the Experimental Surgery Unit, Postgraduate Medical School, London, for their assistance.

M. Ashwini Kumar*
I. MacIntyre

Department of Chemical Pathology,
Postgraduate Medical School,
London W.12.

* Commonwealth Scholar from India.

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Cross-relations of *Escherichia* and Human Red Cells

IT is well-known that certain strains of *Escherichia coli* (particularly 086:*B*7) are antigenically related to human red cell group A and group B substances. There have been differences in the results reported by different workers. Springer[1,2] found only anti-B activity in the sera of rabbits injected with *Escherichia coli* 086:*B*7 which was phenolized and then vacuum-dried. Using both living and treated preparations, Gonano *et al.*[3] showed both anti-A and anti-B activity, but in rather low titre, and their absorption studies gave rather disappointing results.

By the use of trichloroacetic acid extracts of *Escherichia coli* 086:*B*7, we were able to obtain sera with much higher anti-A and anti-B titres than resulted from the use of either formalinized or boiled-whole organisms. This suggests, *inter alia*, that the location of the antigens in the bacterial cell wall may be of importance[4]. The use of antigenic extracts also makes possible more detailed study of the antigenic relations and it is hoped to confirm and expand our impression that the cross-reacting *Escherichia* antigens are rather minor components of the cell.

B. J. TEN RAA
C. R. MACPHERSON

Department of Pathology,
Ohio State University,
Columbus 10.

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Male Specific Hæmagglutination in Mice

IT has been found that male skin grafts are rejected by female mice in the *C57BL* strain[1]. On the other hand, it has been well ascertained that an introduction of the histocompatibility antigens into inbred mice can elicit humoral antibodies to cause hæmagglutination of the donor red cells. This communication shows that the sera of female mice sensitized with male tissues can agglutinate specifically blood cells of intact male mice. *DD* strain mice were used for experiments. The female animals were subjected to the following treatment: A subcutaneous injection of 0·2 ml. testis-Freund's adjuvant emulsion per week for 4–6 weeks. Each of the injections contained 15–33 per cent testis homogenate. One to two weeks after the last injection the antisera were collected from the sensitized female mice, and kept at –20° C until the use for the present hæmagglutination test.

Blood cells from the intact male or female mice were collected in heparinized Tyrode solution, washed twice with it, and finally with Tyrode solution adjusted to *p*H 7·35. These cells were suspended in Tyrode solution to 2·5 per cent. For the test, the antiserum was thoroughly mixed up with the blood cell suspension in equal volume on a hollow slide. The mixture was incubated at 18° C. After 90 min, the hæmagglutination reaction was examined. For control, similar tests were performed to all the conceivable combinations of the sera and the blood cells of the non-sensitized *DD* mice.

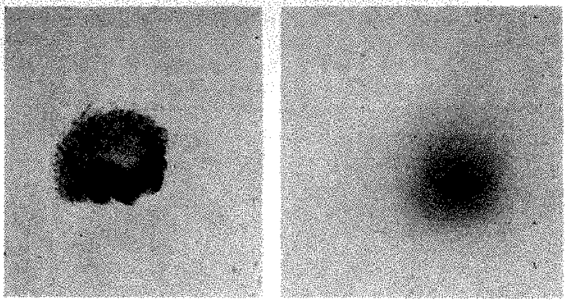
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Fig. 1. Hæmagglutination of intact male blood cells suspended in antisera of female sensitized with testis; (1) positive (*j*—6 antisera × ♂*c* blood cell); (2) negative (*j*—6 antisera × ♀*c* blood cell)

Out of 40 sensitized females remarkable spleen hypertrophy occurred in 20 animals, of which 7 animals had clear hæmagglutinations. As shown in Table 1, the sera from these 7 specimens agglutinate the blood cells of all males tested, but fail to react with those of females, except in 2 cases (Fig. 1). It is worth mentioning that no instance of reverse reaction was seen at all. Every reaction shown in Table 1 was easily reproducible. In the control series, using sera from non-sensitized animals, no hæmagglutination was detectable at all.

These results may suggest that the testis homogenate used for the injection might contain male specific antigen(s), against which the recipient female mice can produce humoral antibody(s) to agglutinate the blood cells of the male mice specifically.

It is clear that the 7 antisera shown in Table 1 were able to discriminate male blood cells from those of female; in other words, some immunological characteristics of blood cells differ between the sexes. (Such an exceptional case as ♀*b* obviously needs further investigation.)

We thank Prof. S. Asayama of this University for his valuable criticism, and Prof. T. S. Okada of the University of Kyoto for his advice.

MITSURU FURUSAWA
MINORU KOTANI
HIROSHI TAKEUCHI

Department of Biology,
Faculty of Science,
Osaka City University,
Osaka.

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Table 1. HÆMAGGLUTINATION CAUSED BY ANTISERA FROM THE FEMALE SENSITIZED WITH TESTIS HOMOGENATE IN *DD* STRAIN MOUSE

Antisera	Intact blood cell															
	♂								♀							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*
c—1					+	+	–*	–*					–	–	–	–
h—1			+	+							+	–				
j—3		+ + +	+ + +		+ + +	+	±	+		+ + +	–		–	–	–	–
j—6		+ + +	+ + +		+ + +	+ +	±	+ +		+ + +	–		–	–	–	–
l—4	+								–							
n—1	+ + +								–							
n—3		+ +	+		+					+ +	–		–			

+ + +, + +, +, ±, Degree of hæmagglutination; –, absence of hæmagglutination.
* Absence of hæmagglutination may be due to weak agglutinating capacity of the antiserum (*c*—1).

PATHOLOGY

Induction of Fibrosarcoma in the Primate *Tamarinus nigricollis*

ATTEMPTS to induce malignant neoplasms in monkeys have not met with the success that has been achieved in other species. The rarity of spontaneous neoplasms in monkeys suggests that these animals are more resistant to the development of malignant disease than man and other species. There have been no specific references, however, to the use of the South American marmoset, *Tamarinus nigricollis*, in the experimental induction of neoplasms. A recent review by Kent[1] indicates that attempts at the production of malignant disease by the application of polycyclic hydrocarbons in monkeys have failed.

We injected 2 mg of 2-methylcholanthrene in olive oil subcutaneously into the forearm of 5 marmosets of the species *T. nigricollis*. Three animals, which died of upper respiratory infection approximately one month following the application of the carcinogen, showed no changes at the site of the implantation. One animal is still alive and exhibits no changes at the site of the methylcholanthrene implantation. One animal died of an upper respiratory infection 10 months following the injection of the methylcholanthrene. Gross examination of the skin over the site of the injection in the animal showed many small (0·5–1 mm) papules on the surface of the skin. When the skin was dissected over this area the subcutaneous tissue, which usually is less than 1 mm thick, was approximately 4 mm thick. It was firm and appeared to be attached to the underlying muscle. Histological examination of this material revealed numerous areas of pseudo-horn cysts as well as hyperkeratosis, acanthosis and papillomatosis (Fig. 1). This epithelial change is similar to, if not identical with, seborrhœic keratosis of man. The dermis rests on a highly cellular tumour composed of fibroblast cells. The nuclei vary in size, shape and staining qualities. Some nuclei appear spindle-shaped and others are round or oval, and occasional typical as well as atypical mitotic figures are seen (Fig. 2). This lesion has the histological appearance of fibrosarcoma, although it did not metastasize.

Because this type of reaction to methylcholanthrene is apparently rare in primates, I think it is important to report the finding now. Further work concerning the production as well as the transplantability of tumours produced in marmosets by methylcholanthrene and other polycyclic hydrocarbons is in progress.

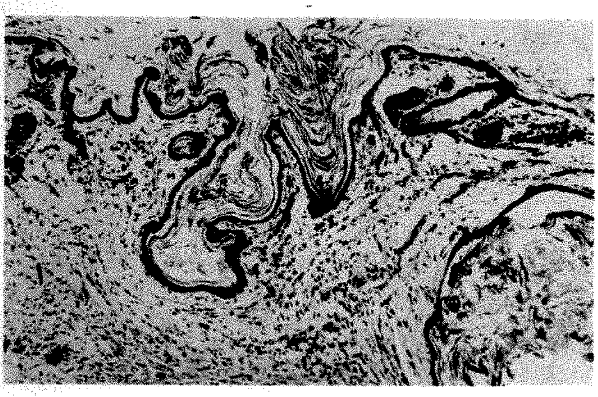

Fig. 1. Photomicrograph of skin illustrating the seborrhœic keratosis-like pattern of the epithelial change

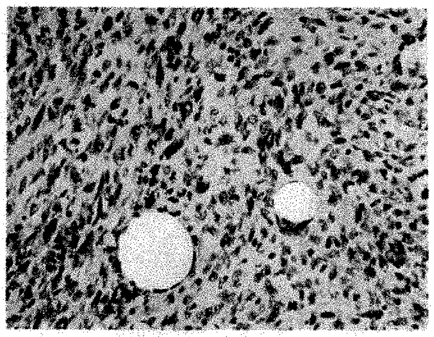

Fig. 2. Photomicrograph of the tumour showing pleomorphic fibroblastic cells with occasional mitotic figures

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BARNET M. LEVY

Dental Branch,
University of Texas,
Houston 25.

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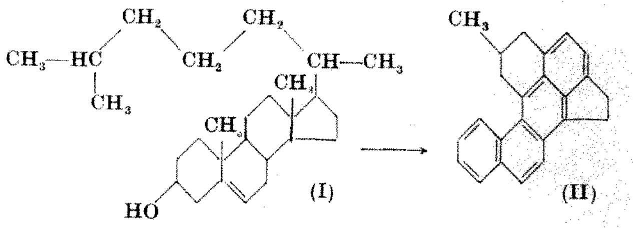
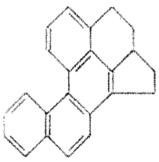
Particular Carcinogenic Activity of *F*-nor-Steranthrene

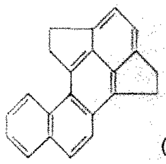
SINCE the discovery of the polycyclic chemical structure of cholesterol on one hand, and, on the other, of the carcinogenicity of 3-methylcholanthrene, it has often been postulated that certain types of cancer might be due to some abnormality in the metabolism of cholesterol and bile acids, resulting in the formation in the body of one or several carcinogenic polycyclic hydrocarbons[1]. Along these lines, Bergmann[2] pointed out the theoretical possibility for cholesterol (I) to be cyclized, with dehydration and dehydrogenation, into the hypothetical hexacyclic hydrocarbon (II), which he named 'steranthrene'



(since, the word 'steranthrene' has been used to designate the non-methylated basic hydrocarbon). Although experimental evidence for the existence of such a biochemical transformation is still lacking, it was of great interest to examine hydrocarbons of the steranthrene group for carcinogenicity. One of these hydrocarbons, 'angular' steranthrene (III), was recently investigated in mice by Dannenberg[3], and found to produce spindle-cell sarcomas by injection and epitheliomas by painting the skin; the substance showed approximately the same degree of activity as 3,4-benzopyrene and 3-methylcholanthrene. We report here the results of our experiments on another such hydrocarbon, *F*-nor-steranthrene (IV), the synthesis of which has recently been described[4].



(III)



(IV)

Mice belonging to strain XVII *nc/Z* (Radium Institute) were divided into four groups: 8 female mice aged 6 months; 7 females of 3–4 months; 7 males of 6 months; and 7 males of 3 months. They all received, by subcutaneous injection in the same area of the flank, 3 injections of 0·6 mg of substance (IV) in 0·2 c.c. olive oil, one month elapsing between each injection. Table 1 gives the results with the three first groups; the fourth group had to be killed before any tumour had appeared because of an outbreak of severe infection in the cage.

These results are of two-fold interest. They show: (1) That *F*-nor-steranthrene is an extremely potent

Table 1. CARCINOGENIC ACTIVITY OF *F*-NOR-STERANTHRENE

No. of animals	Date of appearance of first and last tumours	No. of animals developing tumours	Length of survival	Type of tumour
Group 1. ♀ Mice aged 6 months at start of experiment				
8	70–90 days	6	81–110 days	Mammary epitheliomas, 4 Epithelioma + sarcoma, 1 Sarcoma, 1
Group 2. ♀ Mice aged 3–4 months				
7*	85–90 days	6	100–126 days	Mammary epitheliomas, 2 Epitheliomas + sarcomas, 3 Sarcoma, 1
Group 3. ♂ Mice aged 6 months				
7	80–100 days	7	100–132 days	Sarcomas, 7

* One animal still alive with no tumour after 6 months.

carcinogen with a purely local action; given by subcutaneous injections, it induced the early development of fast-progressing sarcomas which provoked death in about 15 days; (2) that in the female mice, the cancer most frequently obtained and earliest to appear is an epidermoid parakeratotic epithelioma which starts in the excretory ducts of the mammary gland, and which is often associated with a sarcoma. All the stages of epidermoid metaplasia of the mammary ducts are observed, terminating in cancerization.

It should be noted that no spontaneous mammary cancer has ever occurred in the strain used, and none of the numerous carcinogenic hydrocarbons hitherto tested on mice of this strain had produced a mammary tumour; the only mammary carcinomas observed in this strain had been induced by X-rays and, as with *F*-nor-steranthrene, they were epidermoid epitheliomas of the mammary ducts[5].

We thank the National Cancer Institute (U.S. Public Health Service, Bethesda) for a grant.

A. LACASSAGNE
E. BUCHTA
D. KIESSLING
F. ZAJDELA
N. P. BUU-HOÏ

Institute du Radium,
de l'Université de Paris,
26 rue d'Ulm, Paris (Ve),
France,
and
Institut für Organische Chemie
der Universität,
Erlangen, Germany.

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Drastic Morphological Change without Loss of Viability in Ehrlich Ascites Tumour Cells after Incubation with Strongly Diluted Formaldehyde

As is repeatedly stated[1-4], living virulent Ehrlich ascites tumour cells (EATC) incubated several hours at 37° C in 0·9 per cent sodium chloride show normal appearance (round cell forms, Fig. 1) and do not lose their viability and virulence. But living virulent EATC, incubated at 37° C, 1–2 h, with strongly diluted watery formaldehyde solution, show a peculiar drastic reaction of their aspect without dying off. This reaction differs in principle from the damage caused by anti-Ehrlich-immuno serum, or severe osmotic damage which both lead to death of the cells.

Formaldehyde (35 per cent formalin) is diluted with 0·9 per cent sodium chloride or Krebs-Ringer phosphate buffer. The reaction shows up most clearly at a concentration of 3×10^{-3} M formaldehyde (0·0096 per cent solution). At 5×10^{-3} M the effect is much weaker.

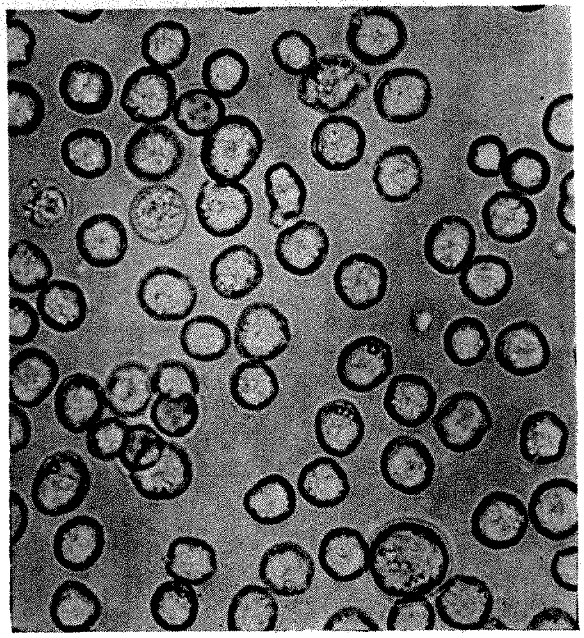

Fig. 1. Control: EAT-cells incubated 50 min in 0·9 per cent sodium chloride solution, 37° C. (× 610)

Sixty million virulent tumour cells (seventh–eighth day after transplantation) suspended in 0·9 per cent sodium chloride were incubated in 1·5 ml. of the formaldehyde solution of the concentration already mentioned at 37° C. After 10–25 min a part of the cells, increasing from 8 to 40 per cent, form short spicules. This gives cells the appearance of a thorn-apple (Fig. 2). Many spicules resemble fine droplets; the longer ones being swollen at their ends like clubs. On further incubation, the number of the thorn-apple-like deformed cells increases strongly; at the same time the spicules become longer and may change to long finger-like processes that may be nearly as long as the diameter of the cells. The longer processes may become curved or angular (Fig. 2, 2*A*); irregular swellings appear along the shaft, but especially at their ends.

The short, and also the longer, processes then grow into spherical drops of different sizes surrounding the cells in all directions.

For this phenomenon, that is, the formation of drops on the cell surface, we suggest the term 'stalagmosis' (stalagma = drop) (Fig. 3). As the size of the cells thus increases strongly, stalagmosis apparently is due to an intake of fluid, especially in the peripheral parts.

The maximal alteration of the cell (formation of 'thorn-apples' and drops) occurs after 50–60 min of incubation. At this time, most of the cells are surrounded on their surface with spicules, longish processes and drops of small to medium size. The content of the small processes and drops usually is homogeneous, but they often contain also vacuoles and—at the base—granules of cholesterol-esters (Fig. 3). The drop-like processes of the cells are originally always attached to the cell-body by narrow stalks.

In spite of the drastic alteration of the cells and the increase of the cell volume due to the processes and drop formation, formaldehyde of such small concentration does not cause any serious damage to the cell. This can be shown by the first fact, that added trypan blue does not stain the altered cells after incubation for 1 h (as in controls, there is only 5–8 per cent blue cells).

The viability and the unchanged virulence of the altered cells can be proved by the second fact, that their transplantation intraperitoneally into healthy mice leads to the death of the animals at the same time as in control animals, injected with untreated tumour cells (death of the mice between the twelfth and fourteenth day after transplantation with 3–5 ml. of ascites tumour; the mean survival time of control mice was 13 days).

After several hours of incubation of the EATC in the diluted formaldehyde solution the greater part of the deformed cells show morphologically a diminution of all processes. The cells which are still alive become spherical again, thus giving the usual aspect of EATC. Only in a part of the cells do the narrow processes and small droplets change into bigger drops. They continue swelling and finally rest with a broad base on the cell surface. If the death of the cells occurs after an incubation of 5 h and more, only about a quarter of the cells show diffuse swelling. The uptake of trypan blue slowly increases only after hours.

It was never observed that, using this concentration of formaldehyde, projections and drops separated themselves from the cells ('stalagmoptysis') as they do under the action of higher aliphatic aldehydes. This phenomenon will soon be described in detail elsewhere.

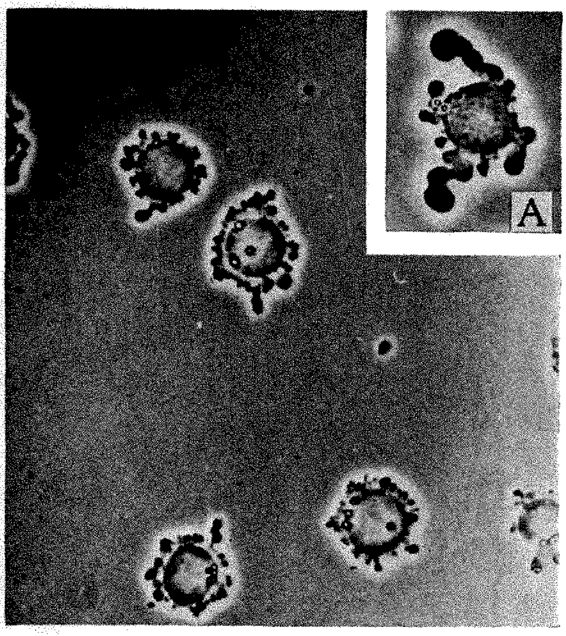

Fig. 2. EAT-cells incubated 20 min at 37° C in the diluted formaldehyde sodium chloride solution. All cells show spicules on their surface: 'thorn-apple' (× 690). *A*, Thorn-apple-like cell with long curved processes (× 710)

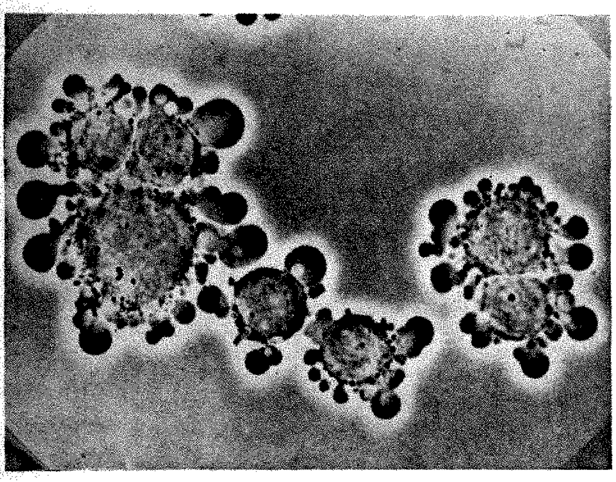

Fig. 3. EAT-cells, incubated 50 min 37° C in the diluted formaldehyde sodium chloride solution. 'Stalagmosis': all cells show small droplets and drops on their surface; many of them contain vacuoles or cholesterol ester-granules on the bases. (× 945 phase contrast)

The foregoing changes in morphology of the cell caused by highly diluted formaldehyde thus differ, both morphologically and biologically, from the irreversible alteration of the EATC due to osmotic disturbances (distilled water) or to anti-Ehrlich-immuno serum[4–6] since these agents cause in a few minutes diffuse membrane damage with much stronger swelling and enlargement of the cells whereby big and circular clear cytoplasmic 'blebs'[7] and 'blisters'[8] are formed surrounding the cytoplasma like yards (swelling yards). The result is the death of the cells through lysis within a short period of time[5].

M. Ratzenhofer
J. Zangger

Institute of Pathological Anatomy,
University of Graz, Austria.

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IMMUNOLOGY

Antibodies in Thymocytes

Fragraeus was not able to show plasmocytes in normal thymus[1]; nor did she find plasmocytes in thymus after antigen injections[2]. After removal of the thymus in adult rats the antibody production is unchanged[3]. In spite of these results which ascribe no important influence on antibody production by the thymus there are still several clinical signs which lead to the conclusion that the thymus takes part in antibody production. Milton[4] has noticed a strong barrier between thymus and blood circulation; he found that this barrier must be damaged so that antigens can reach thymocytes. After thymectomy of newborn 5–12 days old mice Milton found no germinal centres nor plasmocytes in lymph nodes. The purpose of this investigation was to establish: (1) if there are cell-fixed antibodies in thymocytes; (2) if there is a difference between an antibody stimulation with pure antigens and antigens mixed with Freund's adjuvant and alum precipitated antigens.

For every kind of immunization, 10 male rabbits of 2,000–3,000 g body-weight were used.

Group I: Injection of bovine albumin three times a week. First injection (containing 3 mg albumin) was applied intraperitoneally; the later injections contained 1·5 mg and were given subcutaneously. After a course of 4 weeks' injection the animals received a booster injection of 3 mg albumin intraperitoneally 14 days after the last injection. Four days later the animals were killed and the blood and different organs were collected. Groups II and III received the same course of immunization but they were given only 1 mg protein. This protein was mixed with Freund's adjuvant in Group II and alum in group III. Group IV served as untreated controls.

The thymus was removed immediately after death of the animals and cut in slices 2 mm thick. These slices were incubated with Krebs–Ringer solution. Thirty min later, the cells were isolated by filtration through two layers of gauze and washed 4 times with Krebs–Ringer solution. The concentration of the cells was fixed to 10 million per mm^3. In every case the peripheral blood was controlled for cellular composition.

For measuring antigen absorption on thymocytes, one γ iodine-131 marked bovine albumin dissolved in 0·2 ml. Krebs–Ringer solution was added to 1 ml. of cells and this mixture was placed in an incubator for 1 h at 37° C.

Afterwards the cells were washed in Krebs–Ringer solution 4 times. After the last centrifugation the cells were resuspended in 1 ml. and their radioactivity determined in a scintillation counter. In every series 3 and 4 cases were investigated. The control animals were killed at the same time to maintain the same condition in respect to biorhythmical differences.

Data given by Sorkin, Rhodes and Boyden[7] have shown that methanol treatment of cells increases the ability of absorption of antibody-containing cells, but does not change the absorption through cells of untreated control animals. The methanol treatment was carried out as follows: after centrifugation of the cell suspensions at 1,200 r.p.m. by 4° C in 1 ml. of absolute methanol. After fixing for 5 min at room temperature, the cells were centrifuged again, twice washed with Krebs–Ringer solution and suspended in 1 ml. buffer solution. Bovine albumin (Swiss Red Cross) was used labelled with iodine-131 following the method of Dubert, Coursaget, Fallot and Royer[8].

For the precipitation test the classical methods of α- and β-precipitation were used[9].

The results are summarized in Figs. 1–3. Fig. 1 shows the absorption of radioactive marked bovine albumin on thymocytes. The amount is expressed in counts per min of 10^6 cells. Fig. 2 refers to the percentage of the absorbed albumin to the resting amount of radioactivity in the supernatant. Fig. 3 shows the absorption in respect to the DNA content; this means radioactivity counts per min with respect to 10 mg of thymus DNA.

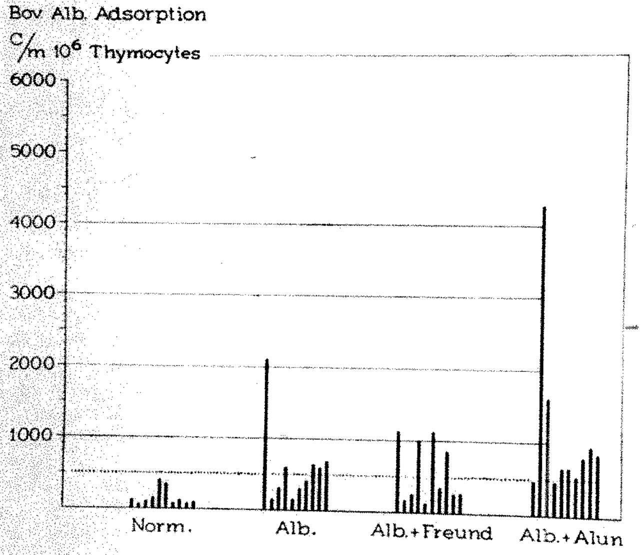

Fig. 1

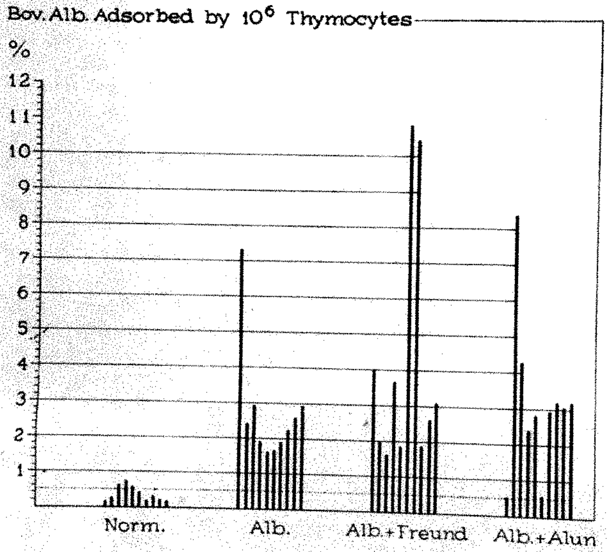

Fig. 2

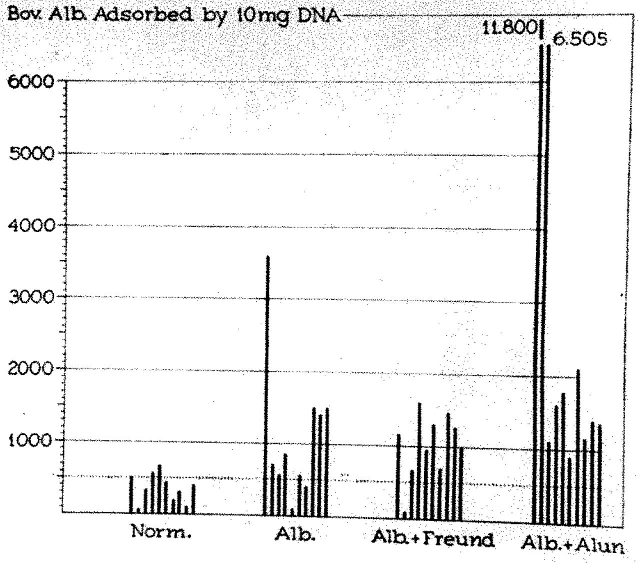

Fig. 3

The figures show clearly that the thymocytes of the hyper-immunized rabbits absorb significantly higher amounts of isotope-marked antigens than thymocytes of control animals. There is no highly significant marked difference between the variously immunized animals.

GABRIELLE HORVÁTH *

Clinique medicale universitaire
et Centre des Recherches
sur les Lymphomes malins,
Lausanne.

* Present address: Laboratorium voor Cyto-histologie, Universiteit van Nijmegen, The Netherlands.

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Experimental Allergic Thyroiditis in the Chicken

SINCE Rose and Witebsky's[1] demonstration of the auto-allergic disease affecting the thyroid gland of the rabbit, experimental allergic thyroiditis has been demonstrated in a number of mammalian species. However, auto-allergic lesions of the thyroid has not been heretofore produced in non-mammalian species. The work recorded here deals with the induction of thyroiditis in chickens by means of homologous thyroid tissue in complete Freund's adjuvant.

Thyroids were removed from normal Rhode Island Red chickens and 1 g aliquot was homogenized at 4° C in 5 ml. of 0·9 per cent saline. The homogenate was centrifuged at 800 r.p.m. for 5 min and the supernatant fluid lyophilized. The antigen-adjuvant emulsion was prepared thus: two parts of lyophilized thyroid extract dissolved in distilled water were mixed with one part of paraffin oil

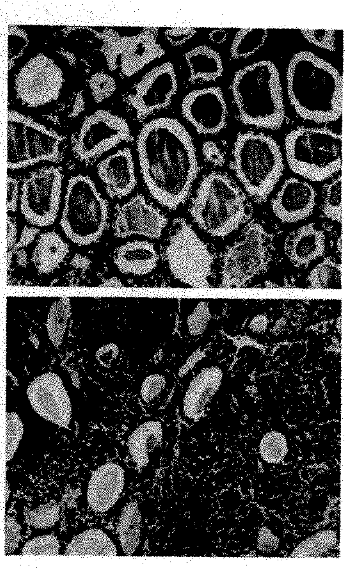

Fig. 1. Above, normal appearance of chicken thyroid; below, severely damaged gland in experimental allergic thyroiditis (× *c.* 20)

and one part of complete Freund's adjuvant. Adjuvant contained 1·5 vol. of 'Arlacel *A*', 8·5 vol. of paraffin oil and 12 mg/ml. of dried *M. tuberculosis*. Rhode Island Red chickens at the age of 10 weeks were injected subcutaneously by way of two foot-pads (a total of 5 mg of lyophilized thyroid extract per chicken). Skin tests were carried out 21 days after the sensitization. For this purpose homologous thyroid extract was prepared as described here and centrifuged for 1 h at 2,500 r.p.m. The supernatant fluid was injected intracutaneously in the wattle (0·1 ml. injection), and the reactions were observed at 24, 48 and 72 h. The birds were bled before death, serum separated, and anti-thyroprotein antibodies determined by passive hæmagglutination reaction[2] using tanned formalinized sheep erythrocytes. The cells were sensitized with saline extract of chicken thyroid in a proportion of 1 ml. of cell suspension to 0·5 mg of antigen. To meet the requirement of fowl antibody for complete precipitation, 8 per cent solution of sodium chloride was used as diluent[3]. The experimentation was terminated 30 days after the challenge injection, thyroid glands were removed, processed through paraffin embedding, and stained with hæmatoxylin–eosin and methyl-green-pyronine. The severity of thyroiditis was graded on an arbitrary scale ranging from ± to + + + +, based on the degree of infiltration of thyroid tissue with mononuclear cells.

Table 1. EXPERIMENTAL ALLERGIC THYROIDITIS, DELAYED HYPERSENSITIVITY AND ANTIBODY FORMATION TO HOMOLOGOUS THYROPROTEINS IN CHICKENS

Chicken No.	Severity of thyroiditis	Delayed sensitivity*	Reciprocal antibody titre
1	+	+ +	0
2	+ +	+	0
3	+	±	0
4	+ +	±	0
5	0	0	0
6	±	0	0
7	+ + + +	+ +	2
8	+ +	±	8
9	+	0	2
10	+	±	8
11	+ + +	+ +	0

* 48-h reaction.

Table 1 records the experimental allergic thyroiditis, delayed hypersensitivity and antibody formation to thyroproteins in chickens. Out of eleven birds, nine developed thyroiditis, and one chicken showed a few small aggregates of mononuclear cells (±). Eight control chickens, inoculated with complete adjuvant alone and killed 30 days later, did not show histopathological changes in the thyroids. There was a good correlation between the severity of disease and the intensity of delayed wattle reactions. Contrary to that, anti-thyroprotein antibody titres could not be correlated with thyroiditis.

Thyroiditis of moderate intensity was characterized by several foci of cellular inflammation located frequently in the peripheral areas of the gland. However, in advanced disease the lesions were quite diffuse (Fig. 1). The predominating cellular elements of a mild lesion were histocytes and lymphocytes, whereas in severe thyroiditis histocytes, lymphocytes, plasma cells, macrophages, polymorphonuclears, epithelioid cells and fibroblasts were found. The inflammatory process brought about the reduction in size of follicles, many of them being filled with histocytes, macrophages and leucocytes. In areas of dense mononuclear infiltration the follicles were totally destroyed. It should be mentioned that the histological appraisal revealed in one case the presence of a small ectopic thymus and a lymphoid mass resembling the germinal centre of the spleen. However, these lymphoid islands were clearly distinguishable from the cellular make-up of the thyroiditis lesions.

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BRANISLAV D. JANKOVIC
KOSANA MITROVIC

Microbiological Institute,
Faculty of Pharmacy, and
Immunology Unit, Institute of Biology,
University of Belgrade,
Belgrade, Yugoslavia.

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RADIOBIOLOGY

Influence of Lactation on the Retention of a Single Dose of Strontium-90 in Rats

IN previous investigations[1–3], it was found that the retention of radiostrontium after the lactation period (21 days *post partum*) was 45 per cent lower than that of virgin control rats when the isotope was injected into the pregnant rats 3 days before delivery. It was not clear, however, how much of this loss was due to a decrease in uptake of strontium-90 into bone or to other processes restricted to the time immediately after uptake or to a process still ruling after this initial period.

In contrast to the experiments already mentioned[1], in the experiment recorded here the isotope was injected within the day after delivery. The phase of initial incorporation into the skeleton was assumed to be finished before the third day after injection. At this time the amount of strontium in blood is less than 0·001 per cent of the injected dose and the retention in bone is declining[4–7]. Therefore, two groups of animals were killed 3 days, two further groups 21 days after injection of the isotope. This experimental arrangement allowed the estimate of net removal of strontium-90 from bone during this period by comparing radioactivity of the femora at these times.

Virgin rats and dams of the same age nursing litters of 4 juveniles were subdivided in groups as indicated in Table 1. The animals were albino rats of the Heiligenberg strain. 10 μc. of practically carrier-free strontium-90–yttrium-90 chloride contained in 0·5 ml. of physiological saline were injected intraperitoneally to each of the adult rats. Both femora of the females were ashed dry. Radioactivities and weights per femur are given in Table 1.

Retention of strontium-90 in the skeleton of the controls was 49 per cent of the injected dose (retention of skeleton = retention of femur × 20) 3 days after injection. At the same time, the nursing animals retained 41·6 per cent of the injected dose. Twenty-one days after the injection the radioactivities were 35 per cent of the injected dose for virgin

Table 1. RADIOACTIVITIES OF THE FEMORA OF NURSING AND VIRGIN RATS AT DAYS 3 AND 21 AFTER INCORPORATION

Experimental groups	No. of rats	Day of death after ^{90}Sr injection	Mean weights of animals at ^{90}Sr injection (g)	Ash weights per femur (mg)	Radioactivities % of injected dose*	
					per femur	per 100 mg ash of femur
Controls (not lactating)	5	3	194	220 ± 6	2·45 ± 0·12	1·09 ± 0·05
	10	21	191	239 ± 5	1·75 ± 0·09 71†	0·76 ± 0·04 67†
Dams (lactating)	5	3	214	246 ± 13	2·08 ± 0·09	0·86 ± 0·08
	7	21	215	220 ± 6	1·04 ± 0·07 50†	0·48 ± 0·03 51†

* Means with single standard deviation. † Percentage of amount of strontium-90 at third day after injection.

rats, 20·8 per cent for nursing rats. The difference of radioactivity relative to the injected dose, therefore, increased from 7 to 14 per cent during this time. Relative to the 3 days' value the retention of strontium-90 decreased 29 per cent within the control group, 50 per cent within the lactating group. Ash-weights of the femora of lactating animals relative to those of controls were found to be reduced at the end of the experiment ($P < 0·05$). The observed increase of the mean ash-weight of the femora of controls and the decrease of that of the nursing rats were not significant ($P < 0·1$). The concentrations of radioactivity decreased 33 per cent in the controls and 49 per cent in the lactating group.

The results demonstrate a considerable capacity of nursing rats to mobilize radiostrontium from their skeleton later than 3 days after injection of the isotope.

W. E. KOLLMER
H. KRIEGEL

Heiligenberg-Institut,
Germany.

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Biological Half-life of Cæsium in Man in Acute and Chronic Exposure

THE biological half-life of an element in man is needed for the calculation of maximum permissible concentrations in air and water of radioisotopes of the element[1]. An important assumption in making such calculations is that the body content during chronic exposure may be predicted by mathematical integration with respect to time of the equation describing the retention following a single acute intake. This hypothesis has never been tested in man. The results of recent investigations here demonstrate the essential correctness of the assumption for radioisotopes of cæsium.

In an investigation of the retention of cæsium in man, ten normal volunteer subjects took by mouth a single dose of 1 mg cæsium as the chloride, in aqueous solution containing about 0·6 μc. cæsium-132 and 0·1 μc. cæsium-134. The retention was determined by excretion analysis and by body radioactivity measurement[2], and during the 200 days that have so far elapsed it could be described by the sum of two exponential functions of time, of the general form:

$$R_t = (1-a)e^{-\lambda_1 t} + ae^{-\lambda_2 t}$$

where R_t is the fractional retention at time t days, a is a constant and λ_1 and λ_2 are biological elimination constants in day^{-1}. These findings are in agreement with those of others who have carried out similar investigations[3]. The values of a, λ_1 and λ_2 observed by us were somewhat variable among the ten individuals, and the means and ranges are shown in Table 1.

Table 1. VALUES OF a, λ_1 AND λ_2 AND THE CORRESPONDING BIOLOGICAL HALF-LIVES FOR TEN NORMAL SUBJECTS, FOLLOWING ACUTE EXPOSURE

	a	λ_1 day^{-1}	λ_2 day^{-1}	Biological half-lives τ_1 days	τ_2 days
Mean	0·928	0·693	0·00779	1·0	89
Range	0·88–0·95	0·577–0·866	0·00537–0·0119	0·8–1·2	58–129

The retention of radio-cæsium has also been determined by body radioactivity measurement in the cases of four men who accidentally acquired small burdens of cæsium-134 or cæsium-137. These measurements were begun too late to observe the short-lived component, but the retention decreased exponentially (for four years in one case) until the content had dropped below the limit of detection (for cæsium-134) or until it was no longer distinguishable from the contribution from fall-out (for cæsium-137). The values of τ_2 ranged from 109 days to 149 days[4,5].

When chronic exposure continues for several biological half-lives, the body content, q, increases to an equilibrium value q_∞ given by the equation:

$$q_\infty = m\left(\frac{1-a}{\lambda_1} + \frac{a}{\lambda_2}\right)$$

where m is the daily intake in the same units as q. From the values in Table 1 we see that:

$$\frac{1-a}{\lambda_1} \ll \frac{a}{\lambda_2}$$

and to a close approximation:

$$q_\infty = \frac{ma}{\lambda_2}$$

The presence in man of fall-out cæsium-137 from the food-chain affords an opportunity for the determination of λ_2 from measurements of q[6,7] and m, provided it can be ascertained that the subject is in equilibrium with the diet, that is, that q is not changing when the measurements are made. An alternative to the difficult determination of the daily intake is to measure the daily total excretion, averaged over several days[4]. This has been done for two of the ten subjects of our study, and the results are shown in Table 2; the results for another subject are also included. In calculating the values for λ_2 for subjects R and M, the values shown for a were those obtained from the study following acute exposure. When individual values for a are unknown, adoption of an average value of 0·93 (as in the case of subject C) introduces no greater error than 5 per cent in the values for λ_2 and τ_2. In April 1959 the levels of fall-out cæsium-137 *in vivo* were increasing[6] and subject R was therefore not in equilibrium with his diet. This had the effect of making τ_2 lower than the true value. It should also be noted that the urinary/fæcal excretion ratios for subjects R and M (8·4 and 5·0 respectively) were the same, within experimental error, as in the acute exposure study (8·6 and 5·3 respectively). This showed that the fraction transferred

Table 2 BIOLOGICAL HALF-LIFE OF CÆSIUM IN THREE MEN CALCULATED FROM BODY CONTENT AND MEAN DAILY EXCRETION OF FALL-OUT CÆSIUM-137

	Subject *R* April 1959	Subject *R* April 1961	Subject *M* April 1961	Subject *C* April 1961
q (mμc)	5 5	2 55	6 4	7 65
m (mμc /day) (urine + fæces)	0 081	0 036	0 051	0 042
a	0 885		0 93	0 93 (assumed)
λ_2 (day^{-1})	0 0130	0 0125	0 0074	0 0051
τ_2 (days)	53	55	94	136
τ_2 (days) (from acute exposure study)	58		100	—

from the gastro-intestinal tract to the blood, of cæsium-137 ingested in food, was at least 0 9

The good agreement between the values for τ_2 determined by the two methods validates the integrated form of the acute exposure retention equation as applied to chronic exposure The detailed results of the acute exposure study will be published elsewhere

J RUNDO
JUDITH I MASON
D. NEWTON
B T TAYLOR

Health Physics and Medical Division,
Atomic Energy Research Establishment,
Harwell, Didcot

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BIOLOGY

Second-day Minimum in the Growth Curve of Mice subjected to Magnetic Fields

IN a previous communication[1] it was shown that strong static magnetic fields retard the growth of young mice The maximum weight difference, relative to identically housed controls, is reached after 10–12 days of residence in a homogeneous field of 4,200 œ strength In the first such experiments, conducted in 1948 in the Institute for Experimental Physics of the University of Budapest, as well as in the twelve subsequent experiments conducted on several hundred mice in the laboratory of the Biomagnetic Research Foundation in Evanston, Illinois, a sharp drop in the weight of the mice was observed on the second day of residence in the field, followed by a steep increase up to the base line

To investigate this 'shock effect' of the magnetic field, six 20-day-old female *IRC* strain mice were conditioned for 18 days to the 3$\frac{3}{4}$ in diameter and 1$\frac{7}{16}$ in high cages, used in the electromagnet and in the dummy magnets alike The cages had automatic water and food supply (Rockland Mouse Diet), the floor of the cages was a copper plate covered with 'Mylar', thermally insulated from the lower pole (or the simulated pole of the dummy magnets) and kept through a thermocirculator at 28° C Between cage wall and floor a circular opening provided free movement for the tails of the mice and helped to sweep excrements and food remnants from the cage area When the Varian 4-in electromagnet was energized, it produced a field of 9,400 œ in the centre of the cage and 8,900 œ, 0 5 in from the cage wall The average paramagnetic strength of the field was, therefore, about 1 5 mega-œ²/cm Water cooling of the electromagnet kept the magnet poles 2° below the room temperature of 23° C The dummy cages, situated 2 ft from the magnet, had identical lighting and ventilation conditions

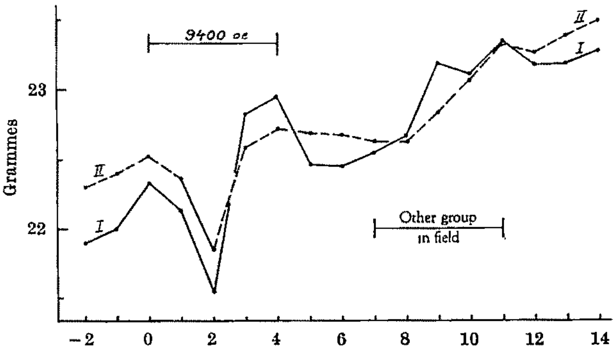

Fig 1 Average growth curve of two groups of mice, alternately exposed each 14 days, for 196 h, to a vertical field of 9,400 œ

The six animals were divided into two groups, three in the magnet cage and three in the dummy magnet cage The following sequence was adopted The magnet was energized from 4 p m Monday until 4 p m Friday, when the magnet group was similarly placed in a dummy magnet cage On the following Monday the previous dummy group was placed in the field from 4 p m Monday until 4 p m Friday and the sequence repeated The weight of the mice was individually measured to 0 1 g every day at 4 p m , when the cages were cleaned

With this schedule, the same group was treated every 14 days for 196 h in the field The 14-day cycle was repeated 5 times and covered the age-period of 5–16 weeks Group I mice started and finished the 5 cycles one week earlier than group II mice, therefore, their average age and weight are less and their average growth rate slightly higher Fig 1 shows, for each group separately the average weight variation during the 14-day cycle It can be seen that the second-day minimum is definitely present The average weight difference between zero hour and 48 h in the field is −0 95 ± 0 29 g, and the difference between 48 h and 96 h in the field is +0 99 ± 0 37 g (Both corrected for average daily weight gain) The existence of the second-day minimum is thus established on a probability-level of 1 3,000 The magnitude of the minimum does not decrease in subsequent cycles, proving that the mice do not get adapted to the 'shock' of the magnetic field, if repeated every 14 days It seems that an after-effect of the field prevails for four days after removal, since during this time the normal growth is arrested

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J M BARNOTHY

Biomagnetic Research Foundation

M F BARNOTHY

College of Pharmacy,
University of Illinois

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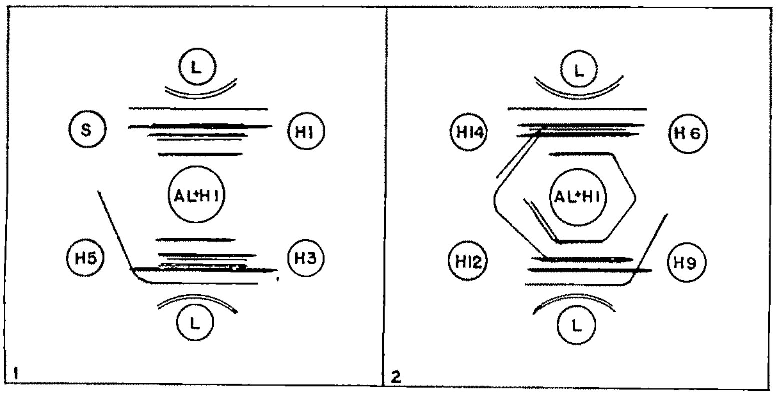
Loss of Liver Cell Antigens in Azo-dye Induced Hepatomas of Rats

THE immunizing ability of several chemically and virus induced tumours and immuno-chemical studies suggest that neoplasms of animals and man are characterized by a changed antigenic composition[1-3] As yet however, no regularity in these changes has been observed, probably due to the small amount of data available It remains unknown whether the cell type of a tumour, the carcinogens used for tumour induction, or the genetic composition of the neoplastic cells affect the kind and degree of these antigenic changes To obtain information about some of these problems we compared the loss of liver antigens in fourteen hepatomas Although these tumours were selected on the basis of the histological features characteristic of hepatomas it is doubtful whether they are strictly comparable. First, the deoxyribonucleic acid (DNA) content of the stemline is not the same in different hepatomas This agrees with our previous observation that even two hepatomas of the same liver lobe can consist of cells with different DNA values[4] Secondly, some of these hepatomas consist of a uniform population of cells whereas others are characterized by a heterogenous population of cells with varying amounts of DNA

Antibodies against liver antigens were produced by four weekly subcutaneous injections of liver homogenates emulsified with equal parts of Freund's complete adjuvant (Difco) into rabbits All antisera have been absorbed with rat sera The antigens have been prepared from saline-perfused livers and hepatomas The antigen-antibody reactions were examined in micro-diffusion plates[5], using 1 per cent agar in buffered physiological saline, and plastic templates for the reactants[6] The absorption was done by placing the absorbent antigen into the wells of the templates before the addition of antiserum The hepatomas were induced in male rats of the Sprague-Dawley strain by the feeding of 4-dimethylaminobenzene (DAB) containing diet. The diet was discontinued 3 weeks before samples were taken

The results shown in Figs 1 and 2 indicate that the hepatoma cells have lost some antigens present in the normal liver of adult male rats This conclusion is based on the following observations Anti-liver serum absorbed with normal liver does not produce precipitin bands when tested against liver antigens, rat serum and hepatomas respectively However, anti-liver sera absorbed with hepatoma-($AL+H1$) produced at least five well-defined precipitin bands when tested against normal liver, but none against rat sera (Fig 1), indicating that the obtained precipitin bands represent antigens associated with normal liver The same anti-liver sera ($AL+H1$) was tested against fourteen hepatomas of different rats Seven samples of the result obtained are shown in Figs 1 and 2 The presence of one ($H5$, $H6$), two ($H9$, $H14$) and three ($H12$) precipitin bands indicates that not all hepatomas have lost the same number of antigens

The present results indicate either a loss or a decrease of liver antigens in hepatoma cells This conclusion is based on the assumption that parenchymal cells were the source of hepatomas and of the antibody producing antigens These observations agree with the results obtained by immunofluorescence techniques on a variety of neoplasms including hepatomas of rats[7], kidney carcinomas of hamsters[8], squamous cell carcinomas of human skin[9] and carcinomas of the human intestine[10]. Thus it appears likely that the loss or decrease of antigens in neoplastic cells is a widespread phenomenon. Considering that most, if not all, of the afore-mentioned tumours consist of cells with an abnormal chromosome complement and (or) an abnormal amount of DNA[11], the question arises whether a correlation exists between the loss of antigens and the alteration of a genome In this connexion it is of interest to note that the examined hepatomas differ not only by their DNA modal values but also by the number of antigens lost



Figs 1 and 2 Precipitin bands in micro-double diffusion plates $AL + H1$, anti-liver serum absorbed with hepatoma No 1, L, liver antigen, S, serum of rats, $H1$, $H3$, $H5$, $H6$, $H9$, $H12$, $H14$, antigens of hepatomas of different rats

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V I. KALNINS
H F STICH

Department of Biology,
Queen's University,
Kingston, Ontario, Canada.

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Rapid Changes in Transpiration in Plants

I SHOULD like to put forward a different interpretation for the experiment illustrated in Figs 2 and 3 of the recent article in *Nature* under the above title[1] It may be presumed that at the start of the second part of the experiment (Fig 3, Curve II) the leaves in the cuvette C of Fig 2 were not flaccid or such an important fact would have been mentioned, therefore the suction pressure in the transpiring mesophyll cells must initially have been less than the full osmotic pressure of their vacuolar sap. and this in turn must have had a value much lower than the 40 atmospheres or so exerted by the 1 M sodium chloride solution (here assumed to be at 20° C) which replaced the water in vessel B The latter change therefore caused a considerable suction pressure gradient *down* the plant from the leaf mesophyll cells to the solution in B and water must have moved out of the roots into the salt solution—any increase in water permeability of the roots would only accelerate this water loss At the same time the mesophyll cells continued to transpire, and at an enhanced rate, as shown by the curve They must, therefore, have been losing turgor rapidly

The simplest hypothesis is that the early changes in transpiration rate were due to stomatal movements, and the mechanism suggested for these[2] is based on Darwin and Pertz[3], namely, the increase in suction pressure of the wilting mesophyll cells withdraws water from the epidermal cells, including the subsidiary cells, and the rapid loss of turgor of the latter allows the guard cells to bulge outwards, opening the stomatal pores, closure then follows as the subsidiary cells, by virtue of their increased suction pressure, withdraw water from the guard cells Such 'passive' stomatal opening is not observed with gradual wilting, it may be supposed that under these conditions the suction pressures in subsidiary cells and guard cells remain so near to equilibrium that no opening movement can be detected but only the gradual closure with loss of guard cell turgor Presumably for curve I (Fig 3) the water-strain imposed by dipping the tip of a single leaf in 1 M sodium chloride was much less than that due to the immersion of the whole root-system in it, but the fact that the initial rate of transpiration was equal to the maximum of curve II also suggests that the stomata may already have been as wide open as was mechanically possible

It seems essential to follow the course of stomatal movement, as well as of transpiration, if such experiments are to help elucidate the so-called 'Ivanoff effect'[1] It may well be, for example, that the leaves of conifers, mentioned earlier in the article as not showing this effect on transpiration, do not have the initial 'passive' stomatal opening on wilting because of their rigid structure and thick epidermal cell walls

The 'Ivanoff effect' on transpiration when a leaf is detached in air must be in part due to the initial stomatal opening and in part to release of tension in the xylem sap The latter contribution is, however, likely to be extremely small Even if we suppose that the mesophyll cells were just flaccid before excision, with a vacuolar osmotic pressure of, say, ten atmospheres, and that they at once became fully turgid when the petiole was severed, a simple calculation will show that the vapour pressure in equilibrium with their suction pressure would increase only by 0 8 per cent With an ambient relative humidity of, say, 70 per cent, the transpiration would only increase 2 7 per cent if the stomatal resistance did not change This is obviously an over-estimate, for the cells could not become fully turgid while transpiration continued

O V S HEATH

University of Reading,
Horticultural Research Laboratories,
Shinfield Grange,
Shinfield, Berks

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PROF HEATH's interpretation of my results is based on a hypothesis which, according to my experiments, is no longer valid He assumes that water transport in the plant is mediated by a cell-to-cell mechanism and that it is the suction potential of the mesophyll cells which determines the rate of water movement through the plant I imagine water transport from the root to the atmosphere in easily movable columns in the xylem and cell walls The rate of transport then depends on the gradient of suction potential from the root medium to atmosphere and the permeability or resistance to water movement of the intervening stages The cells of the plant obtain their water from the cell wall reservoir The uptake of water does not depend on the suction potential of the mesophyll cells but on that of the air in the intercellular spaces, which is a function of that in the ambient air and the stomatal resistance However, as the water content of both the mesophyll cells and the guard cells, at equilibrium, is determined by the cell-wall water potential (referred to earlier[1] as the cell-wall water deficit), the impression is obtained that the guard cells are regulated by the mesophyll cells When equilibrium does not occur, as can happen when a stress is suddenly released, the water content of the guard cells and the mesophyll cells may vary independently, if they have different permeabilities The results which have been obtained experimentally agree with the supposition that the guard cells are more permeable than the mesophyll cells

On the assumption that it is the suction potential of the mesophyll cells in the leaf which determines water uptake, Heath has supposed that the addition of sodium chloride to the root medium to raise the osmotic pressure to 40 atm must result in a suction pressure gradient down the plant and water movement in the same direction. On the same assumption, one might expect the same result when a shoot or leaf is cut off or the petiole lifted out of water, since the cut surface is exposed to the much lower water potential of the air However, as shown in my Table 1 and by Allerup and Nielsen[2], movement of water continues up towards the leaf We have many times obtained the transitory increase in transpiration in response to increases in the osmotic pressure of the root medium of 1–5 atm (refs 3 and 4) which could not, even on Heath's supposition, reverse the gradient of suction potential. I would suggest that the suction gradient is always in the direction of water movement up the plant in the examples under discussion because of the much lower water potential of the ambient air

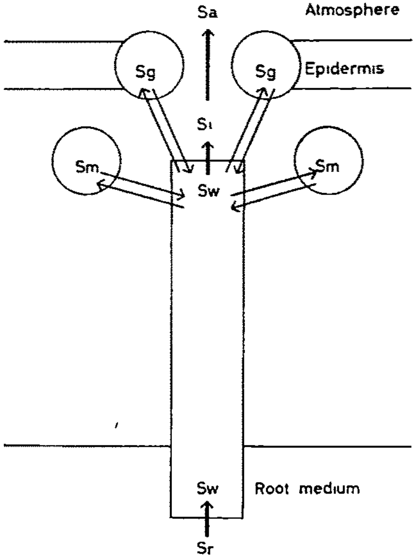

Fig 1 Schematic representation of water transport in a plant Suction potentials in root medium (*Sr*), cell wall system (*Sw*), intercellular spaces (*Si*), surrounding air (*Sa*), guard cells (*Sg*), mesophyll cells (*Sm*)

A large increase in suction potential in the root medium of 40 atm gives rise to a corresponding increase in suction potential of the water in the leaf cell walls and intercellular spaces It is the latter suction potential which causes water stress in the plant and not the suction potential of the vacuolar sap in the mesophyll cells, this increases secondarily In a severed leaf there is no need for full turgidity before the transitory opening of the stomata occurs That turgidity does not in fact increase is shown by the determinations of relative turgidity (Table 1) When the full effect of the sodium chloride is realized, the increase in cell-wall water deficit causes reduction in stomatal aperture As the permeability barrier in the root can scarcely be exposed to the solution of high concentration instantaneously (the water in the AFS will dilute the solution), I suppose that the decrease in water uptake is preceded by a temporary increase due to an increase in root permeability This makes it possible to explain the Ivanoff effect and the increase obtained by addition of an osmotically active substance in the same way

In Fig 1 the proposed idea is summarized in a simplified form The main pathways of water are indicated by the arrows At the root barrier, the evaporation boundary between cell wall and intercellular spaces, and the diffusion barrier in the stomatal openings, the transport is illustrated by single arrows because it can be presumed to take place in practice only in one direction, whereas dynamic equilibria normally exist between the cell-wall reservoir and the vacuoles of both the mesophyll cells and the guard cells Transport to mesophyll cells and guard cells differs only in that the latter takes place faster, facilitated either by higher permeability or a metabolic driving force

HENRY RUFELT

Institute of Physiological Botany,
University of Uppsala,
Uppsala,
Sweden

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MICROBIOLOGY

Accumulation of Oxytetracycline Relevant to its Bactericidal Action in the Cells of *Escherichia coli*

IN the course of work on the effect of oxytetracycline on the growth of *E coli B*-151-1 using a nepherometer, we observed a remarkable increase in turbidity when the cells were incubated at high concentration of oxytetracycline over 100 μg/ml in nutrient broth Results showed that this increase in turbidity was not caused by the increase of number of cells, but was due to a remarkable accumulation of oxytetracycline in the cells of *E coli* Cells of *E coli B*-151-1 or *E coli K*-12 were collected from 15–16 h culture by shaking at 30° C in nutrient broth (*p*H 7 2), and were washed twice with distilled water Usually Monod's test tube was used for both growth and accumulation tests unless otherwise indicated Usually, cells of *E coli* (about 1 mg dry weight) were suspended in 10 ml of medium in 30 ml Monod's test tube, and the tube was shaken at 30° C on a shaking machine The resting cells were incubated with various concentrations of oxytetracycline in nutrient broth (*p*H 7 2) at 30° C, and the growth was measured using a Kotaki nepherometer The amount of oxytetracycline was expressed as that of its hydrochloride

The increase in turbidity began immediately as if the cell growth were promoted in the presence of high concentration of oxytetracycline (100–400 μg/ml) Microscopic counting analysis, however, showed that the number of cells did not increase during the incubation The cells incubated with high concentration of oxytetracycline became yellow because of an abundant accumulation of oxytetracycline, and they were found to have higher electron density than the normal cells by electron microscopic examination Further experiments were carried out using a nitrogen-free simple medium containing 1 per cent glucose, 0 2 per cent K_2HPO_4 and 0 02 per cent $MgSO_4 7H_2O$ The *p*H of the medium was adjusted to 6 5 As shown in Fig 1, the increase in turbidity was remarkable when the cells were incubated with glucose, K_2HPO_4 and $MgSO_4$ Without glucose or $MgSO_4$ the increase in turbidity was little When the heat-treated cells (1 min in boiling water) were used instead of intact cells, we did not observe any appreciable increase in turbidity, and the same result was obtained when the incubation was carried out without the cells The presence of K_2HPO_4 seemed unnecessary for increase of turbidity since a large amount of such increase also occurred in the presence of glucose and $MgSO_4$ of the *p*H of the medium was about 6 5 Usually K_2HPO_4 was used for its buffer action

After incubation, the cells were centrifuged and washed with distilled water once and were then suspended in 5 ml of distilled water When the cells were heated in the boiling water, a large decrease in turbidity was observed only for the cells which were incubated with high concentrations of oxytetracycline in the medium containing glucose, K_2HPO_4 and $MgSO_4$ Electrophotometric analysis showed that the absorption spectrum of the boiled extract and that of heated oxytetracycline hydrochloride solution were almost identical Based on these results, we usually estimated the accumulated oxytetracycline by measuring the optical density at 340 mμ or 260 mμ of the extracts obtained by boiling the cells which accumulated oxytetracycline with water for 5 min and comparing its value (*A*) with that of the standard oxytetracycline solution which was also heated in the same condition The endogenous value (*B*) was determined with the boiled extracts of cells which were incubated without oxytetracycline and was subtracted from the value (*A*) giving the amounts of oxytetracycline accumulated (*A*–*B*) This value well indicated the relative amount of oxytetracycline accumulated, although it may not show the exact amount of oxytetracycline accumulated

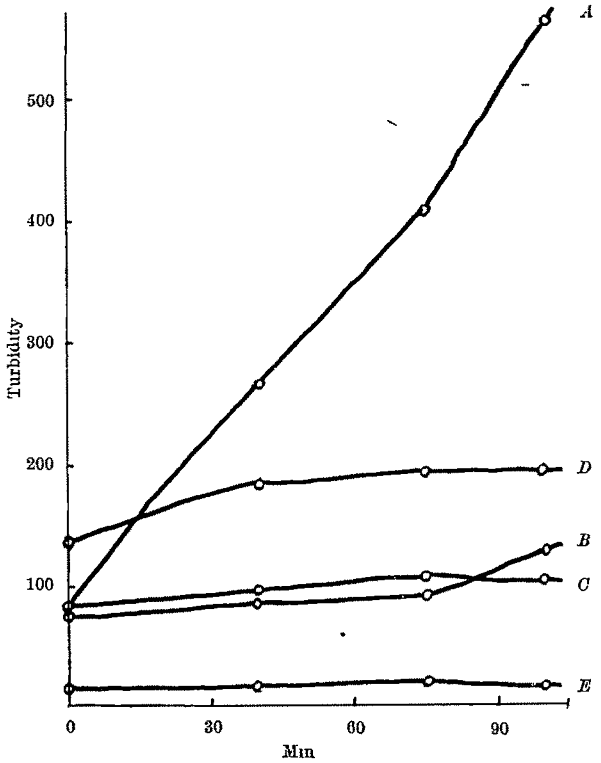

Fig 1 Increase in turbidity of cells of *E coli B*-151-1 owing to accumulation of oxytetracycline Reaction mixture contains 1 ml cell suspension (1 6 mg dry weight) 1 ml oxytetracycline hydrochloride solution (4 mg/ml) and 1 ml of 10% (w/v) glucose, 2% K_2HPO_4 (*p*H 6 5), 0 1% $MgSO_4 7H_2O$, respectively, in the total volume of 10 ml Medium *A*, glucose 1%, K_2HPO_4 0 1%, $MgSO_4 7H_2O$ 0 01%, *B*, glucose 1%, K_3HPO_4 0 2%, *C*, K_2HPO_4 0 2%, $MgSO_4 7H_2O$ 0 01%, *D*, glucose 1%, K_2HPO_4 0 2%, $MgSO_4 7H_2O$ 0 01% (heated cells), *E*, glucose 1%, K_2HPO_4 0 2%, $MgSO_4 7H_2O$ 0 01% (without cells)

Table 1 ACCUMULATION OF OXYTETRACYCLINE IN *E coli B*-151-1

Incubation condition was the same as described in Fig 1 The amounts of oxytetracycline was measured from the value of optical density at 340 mμ of boiled extracts of the cells which accumulated oxytetracycline

Medium	Incubation time	
	40 min	100 min
A	305 μg/mg dry weight	464 μg/mg dry weight
B	35	99
C	70	75

From the results shown in Fig 1 and Table 1, it is apparent that oxytetracycline remarkably accumulated in the cells of *E coli*, and consequently the turbidity of the cells greatly increased when the cells were incubated with high concentration of oxytetracycline in the medium containing glucose, K_2HPO_4 and $MgSO_4$ This may be the first indication of accumulation of large amounts of oxytetracycline in bacterial cells Optimum *p*H for the accumulation was about 6 5 At a low temperature, such as 2° C, the accumulation was very small Since glucose was needed for the accumulation, supply of energy seemed to be necessary This speculation was supported from the experiment using inhibitors Metabolic inhibitors such

Table 2. CORRELATION OF PROMOTION BETWEEN OXYTETRACYCLINE ACCUMULATION AND ITS BACTERICIDAL ACTION

Incubation was carried out using Monod's test tube in aerobic condition at 30° C for 90 min Monod's test tube contains glucose, K_2HPO_4 (*p*H 6 5), $MgSO_4 7H_2O$, oxytetracycline and the cells of *E coli K*-12 (1 mg dry cells) as shown in Table 2 in a total volume of 10 ml

Incubation medium	Oxytetracycline added	Oxytetracycline accumulated per mg dry cells	Viable cells/ml	Viable cells (%)
Glucose 1% K_2HPO_4 0 2% $MgSO_4 7H_2O$ 0 01%	—	—	$3\ 31 \times 10^8$	
	200 μg/ml	190 μg	$1\ 69 \times 10^8$	51
Glucose 1%	—	—	$2\ 94 \times 10^8$	
K_2HPO_4 0 2%	200 μg/ml	53 μg	$2\ 38 \times 10^8$	81
K_2HPO_4 0 2% $MgSO_4 7H_2O$ 0 01%	— 200 μg/ml	— 27 μg	$3\ 26 \times 10^8$ $2\ 94 \times 10^8$	90

as 2,4-dinitrophenol (10^{-4} M) and sodium azide (10^{-2}M) inhibited the accumulation, while they did not inhibit the oxidation of glucose by the resting cells and, moreover, promoted it at these concentrations

The next experiment was carried out to clarify whether the bactericidal action of oxytetracycline was strengthened in such a condition in which the accumulation of oxytetracycline was greatly promoted The incubation conditions and the results are given in Table 2 Viable cells were counted by the usual plate culture method As shown, bactericidal action of oxytetracycline was greatest in the presence of glucose, K_2HPO_4 and $MgSO_4$ at 30° C From these results, it is assumed that permeation and accumulation of oxytetracycline in the cell are relevant to the bactericidal action of oxytetracycline and are dependent on the energy-yielding system If supply of energy is necessary for the entry and accumulation of oxytetracycline, it is curious that bacteria take antibiotics into the cells using energy Perhaps bacteria can absorb the antibiotics at random since they cannot discriminate between such antibiotics and a certain nutrient as in the case of amino-acids and their analogues Recently, Hancock has reported the decreased uptake of streptomycin by the cells in the conditions such as anaerobiosis and presence of carbon monoxide[1]

KEI ARIMA
KAZUO IZAKI

Department of Agricultural Chemistry,
Faculty of Agriculture,
University of Tokyo

[1] Hancock, R , *J Gen Microbiol* , 28, 493 (1962)

VIROLOGY

Isolation of Louping ill Virus from the Red Grouse

WILD birds play an important part in the build-up and dissemination of some arboviruses[1] In the sub-group, the tick-borne encephalitis complex, evidence has been obtained from the U S S R [2], Czechoslovakia[3] and Holland[4] that some avian species are infected in Nature or can be infected with members of this group We report here the isolation from the red grouse (*Lagopus scoticus*) of louping ill virus, one of the members of the tick-borne complex, together with the results of some preliminary experiments on the susceptibility of this species to infection with louping ill virus

In June 1962 we examined a red grouse which had been found moribund on a hill in Morayshire In the course of serological investigations it was found to have a hæmagglutination inhibiting antibody titre of 1 80 against 4 units of louping ill virus antigen, and virus (*GRA*) was recovered from a 10 per cent brain emulsion in saline inoculated intracerebrally into suckling mice No virus was recovered from liver, spleen or lung During the period August–October 1962 we examined blood samples from 181 grouse killed over three areas in North Scotland —Speyside around Grantown-on-Spey, Morayshire, Braemar area, Aberdeenshire, and east Sutherland Louping ill is known to be endemic in parts of the first and last of these areas All the samples were from shot birds and blood was collected on filter paper strips The eluates were tested against 4 units of antigen for hæmagglutination inhibiting antibody The results are shown in Table 1

Table 1 HÆMAGGLUTINATION INHIBITING TITRES OF 181 RED GROUSE AGAINST LOUPING ILL VIRUS ANTIGEN

Area	No examined	Positive > 1 10	Negative	Reciprocal antibody titres 10–40	40–80	80–160
Speyside	88	10	78	7	2	1
Braemar	47	3	44	3	0	0
Sutherland	46	4	42	3	1	0

Some of the birds from the Speyside group were sent to the laboratory and virus (*GRB*) was isolated from the brain of one bird (hæmagglutination inhibiting titre 1 80) Both viruses isolated were bile-sensitive

The results of serological tests with these two isolates *GRA* and *GRB* in comparison with the mouse adapted (limb) strain of louping ill are shown in Table 2 We have failed so far to produce a hæmagglutinating antigen from one of them (*GRB*) by standard methods[5]

Table 2 SEROLOGICAL COMPARISON OF VIRUSES *GRA* AND *GRB* WITH LIMB STRAIN OF LOUPING ILL

Serum*	Virus	Hæmagglutination inhibiting	C F (fixation area)†	Neutralization Suckling mouse	Plaque	Metabolic inhibition
Limb	Limb	640	64/32	●	+	+
GRB	Limb	640	64/32	±	+	+
Limb	*GRB*	●	128/64	+	●	+
GRB	*GRB*	●	128/64	●	●	+
Limb	*GRA*	320	32/64	+	●	+
GRB	*GRA*	160	64/32	+	●	+

* Antisera made by inoculation of sheep with mouse brain virus suspension

† Highest dilution of serum fixing complement with any dilution of antigen/highest dilution of antigen reacting with any dilution of serum

Two grouse *X* and *Y*, one a wild bird, the other hatched in captivity and maintained at the Nature Conservancy Unit of Grouse and Moorland Ecology, Blackhall, Banchory, Kincardineshire, were made available to us by Dr D Jenkins, Natural History Department, Marischal College, Aberdeen, for experimental purposes A persistent viræmia developed in both those birds inoculated with the standard limb strain and they died within 8 days of inoculation (Table 3)

Table 3 INFECTION OF TWO RED GROUSE WITH LOUPING ILL VIRUS

	Grouse *X*		Grouse *Y*	
Pre-inoculation hæmagglutination inhibiting titre	Nil		Nil	
Subcutaneous inoculation dose of louping ill (limb)	25,000 mouse LD_{50}		2,500 mouse LD_{50}	
Post-inoculation blood samples (h)	Virus isolated	Hæmagglutination inhibiting titre	Virus isolated	Hæmagglutination inhibiting titre
7	+	–	+	–
31	+	–	+	–
79	+	1 10	+	1 80
168	Died		+	1 80
192			Died	

At death, grouse *Y* had 3×10^5 mouse LD_{50} virus/g liver, 3×10^5 mouse LD_{50} virus/g spleen, and 3×10^6 mouse LD_{50} virus/g brain tissue Histological sections of the brain examined showed marked congestion of the blood vessels but no obvious damage to nerve cells

We conclude that the red grouse can harbour louping ill virus in Nature, that viræmia sometimes occurs and in some circumstances the infection can be lethal to this species

These strains are held by Dr C. E Gordon Smith, Arbor Virus Research Unit, London School of Hygiene and Tropical Medicine.

H Williams
H. Thorburn
G S Ziffo

Bacteriology Laboratory,
Northern Regional Hospital Board,
Inverness

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[4] Van Tongeren, H A E , and Timmers, W C , *Arch gesamte Virusforschung*, 10, 606 (1961)

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PSYCHOLOGY

Effects of Field Brightness on an Induced Negative After-image Phenomenon

If a coloured stimulus, a white stimulus, and relative absence of stimulus are repeatedly presented in that order for 0 017, 0 10 and 0 083 sec respectively, there are values of stimuli brightness at which only the complement of the coloured stimulus is perceived This effect, first reported by Bidwell in 1897[1], has facilitated investigations of after-images, the decision as to which of two complementary colours is seen is more accurately made than are decisions as to the colour, intensity, or duration of after-images The study of induced negative after-images is of interest, as these phenomena depend on the relation between excitatory and inhibitory retinal reaction and may be used in physiological and pharmacological studies

In 1947 Lehmann[2] devised apparatus by means of which the brightness of the coloured stimulus was maintained at a fraction of that of the white stimulus and the effective illumination below which only the coloured stimulus and above which only its complement was seen Both Lehmann[2] and Kaplan[3], who used somewhat similar apparatus, determined that this threshold could be altered by pharmacological agents The equipment used in these investigations permitted the definition of only one set of brightness values of coloured and white stimuli at which reversal of colour perception occurs Keeler[4] constructed apparatus that would permit independent control of the brightness of the coloured and white stimuli and determined the curve of brightness of coloured versus brightness of white stimuli at which colour reversal occurs (A typical such curve is shown in Fig 1 as 'brightness of field' = 1/2 white stimulus)

In all of the apparatus in the investigations noted, the coloured-white-'black' stimuli cycle was presented on a field the brightness of which was a function of the brightness of the white stimulus (As the white stimulus is presented half the time, effective field brightness is about half that of this stimulus) In this circumstance, a change in brightness of the white stimulus changes field brightness which, in turn, alters the reactivity of that part of the retina receiving the image of the stimuli cycle This might be empirically satisfactory for some purposes, but the presentation of the stimuli-cycle on a field of constant brightness would give additional information as to the relations between white and coloured stimulus required for reversal of colour perception

The independent control of field brightness was also desirable, as this would make it possible to maintain the coloured stimulus at a constant value and determine any effects of field brightness on the brightness of the white stimulus necessary for reversal of colour perception

The equipment which generates the stimulus sequence[4] consists essentially of a whirling episcotister with a 180° white segment, a 130° black segment, and a 30° aperture through which an opal-glass target may be seen The episcotister is illuminated from above, the intensity of this illumination determines the brightness of the white stimulus The coloured stimulus is produced by trans-illuminating the opal-glass target The black segment provides relative absence of stimulus An aperture smaller than the meiotic human pupil in the eyepiece prevents changes in the subject's pupillary diameter from affecting retinal illumination. This equipment was revised by interposing a white screen, illuminated from above, with a 1° central aperture between the eyepiece and the episcotister A diaphragm, between the screen-illuminant and the screen, controls field brightness The system, as seen from the eyepiece, consists of a 1° central area in which the coloured-white-'black' cycle is presented visualized on a 5° white field of independently controlled brightness

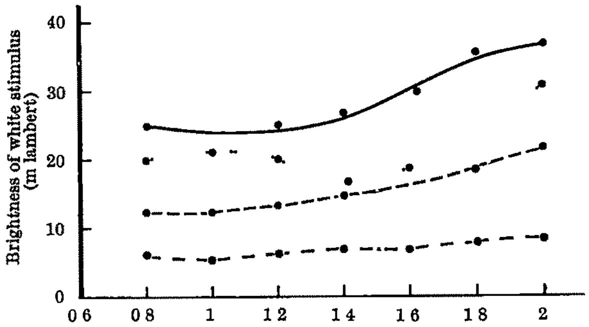

Fig 1 Effects of field brightness on the brightness of red and white stimuli at which, when the brightness of the red stimulus is held constant and that of the white stimulus increased, green rather than red is first perceived Red (625 mμ), white (3,000° K) and black stimuli are repeatedly presented in that order for 0 017, 0 10 and 0 083 sec respectively to the central 1° of a 5° white (3,000° K) field. ——, 50 m lamberts, , half-white stimulus, – – –, 25 m lamberts, – –, O

Some results obtained are presented in Fig 1, the curve generated when the brightness of the white stimulus determines that of the field is also included An orderly array of curves of brightness of the coloured stimulus versus the brightness of the white stimulus necessary for reversal of colour perception is generated With a coloured stimulus of constant brightness, an increase in field brightness requires an increase in the brightness of the white stimulus for reversal of colour perception The slope of the curves with constant field brightness is less than that in the situation where the white stimulus also determines the field This may be explained on the basis that, in this latter circumstance, an increase in the brightness of the coloured stimulus will necessitate an increase in the brightness of the white stimulus and thus, in field brightness which will require a yet further increase in the brightness of the white stimulus

The results shown are for one subject typical of six All determinations were made with a red (625 mμ) stimulus, the brightness of the white stimulus was increased until green rather than red was perceived Absolute values vary among observers and in the same observer over time, but the relations among stimuli and field brightness noted are consistent

Most significantly, these data indicate that the device may be quite responsive to changes in the sensitivity of the part of the retina which receives the image of the field, such changes would be equivalent to changes in field illumination This is crucial in the interpretation of drug-induced changes in the colour reversal threshold Even when the stimuli cycle is presented to the fovea (and thus to the cone-based systems) changes in the reactivity of the peri-foveal region (and thus rod-based systems) may alter the endpoint

Martin H Keeler

Department of Psychiatry,
North Carolina Memorial Hospital,
University of North Carolina, Chapel Hill

[1] Bidwell, S , *Proc Roy Soc* , 61, 263 (1897)

[2] Lehmann, H E , *Science*, 112, 199 (1950)

[3] Kaplan, S , *Psychiat. Res Rep Amer Psychiat Assoc* , 12, 104 (1960)

[4] Keeler, M H , *Nature*, 196, 1343 (1962)

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 14

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p m —Dr Brian Ackner "Disorders of Personality" *

Institution of Electrical Engineers (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Patents and British Industry Today", opened by Mr P Ford

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Modern Advances in the Design and Use of Automatic Vending Machines"

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 6 p m —Mr Eric Shipton, C.B E "Patagonian Adventure" (colour films with commentary)

Society of Chemical Industry, London Section (joint meeting with the Surface Activity Group, at 14 Belgrave Square, London, S W 1), at 6 p m —Dr J M Corkill "The Structure of Thin Detergent Films"

Plastics Institute, Engineering Sub-Group (at "The Ring", Blackfriars Road, London, S.E 1), at 6 30 p m —Mr. P J Gough "Toolmaking by Spark and Electrochemical Erosion".

Tuesday, October 15

The Wellcome Trust (in the Wellcome Historical Medical Library, The Wellcome Building, Euston Road, London, N W 1), at 1 p m —Dr Edwin Clarke "Hippocrates as a Clinician" * (First of twelve lectures on "The History of Medicine" Further lectures on October 17, 22, 24, 29, 31, November 5, 7, 12, 14, 19 and 21)

University of London (at Queen Mary College, Mile End Road, London, E 1), at 5 p m —Prof M F Hawthorne (University of California) "Recent Advances in the Chemistry of the Higher Hydrides of Boron" (Further lecture on October 17)*

University of London (at the Institute of Education, Malet Street, London, W C 1), at 5 15 p m —Dr D M Lee "Perception, Intuition and Insight".*

Institution of Civil Engineers (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr P S A Berridge "Factors Governing the Choice between Repairing, Strengthening and Reconstructing Railway Girder Bridges".

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Dr J R Mortlock "Computers for Engineers" (Chairman's Address)

Royal Institution (at 21 Albemarle Street, London, W 1), at 5 30 p m —Sir Lawrence Bragg, F R S "Electrical Charges and Electrical Currents" (Afternoon Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties Further lectures on October 16, 22 and 23)

University of London (in the Physiology Lecture Theatre, Royal Free Hospital School of Medicine, Hunter Street, Brunswick Square, London, W C 1), at 5 30 p m —Prof P K Anokhin (Moscow) "New Data of the Specific Character of Ascending Activation on Brain Cortex" *

Institution of Mechanical Engineers, Applied Mechanics and Steam Plant Group (at Rugby College of Engineering Technology, Rugby), at 6 30 p m —Mr M B Coyle and Mr S J Watson. "The Fatigue Strength of Turbine Shafts with Shrunk-On Discs".

Wednesday, October 16

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 2 p m —Dr J R Hobbs "Immunoglobulins and their Paraproteins" *

Institute of Navigation (at the Royal Geographical Society, 1 Kensington Gore, London, S W 7), at 4 p m —Annual General Meeting Mr D G King-Hele "Theories About the Shape of the Earth" (Third Duke of Edinburgh's Lecture).

Royal Society of Medicine, Comparative Medicine Section (at 1 Wimpole Street, London, W 1), at 5 p m —Mr K C Sellers "Breeding for Feeding, Present and Future Food Animal Populations" (Presidential Address)

University of London (in the Physiology Lecture Theatre, Royal Free Hospital School of Medicine, Hunter Street, Brunswick Square, London, W C 1), at 5 30 p m —Prof P K Anokhin (Moscow) "New Information on the Mechanisms of the Conditioned Reflex" *

British Coal Utilisation Research Association (at the Institution of Civil Engineers, Great George Street, London, S W 1), at 5 30 p m —Dr D T A Townend, C B E "Some Reflections on Combustion" (Twelfth Coal Science Lecture)

Society for Analytical Chemistry, Microchemistry Group (at "The Feathers", Tudor Street, London, E C 4), at 6 30 p m —Discussion Meeting on "The Determination of Boron" introduced by Dr H J Cluley and Mr M R Hayes

Thursday, October 17

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr T A Quilliam "Information from the Skin—How is Vibration Detected?" *

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Dr M J Jaycock and Dr R H Ottewill "Adsorption of Ionic Surface Active Agents by Charged Solids", Prof I N Plaksin and Mr R Sh Shafeev "Influence of Surface Properties of Sulphide Minerals on Adsorption of Flotation Reagents", Prof R Higgins and Dr T J B Jones "Viscosity Characteristics of Rhodesian Copper Smelting Slags"

Linnean Society of London (at Burlington House, Piccadilly, London, W 1), at 5 p m —Miss A G C Grandison "Herpetological Travels in Nigeria", Dr D C Smith "Some Recent Studies in the Biology of Lichens"

University of London (at Goldsmiths' College, New Cross, London, S E 14), at 5 p m —Prof. Herbert Dingle "The Impact of Science in the Humanities" *

Institution of Civil Engineers (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Informal Discussion on "Recent Developments in Piling Theory"

University of London (in the Beveridge Hall, Senate House, London, W C 1), at 5 30 p m —Sir Charles Dodds, F R S "Stilbœstrol and After" * (First of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

British Institute of Radiology (at 32 Welbeck Street, London, W 1), at 6 p m —Discussion Group meeting on "X-Ray Departments and the Ministry of Health" 8 p m —Prof C B Allsopp "Nothing New?" (Presidential Address)

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S W 1), at 6 p m —Dr J D Watt "The Composition and Pozzolanic Properties of Pulverised Fuel Ash"

Royal Institute of Chemistry, London Section (joint meeting with the Brighton College of Technology Chemical Society, at the College of Technology, Lewes Road, Brighton), at 7 p m —Prof R N Haszeldine "Carbanions to Carbenes"

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W 1), at 7 30 p m —Dr Charles Wilcocks Presidential Address

Friday, October 18

Biochemical Society (in the Department of Physiology and Biochemistry, The University, Southampton), at 9 15 a m —Colloquium on "Comparative Biochemistry and Physiology" 2 15 p m —Scientific Papers

University of London (in the Physiology Lecture Theatre, Royal Free Hospital School of Medicine, Hunter Street, Brunswick Square, London, W C 1), at 5 30 p m —Prof P K Anokhin (Moscow) "Neurophysiological Bases of the Therapeutic Action of Anæsthetic and Psychotropic Drugs" *

Occam Society (at the Northampton College of Advanced Technology, St John Street, London, E C 1), at 6 15 p m —Discussion on "The Philosophical Importance of the Unconscious" opened by Mr Paul de Berker

Royal Institution (at 21 Albemarle Street, London, W 1), at 9 p m —Prof S K Runcorn "How the Continents Drift"

Friday, October 18—Saturday, October 19

Faculty of Radiologists, Radiological Section of the Royal Society of Medicine, and the British Institute of Radiology (in the Barnes and West Halls, Royal Society of Medicine, 1 Wimpole Street, London, W 1 (Radiodiagnostic Meetings), and Cowdray Hall, Royal College of Nursing, 1a Henrietta Place, London, W 1 (Radiotherapy Meetings))—Meeting on "The Planning of Diagnostic X-ray and Radiotherapy Departments"

Saturday, October 19

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Dr Eva Crane "Dances and other Forms of Communication Between Bees" *

Monday, October 21

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Dr J Metivier and Dr B J Heywood "Vamidothion—a New Systemic Insecticide"

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W C 2), at 6 30 p m —Mr R C Foss "Feedback" (Chairman's Address)

Royal Geographical Society (at 1 Kensington Gore, London, S W 7)• at 8 15 p m —Mr Tom Harrisson "Inside Borneo"

Monday, October 21—Tuesday, October 22

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at the Royal Commonwealth Society, Craven Street, London, W C 2), at 11 15 a m on Monday and 10 a m on Tuesday—Symposium on "Residual Porosity in Powder Metallurgy"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Senior Lecturer (with an honours degree in mathematics and preferably some lecturing or industrial experience) in Mathematics—The Director of Education, The Polytechnic, 309 Regent Street, London, W 1 (October 18)

Electronic Computer Operator (education to G C E "A" level standard in mathematics, and preferably previous experience on digital computers), to be concerned with the preparation and running of programmes on the KDF9 computer which is to be installed in the College later this year—The Secretary, Royal College of Advanced Technology, Salford 5, Lancs, quoting Ref MA/11 (October 21)

Assistant Lecturer (preferably with an interest in one of the following fields taxonomy, ecology, plant physiology or microbiology (bacteriology)) in Botany—The Secretary, Birkbeck College (University of London), Malet Street London, W C 1 (October 28)

Research Assistant (with experience in embryology and/or biophysics) in the Department of Zoology, for work on the effect of drugs on the uptake and distribution of water in the developing embryo—Dr P H Tuft, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh (October 31)

Senior Lecturer, a Junior Lecturer, and a Graduate Assistant in the Department of Electrical Engineering, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, October 31)

Lecturer in Veterinary Physiology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 1)

Scientific Officer/Senior Scientific Officer (with a good honours degree in physiology or biochemistry, and preferably an additional veterinary qualification and research experience in the field of animal metabolism) in the Physiology Department—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen (November 1)

Lecturer (preferably competent to lecture in logic, including traditional logic) in Philosophy at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 5)

Lecturer and an Assistant Lecturer (with research interests in the high energy physics field) in the Department of Physics—The Registrar, The University, Manchester 13 (November 15)

Lecturer or Assistant Lecturer (preferably with interests in the field of cytology or cytogenetics) in Botany—The Secretary, The University, Aberdeen (November 18)

Chair of Medical Microbiology at St Thomas's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W C 1 (November 20)

Senior Lecturer (with a Ph D in physics or equivalent qualification, and preferably some teaching experience) in Physics or Theoretical Physics in the School of General Studies, Australian National University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Australia, November 25)

Assistant Professor (with a Ph D or equivalent in animal physiology, and preferably a minor in biochemistry) of Animal Physiology, for duties which include fundamental and applied research with farm and laboratory animals with emphasis on environmental physiology, teaching, and limited extension work—Head, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (November 30)

Chair of Electronics—The Secretary, The Queen's University, Belfast, Northern Ireland (November 30)

Hospital Microbiologist (medical graduate with suitable experience in microbiology), to assist the Director (Prof N F Stanley) in the administration of the routine laboratory and clinical services of the Hospital Department of Microbiology, to act in the absence of the Director, and to carry out special investigations from time to time—The Administrator, Royal Perth Hospital, Perth, Western Australia (November 30)

Lecturers or Senior Lecturers (2) in Psychology at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, November 30)

Senior Lecturer in Statistics at the University of Melbourne—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, November 30)

Biochemist (with a first- or second-class honours degree in chemistry, or veterinary qualifications with additional specialization in biochemistry, and preferably experience of the application of modern biochemical techniques to the study of animal diseases), with the Government of Uganda, to work with members of the research staff in establishing modern biochemical techniques for research into adaptation and diseases of domestic livestock in Uganda—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, Victoria, London, S W 1, quoting Ref RC 217/153/04/61

Executive Officer (male, preferably not under 30, and fully conversant with the technical aspects of medical laboratory practice and equipment), for work at the Medical Research Council's Central Store, Colindale Avenue, London, N W 9—Medical Research Council, 20 Park Crescent, London, W 1, quoting Ref E 10/29

Head (with good qualifications and experience) of the Science Department—The Headmaster, The School, Oundle, Peterborough, Northants

Post-doctoral Fellow (with a sound background in physical organic chemistry and/or modern analytical techniques) in Hæmoprotein Chemistry—Dr G R Schonbaum, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada

Research Assistant (with a good honours degree in chemistry or physical chemistry (or a good G R I C), and preferably some research experience), for work on the cross-linking of high polymers in solution—Prof R H Peters, Department of Polymer and Fibre Science, The Manchester College of Science and Technology, Sackville Street, Manchester 1

Research Fellow in the Department of Anatomy, to study the physiology of reproduction and in particular to investigate the control of fertility in primates and lower mammals—The Assistant Registrar, The Medical School, The University, Birmingham 15

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Scottish Plant Breeding Station Record 1963 Pp 181 (Pentlandfield, Roslin Scottish Society for Research in Plant-Breeding, 1963) [128

Ambassade de France Service de Presse et d'Information The French Budget and Finance Act of 1963 Pp 20 (London Ambassade de France, Service de Presse et d'Information, 1963) [128

British Museum (Natural History) Economic Series, No 15 Common Insect Pests of Stored Food Products a Guide to their Identification By Dr H E Hinton and Dr A Steven,Corbet Fourth edition Pp 61 (London British Museum (Natural History), 1963) 3*s* 6*d* [128

Department of Scientific and Industrial Research Automation at Low Cost—2 Temperature and Humidity Pp 16 (London Department of Scientific and Industrial Research, 1963) [128

Forestry Commission Report on Forest Research for the year ended March, 1962 Pp ix+194+12 plates 13*s* net Bulletin No 35 Pruning Conifers for the Production of Quality Timber By D W Henman Pp vii+55+7 plates 6*s* 6*d* net Forest Record No 22 Shelterbelts for Western Hill Farms By W A Cadman Pp 31 3*s* net Forest Record No 49 The Development of Douglas Fir Plantations in Relation to Site Conditions By W R Day Pp 8 1*s* net Forest Record No 50 Wind-Loosening of Young Trees on Upland Heaths By M V Edwards, J Atterson and R S Howell Pp 16 2*s* net (London H M Stationery Office, 1963) [128

Admiralty Hydrographic Department Admiralty Marine Science Publications No 6 Hydrographic Analysis of the Sandbanks in the Approaches to Lowestoft Harbour By R L Cloet Pp iii+15+22 illustrations+12 graphs No 7 Mean Sea Level in the British Isles By Commander D L Gordon and Commander C T Suthons Pp i+8 (London Admiralty Hydrographic Department, 1963) [128

Building Research Station Digest (Second Series) No 37 Jointing with Mastics and Gaskets—2 Pp 4 (London H M Stationery Office, 1963) 4*d* [128

Department of Scientific and Industrial Research and Forestry Commission Forest Products Research Special Report No 18 Bark Form and Wood Figure in Home Grown Birch By R J Newall and A S Gardiner Pp vi+28+8 plates (London H M Stationery Office, 1963) 4*s* net [128

Other Countries

Population Reference Bureau, Inc *Population Bulletin*, Vol 19, No 4 (July, 1963) The Population Problem—Towards a Solution Pp 81–108 (Washington, D C Population Reference Bureau, Inc , 1963) 50 cents [78

Zoology Publications from Victoria University of Wellington No 31 (March 15, 1963) Some Studies on the New Zealand Oysters By P J Hollis Pp 28+3 plates No 32 (April 30, 1963) The Spatangid Echinoids of New Zealand By H Barraclough Fell Pp 8+6 plates (Wellington Victoria University of Wellington, 1963) [78

Smithsonian Miscellaneous Collections Vol 146, No 2 A Contribution Toward an Encyclopedia of Insect Anatomy By Robert E Snodgrass (Publication 4544) v+48 (Washington, D C Smithsonian Institution, 1963) [78

National Science Foundation, Washington NSF 63–23 Profiles of Manpower in Science and Technology Pp 36 (Washington, D C National Science Foundation, 1963) [78

Smithsonian Institution Bureau of American Ethnology Bulletin 185 River Basin Surveys Papers, Numbers 26–32 Edited by Frank H H Roberts, Jr (Inter-Agency Archaeological Salvage Program) Pp xii+344+57 plates (Washington, D C Government Printing Office, 1963) [78

Records of the Australian Museum Vol 25, No 12 (9th August 1962) Two Spirorbid Tubeworms (Serpulidae, Polychaeta) from Eastern Australia By B Wisely Pp 243–248 (Sydney The Australian Museum, 1962) [78

Annals of the New York Academy of Sciences Vol 108, Article 2 Life-Like Forms in Meteorites and the Problems of Environmental Control on the Morphology of Fossil and Recent Protobionta By E Anders and 26 other authors Pp 339–616 (New York New York Academy of Sciences, 1963) 4 dollars [78

The Research of Reactor Centrum Nederland—General Survey of Activities Pp 35 ('s-Gravenhage Reactor Centrum Nederland, 1963) [78

United States Department of Commerce Coast and Geodetic Survey Publication 60–3 Geodetic Operations in the United States and in other Areas Through International Co-operation, January 1, 1960 to December 31, 1962. By F R Gossett Pp vi+32 (Washington, D C Government Printing Office, 1963) 30 cents [78

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Outline of The Institute of High Speed Mechanics, Tohoku University Pp 64+21 plates (Sendai, Japan The Institute of High Speed Mechanics, Tohoku University, 1962) [78

Republic of the Sudan Ministry of Health Annual Report of the Government Analyst for the year 1960–1961 Pp 21. (Khartoum Ministry of Health, 1963) [78

Smithsonian Contributions to Astrophysics Vol 8, No 2 The Relative Positions of Sunspots and Flares By John G Wolbach Pp 101–118 (Washington, D C Government Printing Office, 1963) 20 cents [78

Annals of the New York Academy of Sciences Vol 107, Article 3 (July 8, 1963) New Reflections on Monoamine Oxidase Inhibition By E A Zeller and 74 other authors Pp 809–1158 (New York New York Academy of Sciences, 1963) 5 dollars [128

Smithsonian Institution Bureau of American Ethnology Bulletin 188. Shonto—a Study of the Role of the Trader in a Modern Navaho Community By William Y Adams Pp xi+329+10 plates (Washington, D C Government Printing Office, 1963) 2 dollars [128

United States Department of the Interior Geological Survey Water-Supply Paper 1330-F Water Requirements of the Styrene, Butadiene and Synthetic Rubber Industries By Charles N Durfor Pp v+221–285 Water-Supply Paper 1539-P Geology and Ground-Water Resources of Dougherty County, Georgia By Robert L Wait Pp vi+102+plates 1–6 Water-Supply Paper 1544-D Application of Electrical and Radioactive Well Logging to Ground-Water Hydrology By Eugene P Patten, Jr , and Gordon D Bennett Pp iv+60 Water-Supply Paper 1613-A Geology and Ground-Water Conditions in Southern Nassau and Southeastern Queens Counties, Long Island, N Y By N M Perlmutter and J J Geraghty Pp vi+205 Water Supply Paper 1619-J Ground-Water Geology of Edwards County, Texas By A T Long Pp iv+29+plates 1–5 Water-Supply Paper 1619-Q Reconnaissance Study of the Chemical Quality of Surface Waters in the Sacramento River Basin, California By Robert Brennan Pp iv+44+plates 1–3 (Washington, D C Government Printing Office, 1963) [128

Smithsonian Institution United States National Museum—Museum of History and Technology Bulletin No 235 American Military Insignia 1800-1851 By J Duncan Campbell and Edgar M Howell Pp xv+124 (Washington, D C Government Printing Office, 1963) 2 dollars [128

World Health Organization Public Health Papers, No 20 Preparation of the Physician for General Practice Papers prepared for a European Conference on the Training of the Doctor for his Work in the Community, Edinburgh, 1961 By R Berfenstam and 7 other authors Pp 114 (Geneva World Health Organization, London H M Stationery Office, 1963) 4 Sw francs, 6*s* 8*d* , 1 25 dollars [128

National Science Foundation, Washington Weather Modification Fourth Annual Report for Fiscal Year ended June 30, 1962 (NSF 63-29) Pp v+84+7 plates (Washington, D C Government Printing Office, 1963) 50 cents [128

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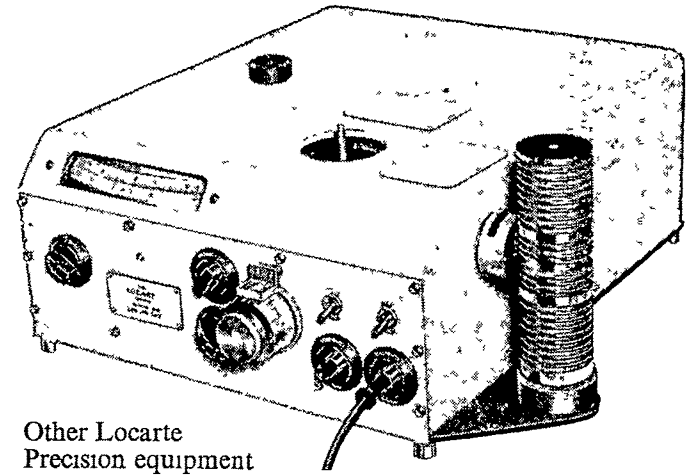

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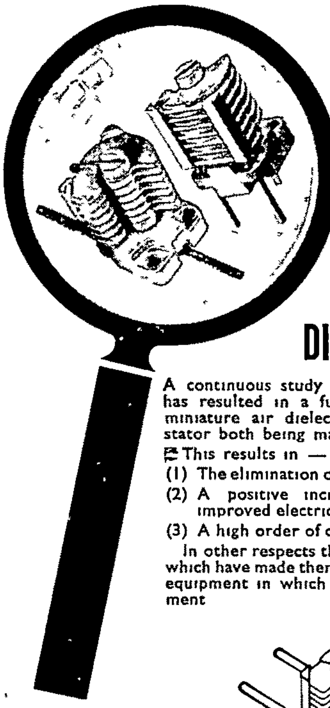

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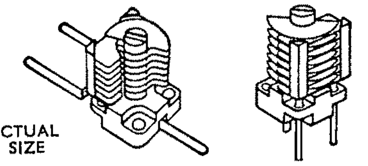

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THE IRISH MEAT RESEARCH INSTITUTE

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DIRECTOR

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Intending candidates are invited to submit full personal details with copies of publications, particulars of experience, etc , to The Secretary, The Irish Meat Research Institute, 7 Gardiner Row, Dublin, 1, not later than October 31, 1963

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Details of conditions of appointment may be obtained from the Agent-General for New South Wales, 56-57 Strand, London, W C 2, with whom four copies of applications and names of two referees should be lodged before November 15, 1963, and a copy of the application should be forwarded, by air mail, to the Appointments Section, the University of New South Wales, Box 1, Post Office, Kensington, New South Wales

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UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF ANATOMY
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Further particulars may be obtained from the Assistant Registrar, the Medical School, Birmingham, 15, to whom applications, naming two referees, should be sent as soon as possible

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UNIVERSITY OF ST ANDREWS

Applications are invited for a Post-Doctoral Research Fellowship awarded by the Department of Scientific and Industrial Research and tenable in the School of Natural Philosophy, St Salvator's College, St Andrews The work will be on " Relaxation Time Measurements in Solids by Spin-Echo Techniques " and is part of an expanded programme of research in the field of nuclear magnetic resonance linked with the existing broad-line programme There will be a considerable expansion in all research facilities when a new physics building is occupied in 1965 The appointment will be for a maximum of three years from a date to be arranged and the salary will be in the range £1,000 to £1,100 per annum, with F S S U benefits where appropriate

Applications should be sent as soon as possible, and preferably not later than October 31, 1963, to the Secretary of the University, St Andrews, Scotland, from whom further details may be obtained

---

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Candidates should be able to cover subjects from Machine Tool Technology and Design, Metrology, Quality Control Tool Engineering, Methods Engineering, Metal Forming theory and practice, Applied Control Engineering including Numerical Control of Machine Tools, Precision Casting Salary scale £1,600 to £1,800 per annum according to qualifications and experience This scale is subject to revision following the reference of salaries in Universities and Colleges of Advanced Technology to the National Incomes Commission

Further particulars and forms of application from the Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, to whom applications should be forwarded within fourteen days of this advertisement Please quote 10/63/6

STAFFORDSHIRE COUNTY COUNCIL EDUCATION COMMITTEE

STAFFORDSHIRE COLLEGE OF TECHNOLOGY

Principal R S Paradise, B Sc (Eng) Hons , M I Mech E , A M I Mar E

MATHEMATICS AND SCIENCE DEPARTMENT

Applications are invited for a post of Assistant Lecturer Grade " B," duties to commence on January 1, 1964 Candidates should have a University degree together with the ability to teach Physics to B Sc General Degree level, and to assist with the teaching of Mathematics or Chemistry

Participation in research will be encouraged, facilities and time are available

Salary in accordance with the Burnham Technical Scale for Assistant Lecturer Grade " B " = £830 to £1,760 per annum

In certain circumstances allowances will be made towards the cost of lodging and removal expenses The Authority also has a scheme whereby loans towards house purchase can be made with a repayment period of up to 25 years in approved cases

Forms of application and further particulars may be obtained by sending a stamped addressed foolscap envelope to the Director of Education (Staffing), County Education Offices, Stafford , completed applications to be returned direct to the Principal at the College by October 18, 1963

NEW ZEALAND

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Applications are invited for the undermentioned vacancy Vacancy B13/18/18/2758 Scientific Officer (Paleontologist) N Z Geological Survey, Lower Hutt Salary Up to £1,780 a year according to age and experience and further advancement is on scientific merit Qualifications desired B Sc (Hon) or M Sc with zoology and/or geology as leading subjects Duties To undertake research on the systematics and history of Tertiary molluscan fauna of New Zealand and thus to contribute to their use in stratigraphy Passages Fares for appointee and his wife and family, if married, will be paid Incidental expenses Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S W 1, with whom applications will close on December 18, 1963 Please quote reference B13/18/18/2758 when inquiring

---

UNIVERSITY OF NOTTINGHAM

A vacancy exists at the School of Agriculture for a Technical Assistant in the Department of Agricultural Sciences to work on problems in Micro-Meteorology Duties will include installation and maintenance of instruments, collection of physical data relating to crop environment, and development of original instrumentation Preference given to applicants with H N C in physics, or equivalent qualifications, and experience in use of modern recording apparatus Appointment on one of the following scales £985 to £1,120 per annum, or £760 to £900 per annum, with supplement of £50 or £30 per annum for appropriate qualifications

Applications to the Secretary, School of Agriculture Sutton Bonington, Loughborough

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ROYAL COLLEGE OF ADVANCED TECHNOLOGY

SALFORD

Applications are invited for a post of Laboratory Assistant from persons with experience of animal house maintenance and work in experimental physiology For candidates aged 21 years and over the salary will be on the scale £665 per annum to £730 per annum The post is superannuable

Applications, giving full particulars and the names and addresses of two referees, should be addressed to the Secretary, Royal College of Advanced Technology, Salford, 5, as soon as possible, quoting reference CH/13

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY

(FACULTY OF TECHNOLOGY IN THE UNIVERSITY OF MANCHESTER)

Applications are invited for the following posts of Experimental Officers in the Department of Polymer and Fibre Science (a) One Experimental Officer to work in the field of spectrophotometry This involves the care of the Department's spectrophotometers and other optical measuring equipment The candidate will be expected to assist in research in the area of colour or fibre physics (b) One Experimental Officer to look after the polymer physics equipment and to assist in research work in this field Salary in the scale £880 by £40 to £1,200 Superannuation scheme Family allowances

Further particulars and forms of application may be obtained from the Registrar of the College, The Manchester College of Science and Technology, Sackville Street, Manchester, 1, to whom completed applications should be returned by Saturday, October 26, 1963

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY

(FACULTY OF TECHNOLOGY IN THE UNIVERSITY OF MANCHESTER)

Applications are invited from graduates in physical science or technology for a Lectureship or Assistant Lectureship in the Department of Paper Science, with corresponding title and status in the University of Manchester Industrial and research experience is desirable, and candidates should be prepared to become interested in research in Paper Technology Some private consulting work is permitted Commencing salary will be determined according to experience in the scales : Lecturer, £1,250 to £60 by £1,670 by £80 to £2,150 Assistant Lecturer, £1,000 by £50 to £1,150 Superannuation under the F S S U Family allowances

Application forms and further particulars may be obtained from the Registrar of the College, Manchester, 1, to whom completed applications should be returned not later than Saturday November 2, 1963

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BOTANY

Applications are invited for the post of Lecturer or Assistant Lecturer in Botany Preference will be given to applicants whose interests lie in the field of cytology or cytogenetics The appointee will be expected to take up his duties by October, 1964 or sooner if possible Salary on scale Lecturer, £1,250 to £2,150 , Assistant Lecturer, £1,000 to £1,150, all with placing according to qualifications and experience F S S U , child allowance and removal allowance

Further information and forms of application may be obtained from the Secretary, the University, Aberdeen, with whom applications (eight copies Lecturer, two copies Assistant Lecturer), should be lodged not later than November 18 1963

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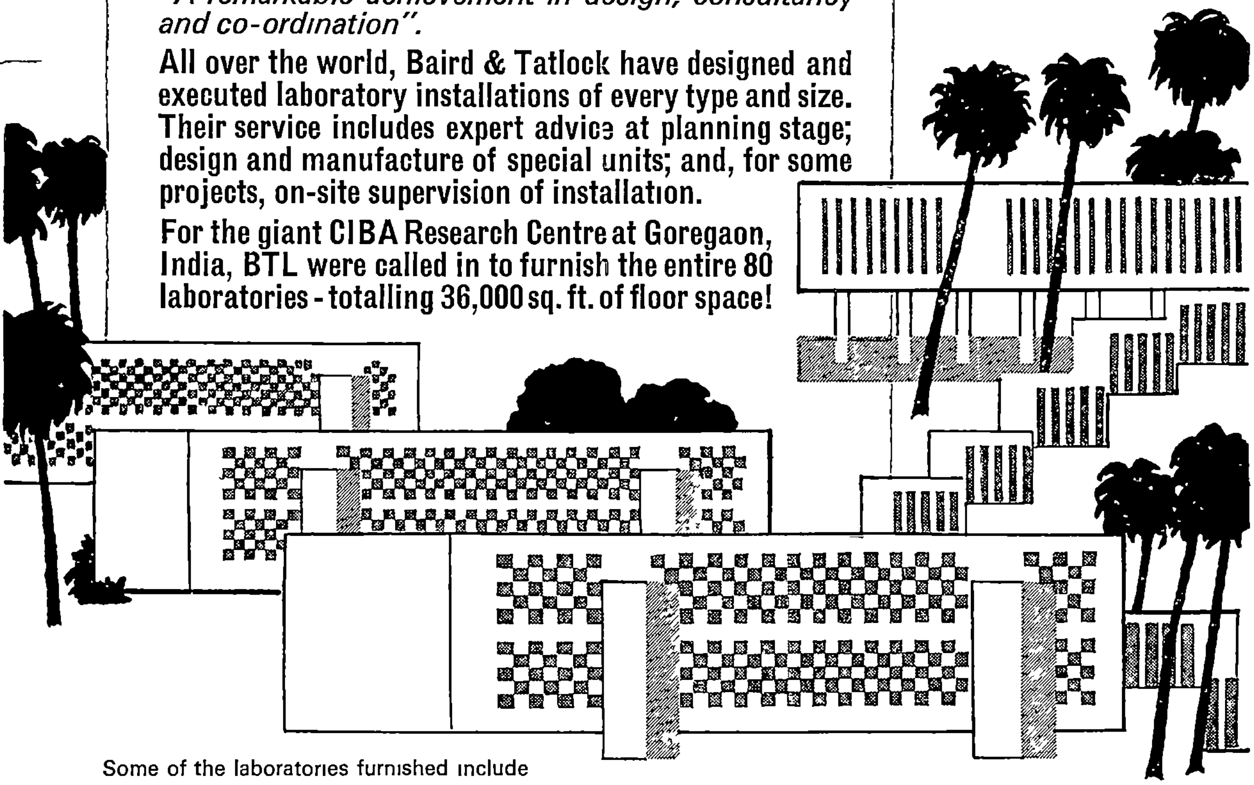

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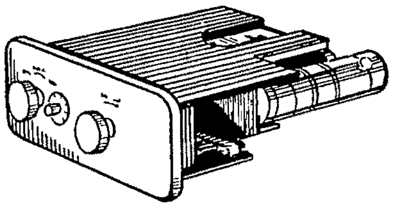

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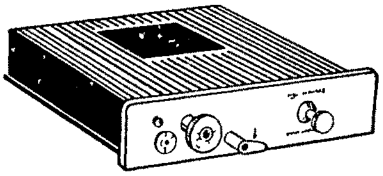

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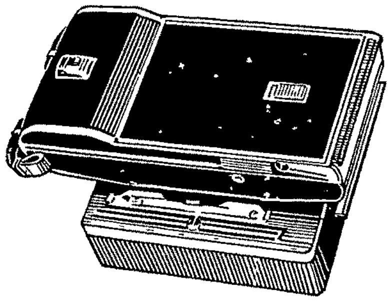

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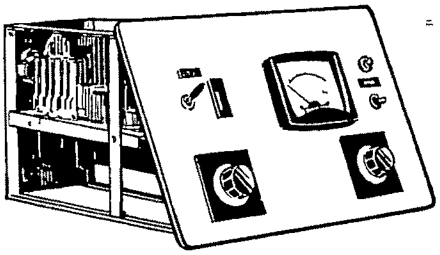

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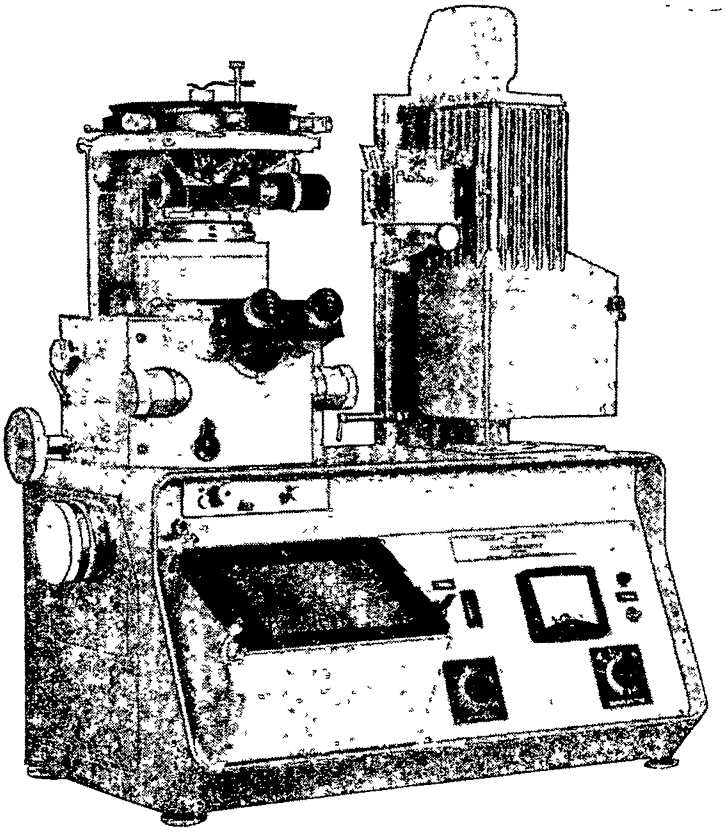

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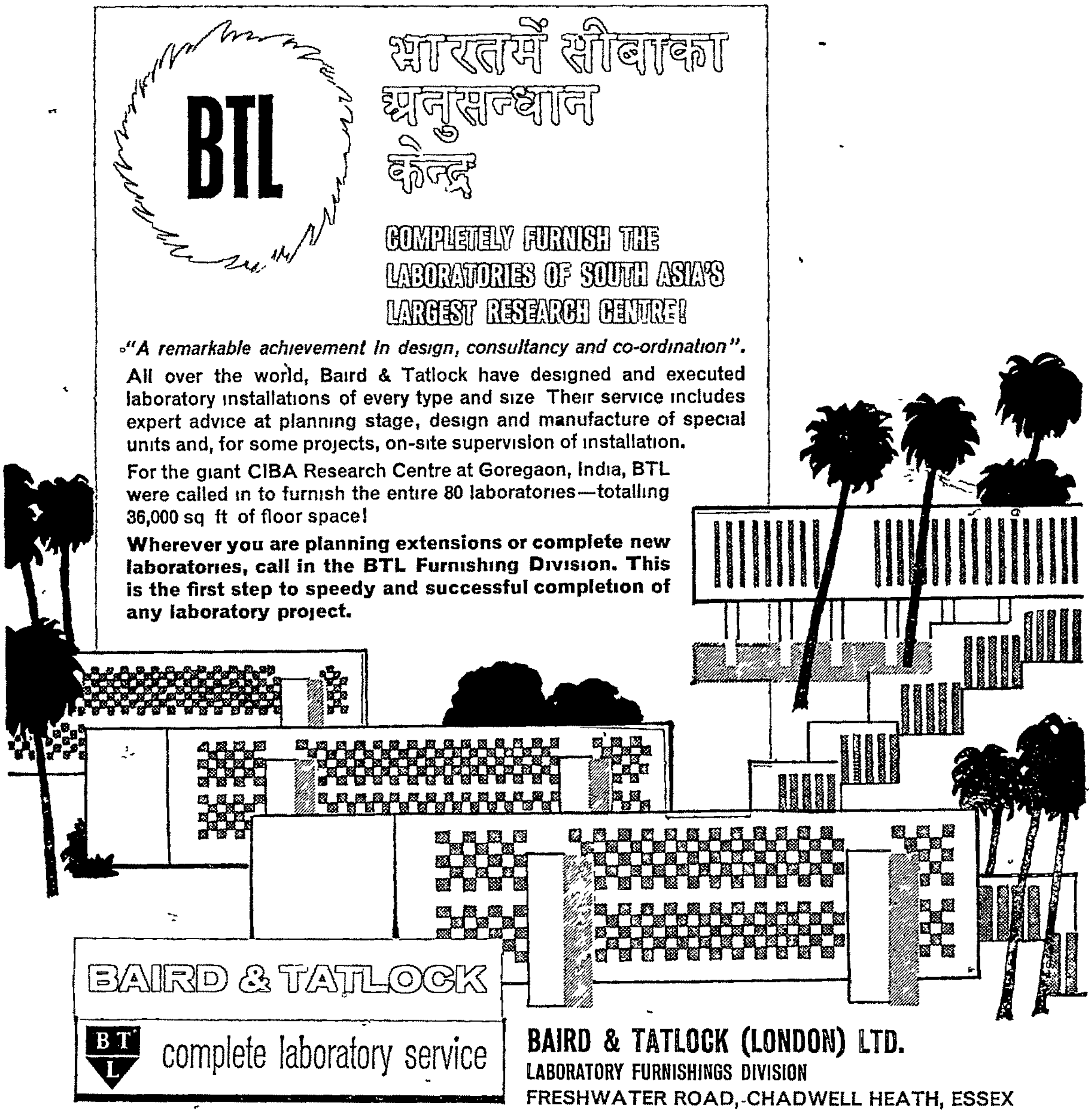

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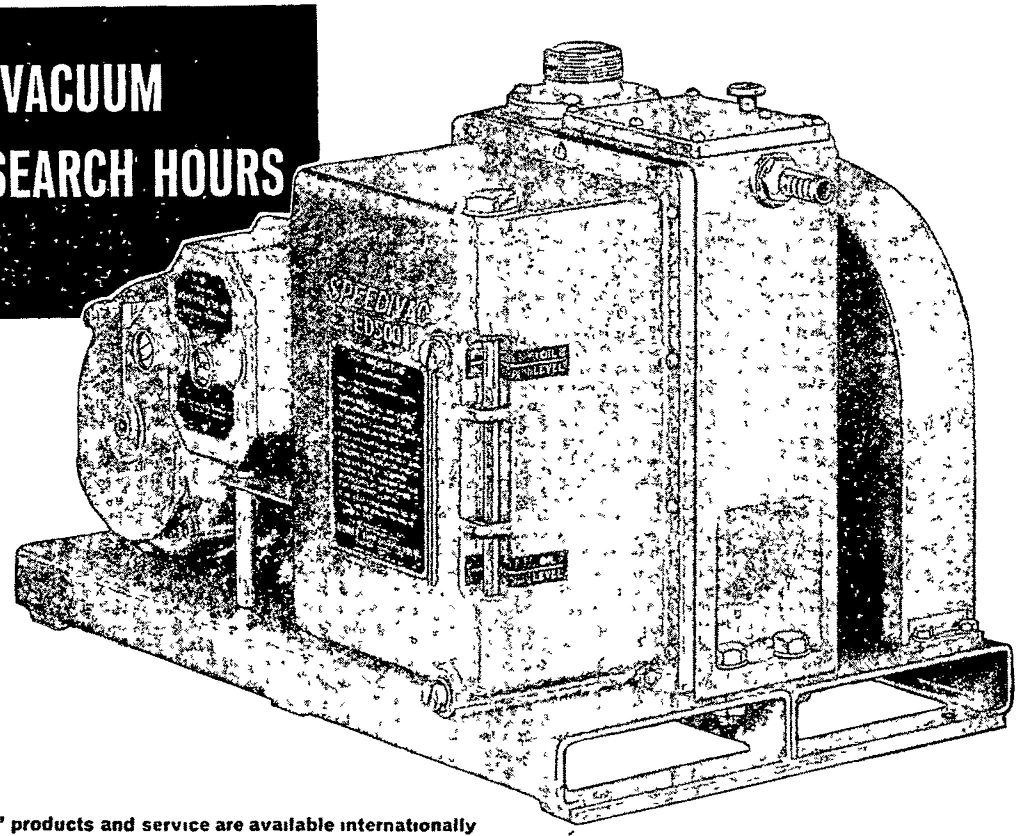

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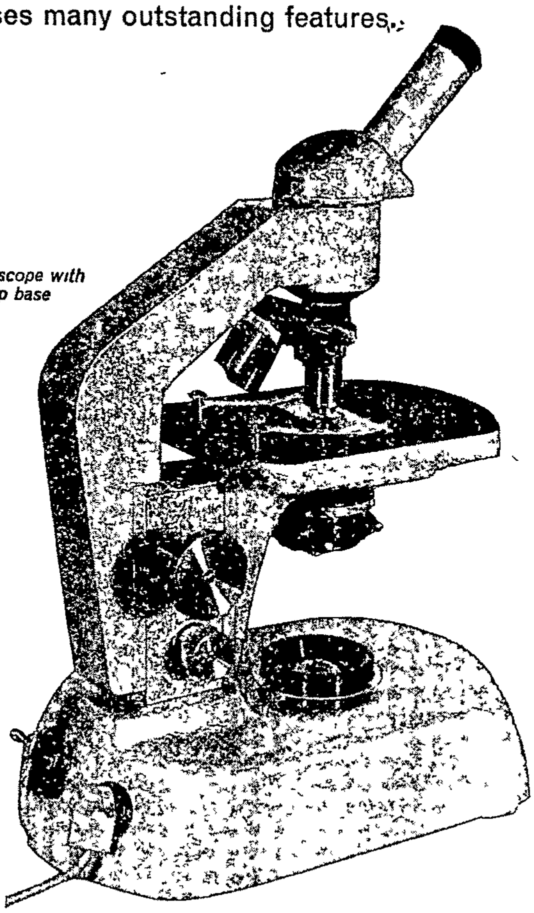

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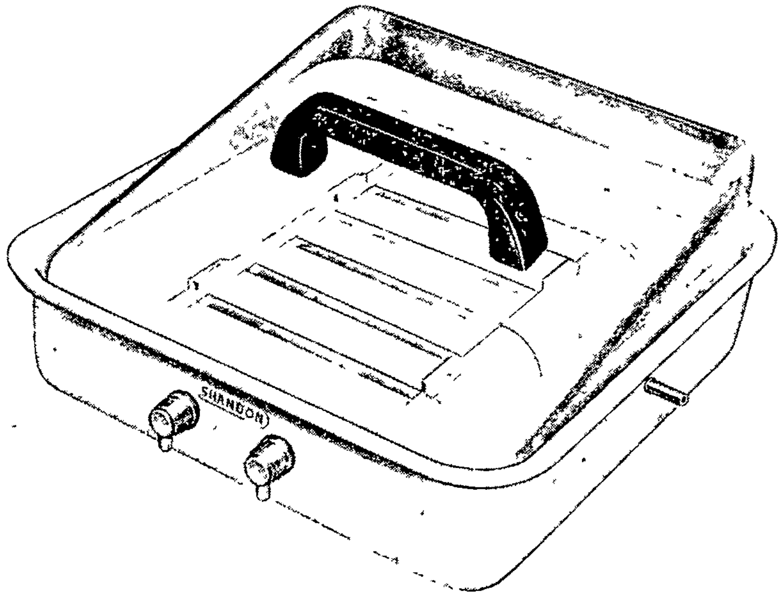

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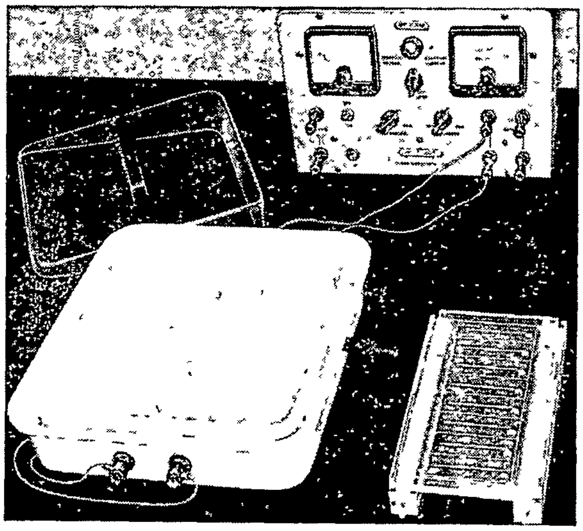

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INTERACTION OF SCIENCE AND THE STATE

SELDOM, if ever, can two major addresses at a British Association meeting have directed such pointed attention to the address of an earlier President as was done at Aberdeen Sir Eric Ashby's tribute (see *Nature*, 199, 877, 1963) to his predecessor was forthright and generous enough, yet a few nights later Prof R V Jones built his evening discourse on "Science and the State" (see *Nature*, 200, 7, 1963) largely around the presidential address which Lyon Playfair delivered when the Association met at Aberdeen in 1885—quoting incidentally the passage from the presidential address of the Prince Consort at the first meeting of the Association in Aberdeen in 1859, which Playfair took as his own text Prof Jones displays the range and versatility of Playfair's interests and mind, recalling that it was pointed out to the Government at a conference on the neglect of science in May 1916 that in the whole history of British Governments there had been only one Cabinet Minister who was a trained professional man of science—Lord Playfair

Appropriate as was this tribute to Lord Playfair at this year's Aberdeen meeting, it is equally welcome for its relevance to the present-day debate on the relations between Government and science, and the announcement of Prof Jones's return to the Ministry of Defence for some months, made shortly before his lecture, added interest to his remarks Prof Jones regrets that, in the address over which he runs his critical eye, Lyon Playfair makes no reference either to the relations between science and the Services or to the general question of wisely making and implementing scientific policy These subjects apart, Prof Jones's survey of Lyon Playfair's address demonstrates not merely the impressive range of his interest but the prescience and pertinence even to-day of many of Playfair's comments. Over science and education, the responsibilities of Government for the schools, the balance between classical and scientific studies in the curriculum, the place of science in the universities, the relations between the universities and other research establishments, between science and industry, and the critical importance of fundamental research, Playfair ranged with a vision far ahead of his contemporaries Prof Jones, bringing the historical survey up to date, remarks justly how many mistakes might have been avoided had Playfair's advice been taken

What makes Prof Jones's lecture one of the major events of the Aberdeen meeting is, however, essentially his discussion of the two topics which Playfair omitted, but to that discussion his review of earlier relations between Government institutions and research establishments is entirely relevant Welcoming the recent report of a working party of the Federation of British Industries on *Civil Research Policy* (see *Nature*, 199, 725, 1963), with its awareness of the need for more co-ordinated support of scientific research in the universities, Government establishments, the research associations and industry generally, Prof Jones puts a reasoned argument for such co-ordination Broadly speaking, his argument is an extension in general terms of that advanced regarding physics departments in the universities in the recent report of the committee appointed by the Council of the Institute of Physics and the Physical Society, of which he was chairman (see p 200 of this issue of *Nature*)

Prof Jones stresses the effect on the balance of our research effort to-day of the redistribution of that effort during the Second World War, particularly in any field such as physics in which the supply of scientists is insufficient to meet the demand It was, he points out, a pure accident, though a consequence of the foresight which led Britain to recruit scientists to Government establishments in support of air defence just in time that, whereas in the United States the universities, in which much of the war research effort was necessarily concentrated, increased considerably in strength compared with the Government establishments, the reverse happened in Britain We have yet to take sufficient account of the consequences of a separation of Government establishments from the universities and the creation of conditions which greatly restrict the interchange of staff Moreover, this is quite apart from the fact that Government establishments are now often able to offer not only higher salaries, better equipment and more generous technical assistance than the universities can do, but also the prospect of giving more time to actual research, including fundamental research

Prof Jones recognizes that this position applies more especially to physics in view of its greater involvement with matters of national defence during the past thirty years, but he points out that Britain's smaller resources make it essential for her to be even more careful than the United States in husbanding her scientific potential, both for teaching and for research. It is just as important to realize the teaching potential locked up in the Government establishments as it is to realize the research potential of the universities, and of the colleges of technology also Nor is it a simple matter to secure the most efficient use of Britain's most precious resource—highly trained scientific manpower—even in industry, let alone the universities It is a problem to be tackled not piecemeal but on a national basis, and Prof Jones regards the securing of a better balance between universities and Government establishments and between these and the requirements of industry, the technical colleges and the schools as one of the most important problems confronting both science and the State in modern Britain

Some awareness of the implications of this situation was shown two years ago in the Zuckerman Report, *The Management and Control of Research and Development* (see *Nature*, 193, 710, 1962) However, although Government departments have been reported to be implementing that Committee's recommendations, the results as regards interchange of staff or location of establishments do not as yet appear to have been appreciable For a decade or more, too, the educational potentialities of the research associations have been emphasized in these columns, but so far with little, if any, effect Prof Jones, none the less, sees this as an urgent and critical problem in the expansion of higher education itself as well as in the more effective deployment of Britain's research effort it is to be hoped that neither the Robbins Committee nor the Trend Committee will fail to report clearly and emphatically on the issue

It is from this issue that Prof Jones comes to consider specifically the question of science in the defence effort, and here he welcomes the changes in organization proposed in the recent White Paper on the *Central Organization for Defence* (see *Nature*, 199, 1021, 1963) These changes, he thinks, will prevent the scientific staff attached to the central Chiefs of Staff organization, with no executive duties, from being overburdened by administration, and hence enable it to devote its whole time to the study of the influence of advancing knowledge on the problems of defence—conditions such as were advocated by Sir Henry Tizard in his lecture to the Royal United Services Institution in 1946 Prof Jones believes that the new arrangement increases the strength of the Chief Scientific Adviser, placing him on the Defence Council alongside the Chiefs of Staff of the three Services, and that it is a wise arrangement for those three Services to keep enough of their individualities and traditions to preserve the loyalties which have contributed so much in emergencies to their fighting spirit The merging at the higher levels he regards as assisting national and economic planning, and he also regards science and technology as a main factor in unifying the Defence Services and lowering the barriers between them

Beyond this, Prof Jones makes no reference to the administrative structure or to any relation between the Defence Research Committee and the Advisory Council for Scientific Policy Nor does he refer to the position of the Minister for Science, though he quotes with approval a suggestion made by Colonel Alexander Strange in a lecture to the Royal United Services Institution as long ago as 1871 for a Minister of Science, responsible for all scientific national institutions, and advised by a permanent, independent and highly qualified consultative body and a similar body to advise the Secretary of State for War and the First Lord of the Admiralty He does not comment on Lord Hailsham's recent book, *Science and Politics* (see *Nature*, 198, 1123, 1963), but some criticism of Lord Hailsham's position is implicit in the lecture

Generally, Prof Jones would appear to be satisfied with an organization which ensures the communication of scientific advice effectively at the highest level, as Lord Louis Mountbatten described in his remarks at the 1962 anniversary dinner of the Royal Society. None the less, he does suggest that all is not well with Britain's national organization of science, nor would it appear that he is entirely satisfied with some of the research arrangements contemplated in the new Defence organization Some rationalization is needed, and Britain is in danger of repeating the German error of allowing individual centres to become just too strong and independent for coherent organization and economic direction

It is here that he insists on the need for strong leadership from the Ministry for Science, and he clearly does not share Lord Hailsham's reluctance for central planning and leadership Nor is he unduly concerned that great responsibility must rest on a relatively small number of men This he regards as inevitable, and he points out that, especially in defence matters, executive decisions have often been taken by individuals He would use the committee system where possible, remembering, however, that decisions taken by a Committee are sometimes less sensible than those which any of its individual members would have reached The important thing is that science should be so organized that some of Britain's best men of science are in key-administrative positions in Government so that policy decisions affecting science are not taken by non-scientific men, whose judgment is at the best based on how individual scientists have convinced them

Prof Jones is confident that Britain is still great enough to produce men of the right calibre, and here he agrees with Sir Charles Snow as to the danger of isolation His own solution is to provide a small forum for each of the principal scientific advisers to the Government Each forum would consist in the main of scientists, as nearly as possible the adviser's equal, so chosen that the membership covers the full range of his activities, and including some relatively young men to ensure that the necessary energy, imagination and working contact with the most modern technology are available If the adviser were as wise as he should be he would consult the forum before taking any decision, and while the responsibility would remain his alone, he would take his decision only after having heard the relevant arguments Moreover, he would be able to justify it in any subsequent encounter with his forum

Prof Jones believes that such a forum would provide the only sanctions needed to prevent the individual from abusing his power, beyond those already set by Britain's form of Parliamentary Government—and his suggestion is based on experience with it in a limited field during the Second World War It certainly appears to offer the possibility of avoiding some of those errors of judgment, at least in the scientific and technical field, which Prof Brian Chapman has so strongly indicted in his recent book, *British Government Observed**, and which he rightly insists we can no longer afford The suggested forum has in any event, superficial resemblance to the "cabinet" or "private office" with which, Prof Chapman suggests ministers should be provided to assist forward planning What is curious, however, is that, in the context in which he writes, Prof Chapman gives no attention to this question of the injection of scientific consideration into matters of State, neglect of which might well be the determining factor in the loss of prestige and influence by Britain

This blind spot is the more surprising in that Prof Chapman in his glance at the French administrative system and the recruitment of civil servants, notes with approval the care taken to avoid creating mere technical experts and to provide, on the contrary, men of unassailable technical competence who have also throughout their careers been learning the art, techniques and discipline of general administration Moreover, with this recruitment to high administrative office of men of widely different backgrounds and general administrative experience, Prof Chapman also stresses the deliberate use in European ministries of the cabinet system of a minister's private office, in which the policy-making function is concentrated, as a forcing ground for younger men of talent By such means, European countries have, he believes, created for themselves a reserve of administrative talent unknown to the higher British Civil Service

Prof Chapman makes three main criticisms of Britain's present system and practice of Government, but before considering these and their implications the importance of the mechanism of tendering scientific and technical advice and the stage at which it is injected should again be emphasized While there are undoubted weaknesses in the present structure, there is little evidence to show that the systems in vogue in Europe or in the United States offer any substantial advantage over that of Britain, if it is properly used and staffed The National Centre for Scientific Research in France, which is the chief body responsible for basic research there, is attached to the Ministry of Education and is responsible for the analysis of scientific development for the Government, and the Minister of Education is a member of the Inter-departmental Committee of Scientific Research, of which the Prime Minister is chairman This Committee of Ministers is advised by a Consultative Committee on Scientific and Technological Research, but it is not easy to see from the report on scientific and technological research under the Fourth Plan 1962–65, published in the current issue of *Minerva* (1, No 4, 1963), or from the annual reports of the National Centre, what inherent advantage this system offers over Britain's, if the Office of the Minister for Science were reasonably strengthened and some rearrangements made

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* *British Government Observed Some European Reflections* By Brian Chapman Pp 64 (London George Allen and Unwin, Ltd, 1963) 12*s* 6*d*

Nor does Dr A Hunter Dupree's article in the same issue, "Central Scientific Organization in the United States Government", suggest that Britain has much to gain by adopting the tripartite structure evolved in the Office of the President The Advisory Council for Scientific Policy obviously serves many of the functions of the President's Science Advisory Committee, though Britain has yet nothing quite corresponding to the Federal Council of Science and Technology, and any analogy between the Special Assistant to the President for Science and the Minister for Science could be misleading Dr Dupree, who does not appear to share Prof Jones's view that the American universities emerged stronger from the War, looks to this structure to be effective in making choices between fields correcting the inherent and valuable pluralism of science itself The essence of his review is that every State must achieve for itself a balance between central unity and this essential plurality, which is in harmony with its own traditions and its own institutions He insists that the scientific community itself has to give serious thought to the protection of the institutions through which science manifests its plurality At the same time, because decisions concerning priorities in science have to be made on behalf of the general community, there is also necessary a central scientific organization, aware of the social and political impact of science, and capable of assisting the authorities of the whole community in making such decisions about the organization of research.

Nor is this view of what is essential invalidated either by an article by Prof D Auger on "Scientific Co-operation in Western Europe", which appeared alongside Dr Dupree's article, or by the even more recent report, *Science and the Policies of Governments* *, from the Advisory Group on Science Policy of the Organization for Economic Co-operation and Development While Prof Auger sees some need for further systematization in the scientific and technical fields, he advances no criticism of the national structures which form the basis for international co-operation These seem to be sufficiently harmonious to facilitate and not to hinder scientific work

Likewise the report on the implications of science and technology for national and international affairs recognizes that any policy developments at international level must be firmly based on the right relations between science and policy making at the national level Its arguments for the establishment by all Governments of what is described as a 'science and policy office' and the duties which it suggests for such are familiar to all who have followed the debates in Britain on the functions of the Minister for Science, but it will be noted that they refer particularly to the need for adequate research in the social sciences and the humanities

If there is no convincing evidence in all this that there is anything seriously amiss with Britain's existing structure, though some that could in various ways be modified and improved, the real indictment is exactly that which has been brought against Britain's overseas broadcasting services—that the organization is not sufficiently used and adequately supported It is a question of Government will-power

This question of will-power brings us to the heart of Prof Chapman's main criticisms First, he believes that Britain's loss of prestige and failure to deal adequately with the problems of the modern State are due to failure to revise archaic procedures and to reform the institutions for making policy Next, he believes that this is accentuated by Britain's failure to recruit talent to the work of Government at all levels, from Ministers and Members of Parliament to the higher ranks of the Civil Service and to local government Thirdly, the consequences of all this is the worse because of secrecy which surrounds so much of the work of Government, and particularly the making of policy, so that effective public discussion before decisions are taken and mistakes are made is almost impossible

Prof Chapman recognizes that this last is not solely a question of secrecy but also of the quality of information, much of which falls short of the standard which should be used in British Government The factual material on which policy is based is sometimes bad and sometimes non-existent Although the Plowden Report (see *Nature*, 192, 485, 1961) pointed in the right direction, much more use could be made of the techniques of operational research and computers to provide the data on which decisions should be based and to enable these decisions to be reached much more swiftly.

That issue could be joined with Prof Chapman, and some minor points should not prevent those scientists who are concerned to see that scientific factors receive full weight in the making of policy and taking of decisions from encouraging such a marriage. Prof Jones's proposal of a forum for the scientific advisers is in keeping with Prof Chapman's emphasis on the importance of informed and authoritative discussion. The proposal to open up the top of the Administrative Class of the Civil Service to outside recruitment is scarcely new, but Prof Chapman's recommendations would carry the process a good bit further. The tardiness with which the much more limited recommendations in that direction of the Plowden Report and the Zuckerman Report have been implemented point to a lack of zeal or will-power on the part of the Government The practice and structure of Government may now be receiving more attention than Prof Chapman seems inclined to admit, although he welcomes some of the proposals of the Plowden Committee for reform of the Treasury as likely to improve the quality of the Treasury's contribution to central Government To secure attention in these fields on the scale and with the urgency required, and still more to effect the reforms he suggests in the efficiency of Parliament itself, the creation of strong regional centres of Government or the revision of the laws of official secrecy will require much informed discussion and the creation of a strong body of public opinion To the creation of that opinion, Prof Chapman's little book makes a contribution which may be all the more valuable in that it is timed to inform the discussion in due course of both the reports of the Robbins Committee and of the Trend Committee

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* Organization for Economic Co-operation and Development *Science and the Policies of Governments The Implications of Science and Technology for National and International Affairs* Pp 55 (Paris Organization for Economic Co-operation and Development, 1963)

HIGHER EDUCATION IN PHYSICS

DURING the past few years a spate of reports and letters bewailing the difficulties of academic scientists has reached the journals and the newspaper Press. Although many different views have been expressed, their sheer number shows that there are urgent problems which must be tackled. Two years ago the Institute of Physics and the Physical Society established a committee, under the chairmanship of Prof. R. V. Jones, to study problems facing university physics departments, especially those affecting their growth. This committee submitted some striking evidence to the Robbins Committee on Higher Education in 1962 (see *Nature*, 193, 922, 1962) and has now completed its work.

The final report* deals with a wide range of problems, and the conclusions are well supported by detailed arguments based on data drawn from many sources, including a special enquiry among all university physics departments. Although some of the problems are specific to physics, this report should be closely studied by all scientists and those responsible for the administration of scientific work.

Certain conclusions are clearly of the greatest importance. For example, the physicists are unanimous in their view that there must be active research work, both pure and applied, within all university physics departments, "in order (*a*) to attract physicists of calibre adequate to carry out teaching, and (*b*) to transfuse this teaching with the inspiration of the living and exciting subject that physics is". University research is seriously hampered to-day by lack of skilled technical help and, frequently, by lack of funds for equipment and travel. If the expansion of student numbers, which the Government desires, is to be achieved, then this report makes a convincing case for increased support for research.

At the present time a great deal of basic physics research in Britain, both pure and applied, is carried on in Government research laboratories. Unfortunately, very little of this, often excellent, work benefits either undergraduate or postgraduate education. This is not the case in the United States, where, during the Second World War, the Administration farmed out to research teams in universities problems that would have been tackled by Government establishments in Britain. This difference in approach has had a lasting effect in both countries, but it is now urgent that some way should be found for gradually changing the pattern of Government spending on research in Britain. A larger fraction than at present must be directed to the support of university research.

If the universities are active centres for research and if the research workers can obtain reasonable financial support for projects, which are blessed by their fellow scientists, then it should be possible to recruit the teaching staffs required to implement the Government's target of 150,000 full-time students in universities by 1966–67. To achieve this total, up to 40 per cent of the Ph.D. output must be attracted to the profession of university teacher. As already argued, this requires adequate funds for research, much more technical help than at present and undoubtedly some improvement in the present salary structure.

Despite the 1963 revision of the quinquennial grant (1962–67), it is clear that the University Grants Committee has failed to persuade the Treasury to give the universities an adequate growth-rate for the annual grants. For example, during the 1957–62 quinquennium student numbers increased by 4.5 per cent per annum. This growth was achieved with financial grants increasing at 12 per cent per annum, and there is evidence that standards, such as the staff/student ratio, have declined slightly. The Government plan for the present quinquennium calls for a student growth of 6.2 per cent per annum, but the financial grant grows only at 11 per cent per annum. To quote the report again "if the final numbers are achieved, it will only be at the expense of the staff/student ratio and basic support for research". This must not be allowed to happen. Britain needs to increase opportunities for higher education, but all concerned must be prepared to pay the proper price.

The Office of the Minister for Science should give close attention to this report, and it is to be hoped that the Government will take urgent measures to improve the situation. The long-awaited reports of the Robbins Committee and the Trend Committee on the organization of civil science ought to deal effectively with many of the more serious problems raised by the physicists.

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* *Problems Facing University Physics Departments.* Pp. 26. (London: The Institute of Physics and the Physical Society, 1963.)

BEARING OF MODERN RESEARCH ON EVOLUTION

Evolution

The Modern Synthesis. By Sir Julian Huxley. Second edition with a New Introduction. Pp. li + 652. (London: George Allen and Unwin, Ltd., 1963.) 42*s*. net.

A CLASSIC is defined as a work that defies the passage of time and preserves its intrinsic value and interest, even when the conditions under which it was conceived are no longer the same as those in which it continues to be appreciated. This is the secret underlying the work of Leonardo, Shakespeare, or Brahms, and it means that although a classic may be dated it is not out-dated. In the humanities, works of art are created by human genius and remain as they were although standards of criticism applied to them improve. In the natural sciences, however, although an outstanding analysis or synthesis may be likened to a work of art, it is immersed in a constantly moving stream of progress represented by advances in knowledge in every direction. The great experiments devised and performed by pioneers in science, the discoveries resulting from them and, to some extent, the publications in which they were described, remain to be admired for all time, although possibly now read more often by historians of science than by practising scientists, but text-books or attempts to provide round-ups of the state of science at any given time for the benefit of students wither into obsolescence and fall into disservice at increasing rates as the *tempo* of successful scientific advance increases.

A special position is occupied by Darwin's *Origin of Species*, sometimes regarded as a fine monument of Victorian achievement that some scientists, in the enthusiasm of their pursuit of new lines of research, would be prepared to leave to the historians. This would be a pity, because that extraordinary book, for all the progress that evolutionary studies have made in the past hundred years, continues to provide not only a fundamental framework in which to interpret all phenomena of biology but also general principles on which new programmes for observation and experiment can profitably be based, and, in addition, can still suggest unsuspected new fields for such activities, so wide was the observational catchment-basin and so sound the judgment and uncanny dumb sagacity of its astonishing author

It is against this background and, to a large extent, this heritage that we must consider the uncommon phenomenon of the re-issue, in its original and unaltered text, of a twenty-year-old text-book devoted precisely to this field in which such spectacular advances have been made during these same twenty years Sir Julian Huxley's *Evolution The Modern Synthesis*, which may be regarded as companion, commentary and extension of the *Origin of Species* When it was first published in 1942 the time was appropriate, for the author had (but only recently) had the benefit of the epoch-making integration of the principles of Darwinian selection with those of Mendelian genetics, largely due to the insight and ability of the late Sir Ronald Fisher, whose great *Genetical Theory of Natural Selection* had been published only a few years previously, in 1930 From that time on, the chaos and confusion into which evolutionary studies had fallen in the earlier decades of this century (still detectable in works that refer to the modern theory of evolution as 'mutationist') were dispelled and removed, and the way opened for co-ordinated research by observation and experiment on evolution in every field of biology This great opportunity was brilliantly seized by Sir Julian with the result that his book became the first general text-book to deal authoritatively and comprehensively with the new situation created by the formulation of the synthetic theory of evolution, to show the general principles to be derived from it and therefore to provide the student and the research-worker with an indispensable chart and compass The remarkable thing is that now, in 1963, the same chart and the same compass are no less useful than they were twenty years ago. The book is a classic, and it is to be welcomed that the author and his publisher decided to leave the body of the text just as it was, and to prefix an introduction of 37 pages directing attention to the more important results achieved during the past twenty years Even new principles, such as those of genetic assimilation or allotypy, fall into place

To review such a book, in detail, a second time would be uncalled for (see previous review, *Nature*, 150, 747, 1942), nor is there any need to comment on the selection of new work included in the introduction except to say that it is a remarkable achievement I shall merely add that, although it has been known since 1859 that evolution by natural selection was a unifying concept for all biology, it is only now becoming clear how all-embracing it is, and how closely it knits together the numerous and ever-increasing branches and experimental disciplines of biology The synthetic theory of evolution has also enhanced and raised the scientific status of certain branches of natural history For example, ornithology has been recognized since the days of Gilbert White as a most respected field for amateurs Ornithology, in the form of the birds of the Galapagos Islands, supplied Darwin with the stimulus to question the fixity of species Ornithology revealed the principle of territory and its significance in bird life, worked out by Eliot Howard, and the biology of epigamic characters, courtship and display in birds was the subject of some of Sir Julian's own early researches Paradoxically, the *Origin of Species*, itself the product of pioneer work in the field, sent biologists indoors into their laboratories for half a century, working out the comparative anatomy, embryology, and palæontology of the representatives of the various evolutionary trees, all indispensable work Now, biologists have gone out into the field again, having remembered that Darwin himself was the founding father of ecology, a movement in which Sir Julian has played a leading part During these recent times, ornithology has made giant strides, in species identification, behaviour studies, analysis of bird-song and the modalities of its learning, control of reproductive rate and clutch-size in relation to ecological and climatic conditions, polymorphism not only of adults but also of eggs combined with mimicry in cuckoos and its genetic control, census and measurement of population size, geographical races and spread of range, climes and ecological 'rules', species-formation in ring-species where it can be observed actually in progress, migration and its relation to ecological and hormonal factors, homing and orientation in flight in relation to meteorological and astronomical factors, the application of biochemical technique to egg-albumens as indicators of affinity, and the principle of mosaic evolution as revealed by that most remarkable of fossils, *Archaeopteryx*

Studies in the field can now be accompanied and controlled by exact analysis in the laboratory, which can lead to novel situations, as, for example, the fact that modern techniques have made the sounds of bird-songs more susceptible to exact analysis than visual observation of form, colour and pattern, previously regarded as the chief aids to species-identification All this co-ordination of points of view and cross-fertilization of effort, due primarily to the concept of evolution by natural selection, is an integrative achievement of which biology may well be proud, and physicists perhaps envious This book is the best guide to it GAVIN DE BEER

THE ROWETT INSTITUTE

Progress in Nutrition and Allied Sciences

Being a contribution marking the first fifty years of the Rowett Research Institute Edited by D P Cuthbertson Pp xvii+452 (Edinburgh and London Oliver and Boyd, 1963) 50*s*

IN the fifty years of its growth the Rowett Institute has had two directors The first was John Boyd Orr, who started it from nothing and tended it for 33 years before handing it over to David Cuthbertson, who has achieved the great expansion of its activities recorded in this Jubilee volume of progress It is fitting that the Golden Jubilee Year of the Rowett Institute should be the occasion of the sixth International Congress of Nutrition which is being held in Edinburgh with Lord Boyd Orr as its honorary president and Dr D P Cuthbertson as its president

It is enlightening in this day of costly buildings and equipment to read of the terms of the appointment of Boyd Orr in 1913 to a post for research in animal nutrition from a Joint Committee of the University of Aberdeen and the North of Scotland College of Agriculture, and of his early presentation to that Committee of plans for a much larger project than their ideas and funds originally permitted Although the First World War interrupted the plans, the idea of establishing a Nutrition Research Institute was encouraged from Government and private sources The largest private aid came from Mr John Quiller Rowett, whose name the Institute commemorates The new building was opened by H M Queen Mary in the autumn of 1922 Then the research work begun in the small stone building at the farm at Craibstone could expand and the flow of distinguished workers already associated with the Institute could increase

The history of the Rowett Institute from 1913 until 1945 is lovingly sketched by Lord Boyd Orr in the opening chapter, and its all-too-brief pages indicate how the unflagging pursuit of a vision by a man of courage and persuasive charm can build up a research foundation able to contribute much for the welfare of mankind In this history is included a short account of the setting up of the Imperial Bureau for Nutrition and of the beginning of the journal *Nutrition Abstracts and Reviews* which carries information on animal and human nutrition to all parts of the world. More information on the growth and expansion of the Bureau, now the Commonwealth Bureau of Animal Nutrition, is given by Dr Isabella Leitch in her later chapter on the subject This side of the work of the Rowett Institute owes much to the wide appreciation of human needs by the first director, and it is in itself a great contribution to research and education in nutrition

The development of world nutrition programmes through the Food and Agriculture Organization of the United Nations had its origin in the nutrition work of the League of Nations inspired through the co-operation of the Rt Hon Stanley Bruce, High Commissioner in London for Australia, and Boyd Orr, who could see that the progress of the world depended on the "marriage of health and agriculture" The implementation of the programme begun in Geneva in 1935 led on to the idea of the World Food Board and to the Food and Agriculture Organization The appointment of Sir John Boyd Orr (as he was then) as first director-general of the Food and Agriculture Organization indicated how highly his work for international nutrition was valued The origins and early development of the Organization are recounted by David Lubbock in a lively chapter reflecting his intimate knowledge of this phase of the outward growth of the Rowett Institute.

The new director, who took over the sadly depleted staff and resources of the Institute in 1945, tells the tale of progress during his own time The very great expansion of activities is further demonstrated by the chapters from many present and recent past members of the staff who give specialized accounts of their own fields of research There are now Departments of Applied Nutrition with sub-divisions of Nutrition, Biochemistry and Metabolic Experiments, of Biometrics, of Protein and Lipid Chemistry, of Enzymology, of Microbiology, of Physiology, of Pathology with Helminthology, as well as the Duthie Experimental Stock Farm and the Commonwealth Bureau of Animal Nutrition The head of every one of these diverse disciplines is an expert in his field, and the staffs attached to the departments are clearly producing useful and interesting work as shown by their research papers

It is impossible to comment on all the individual contributions, but the chapter from R L M Synge, a Nobel Prizewinner for his work on partition chromatography, on "Some Nutritional Aspects of the Non-protein Nitrogen Fraction of Plants" is particularly salutary as it puts into perspective the chemical studies of foodstuffs made in the past half-century. Synge is severely critical of many studies on non-protein nitrogen fractions in cabbage and other vegetables and warns nutritional scientists of the dangers of being unaware of the wide assortment of new substances being isolated from foodstuffs by their chemical colleagues One of the problems he uses as an example is the nature of the goitrogenic substance occurring in cabbage

Many of the chapters are concerned with ruminant nutrition and physiology, but pigs and poultry are not neglected nor are the needs of man forgotten Chapters on "Family Diet and Health in Pre-war Britain" and "Studies of Human Growth" show that the Institute is a centre for work on the nutrition and health of the population not only of Britain but also of other countries The whole volume is rounded off by a chapter from Dr Cuthbertson on "The World Food Situation as Related to Knowledge of Science and its Application"

Lord Woolton in his foreword makes a plea for the simplification of scientific language, especially in reports on matters of nutrition, so that unscientific members of the community may read and understand advances in nutritional knowledge Lord Boyd Orr has always had the gift of simple exposition in telling phrase and has tried to impart his ability to those who worked with him The share that his colleagues and their successors have taken in educating the world in nutrition is amply demonstrated in this Jubilee volume

There are a few errors which have escaped the eagle eye of the editor. The most curious is the caption under the frontispiece where Her Majesty Queen Mary is called Her Royal Highness This no doubt will be quickly corrected Others are probably sheer slips in proof reading and it is perhaps unfair to complain of small blemishes on so fine a publication The illustrations of guests at Founders' Dinners, of the Staff in 1951, of the aerial view of the Institute and, best of all, of John Boyd Orr at Craibstone in 1919, are a delightful accompaniment to the essays from past and present staff members. It is good to have the short biographical notes on past staff members who have contributed to the volume

A M Copping

THE WELLCOME HISTORICAL MEDICAL LIBRARY

Catalogue of Printed Books in the Wellcome Historical Medical Library

Vol 1 Books printed before 1641 Pp xv + 407 (London The Wellcome Historical Medical Library, 1962) 210*s* , 30 dollars

Catalogue of Western Manuscripts on Medicine and Science in the Wellcome Historical Medical Library

By S A J Moorat Vol. 1: MSS written before 1650 Pp viii + 697 (London The Wellcome Historical Medical Library, 1962) 210*s* , 30 dollars

THE decision to publish the catalogues of the important medical and scientific collections in the Wellcome Historical Medical Library was taken while Sir Henry Dale was chairman of the Wellcome Trustees It is therefore appropriate that he should—at the invitation of his successor, Lord Piercy—introduce the first-fruits of this great project to the public and tell us something of the scope and character of what is indeed a very unusual library and one of the most interesting charitable bequests of our time

The Wellcome Library was first opened to the public in December 1949, and its extraordinary resources have been made freely available to students and research workers in all fields of learning and from all countries throughout the whole of the period during which these catalogues have been in preparation and in the press The first indication of the range and importance of its collections was given to the outside world by the *Catalogue of Incunabula* (Oxford University Press, 1954), which was the work of its present chief librarian, Dr F. N L Poynter He has also been responsible for the first volume of the Library's *General Catalogue of Printed Books,* which lists all books in the Library printed before 1641, some 7,000 items which include the *editiones principes* and subsequent Renaissance editions of Galen and Hippocrates, a most comprehensive collection of the first editions of Paracelsus, fine copies of great medical landmarks such as Vesalius and Harvey, as well as those for general science such as Galileo and Kepler The second series of this catalogue, covering works published between 1641 and 1850, will occupy four or five volumes and will reveal equal riches for two centuries of unprecedented scientific development, Boyle, Newton, and Lavoisier being represented by first editions of their works alongside those of Malpighi, Lower, Sydenham, Morgagni, Hunter, and Jenner

These treasures have been brought together over the past sixty-five years, the collection built up by the late Sir Henry Wellcome (1853–1936) and his assistants having been expanded with judgment and learning by subsequent librarians Among these was Mr. S A J Moorat, a graduate of Merton, and librarian during 1931–46, a period when the library was still strictly private and generally unknown He is now keeper of Western Manuscripts in the Library, and to him we are indebted for the first volume of a catalogue which describes all the manuscripts written before 1650 Among the 1,500 items contained in 800 volumes (representing only 15 per cent of the collection of Western MSS) are many of the first importance to the history of science and medicine Although concise details of collation, script, etc , are included, Mr Moorat's main object has been to give information on the authorship and subject-matter of these writings The descriptions of individual manuscripts are supplemented by no less than 11 indexes which open these treasures to every aspect of scholarly research

In these two volumes there is a wealth of material for those seeking to show how modern scientific thought emerged from the blend of classical tradition and semi-religious scholasticism that characterized the Middle Ages, and we must be grateful to all who have been concerned in producing what must be widely regarded as a massive contribution to the history of civilization

K J Franklin

RADIOISOTOPES IN BIOLOGY AND MEDICINE

Use of Radioisotopes in Animal Biology and the Medical Sciences

Vol 1. (Proceedings of a Conference held in Mexico City, 21 November – 1 December, 1961) Pp xvi + 563 (London and New York Academic Press, 1962. Published for the International Atomic Energy Agency) 115*s*

VOLUME 1 of *Use of Radioisotopes in Animal Biology and the Medical Sciences* is the first part of the proceedings of an International Atomic Energy Agency Conference held at Mexico City in November 1961. Many conference proceedings are now being published under titles such as this that conceal their true nature, presumably to encourage sales to library committees

Artificial radioactive isotopes have now been in widespread use in biology and medicine for fifteen years, and international and national conferences on their uses have been frequent during this time. In the early years they were very useful, one thinks, for example, of the well-organized meetings in Oxford in 1951 and 1954 The huge United Nations Geneva conferences in 1955 and 1958 were successively less valuable The many isotopes available and the methods available for their use were becoming well known Papers began to be concerned more with investigations and experiments with diverse and valuable results, but in which the use of isotopes was really only a useful incidental, comparable with the use of, say, the microscope, the paper chromatogram or the flame-photometer

The 1961 Mexico City conference was apparently organized because "although the fields (of human and veterinary medicine) use the same tools, the same training and techniques, and often the same experimental animals, opportunities for the exchange of information between the two fields are all too rare" The papers given, however, cover a wide range of subjects and are by no means confined to veterinary medicine, and only in the very loosest sense to animal biology Most of the contributors come from North and South America, with a small number from Europe

There are four introductory review papers by J H Lawrence, D H Copp, A A Kudryatsev and R. F. Glasscock on the use of isotopes in, respectively, medicine, physiology, veterinary medicine and biochemistry Kudryatsev's paper is in Russian and is a review of entirely Soviet work with 64 references, the subsequent discussion is in English As in other fields, there is much work done on the central nervous system, and on conditioned reflexes in particular It is suggested that the weight of farm animals might be raised by small doses of external radiation The other three papers are extremely good and interesting reviews, but confined in turn to work in the English language

The main body of the volume consists of thirty-five papers classified as relating to general physiology, hæmatology and glandular function There are abstracts in English, French, Russian and Spanish and discussions in English

Space will not allow mention of more than a few individual papers In the first general section there is a variety of interesting subjects There is first a paper from Oak Ridge showing many superb autoradiographs of Protozoa incorporating tritiated cytidine, uridine and thymidine There are papers on different aspects of intestinal absorption One of these is on the unfamiliar metabolism of tin in sheep, studied because of the use of tin in a fungicide used on plants such as turnips and mangolds fed to sheep and cows Workers from Chicago gave a valuable paper on the water spaces of the brain as studied by sulphur-35 and carbon-14-labelled compounds, contrasting with results from Moscow outlined in the succeeding discussion There is a similar contrast between papers from Israel and France investigating spermatogenesis by phosphorus-32 in man and animals, respectively.

The third section, headed "Glandular Functions", consists of ten papers on radioiodine and thyroid function and one on the effect of progestogens on the genital tract of guinea pigs as shown by sulphur-35 autoradiography. There are two interesting reports on endemic goitre in the Philippines and Venezuela; it is suggested that there is a genetic basis for endemic goitre and that there may be a relative increase in the proportion of plasma triiodothyronine compared with thyroxine in goitrous patients

This kind of publication can be useful in directing attention to unfamiliar work which will only be published elsewhere in foreign journals not necessarily available in accessible local libraries This particular volume is very well edited and produced and has a limited 'skeleton index' It can be recommended, in spite of earlier remarks regarding its title, to libraries of veterinary science and animal physiology

K E Halnan

THE BRICKS OF MATTER

Atomic Physics Today

By Prof Otto R Frisch Pp vi + 254 + 18 plates (Edinburgh and London Oliver and Boyd, Ltd , New York Basic Books, Inc , 1962) 25*s*

ATOMIC PHYSICS TODAY is based on a number of individual broadcast talks and articles written by Prof Frisch during the past few years These have been revised to bring them into line with present-day knowledge, but it is pleasant to note that the original brightness and spontaneity have been retained, in addition to the self-contained character of each of the sixteen chapters The reader can, with equal satisfaction and profit, either dip into the volume and read any one of the chapters separately or proceed steadily through the book as a whole. The topics discussed cover a wide range They deal not only with the well-established phenomena of nuclear fission, fusion, radioactivity and elementary particles, but also with the rather abstruse and more difficult concepts and theories relating to waves and particles, quanta, nuclear structure, the neutrino, parity, the individuality and origin of particles of matter, and causality

The descriptions given by Prof Frisch are all clearly, simply and lucidly expressed. Nevertheless one is fully conscious that they are exact, authoritative and penetrating The recent developments in nuclear physics which he discusses are those in which he himself has taken an active interest and to many of which he has made important contributions The fascinating way in which he not only outlines the discoveries and observations but also lays bare the underlying fundamental problems is greatly to be admired His enthusiasm very often leads him beyond this, and he does not hesitate to state categorically, where he considers that theory is lagging sadly behind experiment, where new lines of thinking need to be followed, and where there are many puzzles still remaining to be answered Prof Frisch affirms that fission, though it has given mankind the possibility of enormous amounts of power, is conceptually no great discovery The discovery that parity is not conserved is much more important, maybe not technologically, but certainly intellectually To try to find out why this occurs and how it fits into the material framework in which we live is just one of the many examples representative of the delight and pleasure in the search for and the acquisition of new knowledge, which, Prof Frisch concludes, is the driving force which compels him and his physicist colleagues to undertake often arduous and tedious experimental and theoretical research work

Atomic Physics Today should be read by both physicists and other scientists, and also by the adult layman It presents not only an excellent picture of modern physical knowledge, but also serves an important present need for directing attention to the compelling curiosity that urges a man to become a scientist, and on the devotion and enthusiasm with which research work is carried out in our universities and research establishments

S WEINTROUB

A COOKERY BOOK FOR CARBOHYDRATES

Methods in Carbohydrate Chemistry

Edited by Roy Whistler and M L Wolfrom Vol 1 Analysis and Preparation of Sugars. Pp xxii+589 143*s*. Vol 2 Reactions of Carbohydrates Pp xv+572 139*s* 6*d* Vol 3: Cellulose Pp xvi+407 110*s* 6*d* (New York and London Academic Press, 1962–63)

SINCE all cookery books contain sugar recipes it was inevitable that we should have eventually a cookery book for sugars themselves Here we are presented with three volumes out of a projected five or more of a new series which the editors term the "methodologies" required in dealing with carbohydrates

The field of carbohydrates is broadening at an enormous rate, especially on the biological side and, even more important, in regard to industrial applications The editors felt that the time had come when some help must be given to the non-specialists who needed many of the more unusual sugars or their derivatives Accordingly they have secured the ready collaboration of a wide company of the world's leading carbohydrate chemists, who themselves are familiar with the methods or indeed who initiated the techniques

In Volume 1 is described the methods of preparation and analysis of many of the simple monosaccharides There is a preliminary section on carbohydrate laboratory techniques, describing methods of purification, crystallization, etc , and the important and rapidly developing application of gas-liquid partition chromatography to sugar derivatives Important directions too are given for chromatography on paper, extrusion column chromatography, cellulose column chromatography, zone electrophoresis, and carbon column chromatography These are methods which have made an enormous difference to the separation of closely related sugars and their derivatives, and the non-expert and student will be most grateful to have this widely scattered information set down in such a concise manner by the experts Precise descriptions are given in later work for the preparation of 'exotic' sugars such as erythrose, threose, lyxose, ribose, altrose, tagatose, and so on It would seem impossible to go wrong with such precise directions provided Sometimes, however, one is a little disappointed, for example, in the section headed "Streptose", one finds on reading that this is still only a hypothetical substance and only its derivatives can be obtained by the methods provided An excellent description is provided for the labelling of some sugar derivatives and this work could well have been made more extensive A very large and most valuable section is provided on the analysis of sugars, thus the determination of reducing sugars by a wide variety of methods is given in a precise manner, quantitative aspects of chromatography are shown and the determination by means of enzymatic methods and by isotopic analysis Methods of periodic oxidation, often a complex and tantalizing business, are described with great clarity Nothing is left out, not even the relatively indeterminant colour reactions about which Dische has written with great authority The physical methods of measurement of properties, including X-ray, infra-red and diffraction, are also given in great detail Volume 1 is thus of high importance to all chemists and biochemists interested in sugars

Volume 2 deals with the methods involved in the preparation of derivatives of monosaccharides together with a few oligosaccharide compounds Major authorities in the field have again been appealed to and many valuable points of technique, not necessarily published before, are provided The standard pattern of an introductory paragraph, followed by the procedure and the valuable references, is followed A very large amount of information is given on the sugar phosphates which are indeed among the most difficult of organic compounds Valuable methods for the provision of methyl ethers of mono- and di-saccharides, which for a long time represented the most important series of key compounds in the whole of carbohydrate chemistry, are provided There are some omissions such as those of the methods of Kuhn The degradation of sugars, especially the polysaccharides, by means of alkali is becoming of increasing importance, so that the section on the preparation of saccharinic acids will be very valuable for the industrial chemist in particular Extensive information is provided on thiosugars, on isotopic carbon distribution and on products obtained by oxidation and reduction methods in the sugar field

In Volume 3, written almost entirely by industrial chemists, we are given essentially a new book on cellulose Already there are many volumes on this subject, but here we have, written down for the first time, more precise conditions for the most important aspects in the field The isolation of pure cellulose from a variety of sources is concisely described and, following this, methods are given for the chemical analysis of cellulose Up-to-date information is provided on the wide range of physical methods for the analysis of cellulose As workers in the field will know, the assay of crystallinity is of immense importance, and this is particularly adequately described Methods for the degradation of cellulose together with the products identified are also covered in a valuable way

There are some omissions, particularly in the modern work on cross-linking, which is becoming of great importance In all the volumes the methods are given with such detail and precision that they can readily be repeated The extensive cross-references are extremely valuable Some authors unfortunately still use outmoded methods of writing down formulæ

This publication, although expensive, can be very strongly recommended for all chemists and for all libraries

M STACEY

Language, Meaning and Persons

By Prof Nikunja Vihari Banerjee Pp 173 (London· George Allen and Unwin, Ltd , 1963) 30*s*

THIS book, the work of an Indian professor, is a continuation of his earlier *Concerning Human Understanding*, and embodies a serious attempt to present a cure for what, in his view, is the prevailing sickness of philosophy Whether he will find agreement that his patient is ill, or even, if so, that his prescription will be effective or even desirable, is another matter

It would appear that philosophy needs liberation, and this means in particular that it should extricate itself from its subservience to science and logic Which done, it could then apply itself wholly to mankind and the needs of the individual, making him unexceptionally a member of society, inseparable from his fellows Included in this would be a revival of metaphysics

Naturally, such an outlook must reckon with religion sooner or later, and the way in which this encounter occurs is peculiar The problem is faced sympathetically, but a supra-mundane being is considered incapable of dealing with the evils and perplexities of a technological age and the self-directed cravings of men One may well ask how else can humanity be saved if it is forbidden to look outside itself

The discussion includes the epistemological background, imagination, language, meaning and persons, ending with the question of human liberation Incidentally, the writer has the slightly tiresome habit of saying 'generally', when he almost certainly means 'usually'

But this slender volume is provocative in the good sense, and has a healthy reaction against the tendency of philosophy to be a poser of problems and no more

F I G RAWLINS

The Pathology of the Tumours of Children

(Pathological Monographs, No 2) By Prof Rupert A Willis Pp vii+200 (Edinburgh and London Oliver and Boyd, 1962.) 42*s*

TUMOURS of children are a special problem in pathology It is not to be expected that environmental factors, such as are well known to be responsible for cancer in adults, can be the cause of neoplasms in infancy For an explanation of these we must turn to embryology and to someone who is an acknowedged expert in both embryology and pathology Prof Rupert A Willis is obviously the man A few years ago he began a former book entitled *The Borderland of Embryology and Pathology* with this sentence "Almost from the beginning of my work in pathology, the embryological aspects had a special appeal for me, and I felt sure that embryologists and pathologists had much to gain by a better knowledge of each other's subject" This earlier book (published by Butterworth in 1958) has already become a classic and the best guide to the understanding of malformations and errors of development It has paved the way for the latest book by Prof Willis, entitled *The Pathology of the Tumours of Children*

As with all his other books, this is based on personal experience Its nucleus has been a personal histological collection of more than 700 tumours from children A special feature is that cases cited in the text can be identified by a code of initials, and duplicate histological sections and records of all specified cases have been incorporated in the Imperial Cancer Research Fund's Tumour Reference Collection This will be much appreciated, because the histological classification of some tumours of children is difficult for hospital pathologists without special experience In this field even experts often disagree Many instances of differences of opinion are admitted in this book, one typical example being from the "gliomas of uncertain nature" where, in one instance (case 6, p 35), the panel of experts differed greatly in their diagnosis

The value of a monograph such as this depends on three features (1) the authority of the author, (2) the choice of references to other work, (3) the quality of its illustrations In this work, as in all his previous writings, there is no doubt about the authority of the author or the selection of references Moreover, in this latest book, all will agree also that the illustrations are superb They are the work of Mr E V Willmott, of the Imperial Cancer Research Fund

C E DUKES

The Identification of Molecular Spectra

By Dr R W B Pearse and Prof A G Gaydon Third edition Pp xi+347 (London Chapman and Hall, Ltd , 1963) 120*s* net

SINCE this book was first published in 1941—and particularly in the thirteen years since the second edition appeared—advances in the development of spectroscopic techniques and a widening of the fields of research in which spectroscopy plays a dominant part have led to the publication of a vast amount of new data With the same care and precision as marked the previous editions, the authors have collected and collated the data up to the end of 1961 The table of persistent band heads, extending from p 3–50, includes some 350 new entries and is set out with extreme clarity and ease of consultation The bulk of the book (pp 51–320) deals with individual band systems, including 84 molecules not previously included and 135 molecules for which additional or revised data have been obtained In some ten pages are given practical procedure and precautions, followed by a number of actual spectra and an appendix of the persistent atomic lines of each element

The authors are to be warmly congratulated for their assiduity and patience in sorting out and presenting in such an eminently acceptable form information which will prove to be of incalculable worth to all who are directly or indirectly concerned with molecular spectroscopy.

The printing and general arrangement are excellent and make it so easy to use the book For what it contains —and is—this book is good value at £6

W ROGIE ANGUS

The Earth and You

A Geographic Picture of the World We Live In By Dr. Norman J G Pounds Pp 591 (London John Murray, 1963) 50*s* net

THIS is a general account of the way in which climate, soil and mineral resources have affected the life of man It is especially welcome at a time when the growth of the Earth's population and the problem of increasing food production concern all thoughtful people Dr Pounds makes it clear that geographical factors cannot alone explain differences in living standards, he contrasts areas of comparable climate and resources and does not hesitate to point a moral where necessary

He first discusses the Earth as a whole, its structure and climate, criteria of over- and under-population, gross national product, food consumption and the factors which link men in political units There follows an account of the principal regions of the world and the States composing them The reader is made conscious of the difficulties that arise when social, economic or political aims conflict with facts of topography or climate

The book makes interesting reading, is well produced and is illustrated with many diagrams The two-page maps would have been better mounted to 'fold-in' some of the main points of interest are obscured by the fold of the binding

So wide a survey cannot be detailed, but Dr Pounds has provided a supplementary reading list for those who wish to make a more detailed study of a particular area They are nearly all American publications The book will be a welcome addition to a school library or to the possessions of any person who seeks information on matters of general interest

RESEARCH, INNOVATION AND ECONOMIC GROWTH*

By K. PAVITT†

Directorate for Scientific Affairs of the Organization for
Economic Co-operation and Development, Paris

Economic Growth and the Role of the Organization for Economic Co-operation and Development

ECONOMIC growth means an increasing provision of goods and services to satisfy individual and collective human wants. In the past 150 years, certain areas of the world have seen a steady and substantial growth of production per head of these goods and services, in general accompanied by a steady growth in population. This growth has generally gone hand-in-hand with a more equitable distribution of wealth among the population, more opportunities for education and more leisure.

The Organization for Economic Co-operation and Development is an inter-Governmental organization consisting of twenty member-countries from North America, Western Europe and the Mediterranean region. Its aim is to promote economic growth among its members, and to co-ordinate their aid to the economically less-developed countries. A large part of the work of the Organization is therefore concerned with conventional economic and aid policies.

However, the member-Governments of the O.E.C.D. are becoming increasingly concerned about their national efforts in scientific research and development, largely because many Governments are paying increasingly large sums to support research and development, but also because of a general and rather vague feeling that an adequate national effort in research and development is now essential in an industrial society. This concern has led to the creation in many countries of governmental bodies concerned with the national research and development effort. Belgium now has a National Council for Scientific Policy, France, a General Delegation for Scientific and Technical Research, the United Kingdom, an Office of the Minister for Science, and the United States, a President's Office for Science and Technology. Within the past year the Italian, Swedish and West German Governments have established special departments or councils to deal with questions of scientific policy.

Scientific Discovery and Innovation in Economic Growth

The work of the Directorate for Scientific Affairs of the O.E.C.D. has therefore become largely concerned with the contributions which scientific and engineering education, and scientific research and development, make towards economic growth, and with the governmental policies required to ensure that these contributions be effective. This article will briefly set down some of the important problems which have so far been raised by this work.

It must be stressed from the outset, however, that an adequate level of research and development, and rapid innovation, are not the only conditions necessary for economic growth. For the purposes of this article, it will be assumed that the other conditions, such as a stable political system, stable means of exchange of goods and services, an adequate level of demand for these goods and services, and a system ensuring equitable rewards for productive effort and efficiency, already exist.

To say that scientific discovery and innovation are important factors in increasing the productivity of labour is to say nothing new. In the eighteenth century, Adam Smith wrote of "The invention of a great number of machines which facilitate and abridge labour, and enable one man to do the work of many"[1]; and about the sources of invention he wrote that "All improvements in machinery, however, have by no means been the inventions of those who have had occasion to use the machines. Many improvements have been made by the ingenuity of the makers of the machines, when to make them became the business of a peculiar trade, and some by that of those who are called philosophers or men of speculation, whose trade is not to do anything, but to observe everything, and who, upon that account, are often capable of combining together the powers of the most distant and dissimilar objects"[2].

Much more recently, Prof. S. Kuznets gave six lectures on the nature of economic growth since the time when Adam Smith was writing, and concluded that the sustained increases in the productivity of labour could not have been maintained without continuous innovation, that is, the new application of either old or new knowledge to production processes. Since the possible uses of old knowledge were limited, continuous innovation required continuous new additions to knowledge. He went on to point out that many of the industries now important in the advanced economies, for example, in the electrical, chemical and internal combustion fields, were entirely unknown a hundred years ago[3].

As Prof. Kuznets implies, and as Adam Smith dimly foresaw, innovation in the industrially advanced countries has become increasingly dependent on discoveries made by trained scientists and engineers, and on the regular and systematic exploitation of such discoveries in the economy. There is evidence in many countries that the proportion of the total number of patents taken out by trained scientists and engineers is growing. Also, the resources in the economy devoted to 'research and development activity'[4] are growing in most of the industrially advanced countries[5].

One can suggest that the following factors are influencing this trend. First, entrepreneurs have seen from past experience that innovation based on scientific discovery can be profitable, and the widening boundaries of science have given them more and increasingly powerful tools with which to manipulate their physical environment, and to create and improve products and production processes. Secondly, the increasing scale of industrial operations has made it organizationally more efficient for innovational activity—namely, research and development—to be undertaken as a separate, but related, entity. Thirdly, the nature of production processes is increasingly such that persons working in production cannot so easily make any startling innovation or routine improvement; the outside of a length of chemical piping must surely frustrate any would-be inventor. Fourthly, certain companies are using techniques which are at the limits of scientific and technical knowledge, so that improvement and change can come only by finding new knowledge as a result of research and development. Finally, it must be admitted that certain companies undertake research and development because it is fashionable, and with no clear idea of its potential economic usefulness

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* Substance of a paper read before Section F (Economics) of the British Association meeting in Aberdeen on September 3.

† The opinions expressed here are those of the author and not necessarily those of the O.E.C.D. I thank Dr. A. King and Mr. J. R. Gass for their comments and suggestions.

What, then, can statistics tell us of the extent to which technological innovation, and research and development on which it largely depends, have influenced rates of economic growth? (It must be borne in mind that these statistics can measure neither the benefits which research and development bring in the form of improved goods for consumption at constant prices, nor of the improved welfare of the population resulting, say, from medical research)

The importance of innovation has been confirmed by a series of investigations undertaken in the United States which show that only about one-half of the growth of labour productivity over the past 50 years in the United States can be explained by the growth in the amount of capital per man, and by the improved allocation of both labour and capital[6] The remaining growth must be explained by a variety of 'residual factors', including economies of scale, improved health, skill and education of the labour force, improved organization, and technological innovation It should be noted that all the aforementioned factors, including labour and capital, are interdependent, and that conclusions about the individual 'contributions' of these residual factors must consequently be treated with the greatest care

That research and development influences the rate of industrial growth has been confirmed by a British investigation which shows that, over the past 25 years, the industrial sectors in the United Kingdom and the United States, which undertake relatively large amounts of research and development in relation to net output, are those in which total production has tended to grow rapidly and in which labour productivity is high Also, those industrial sectors are the same in both countries[7] The rapid growth would seem to indicate that research and development influences the rate of innovation, and its sameness in both countries that any new breakthrough in scientific knowledge is likely to benefit some economic sectors more than others

Nevertheless, it has been shown that even within an industry there tends to be a correlation between the amount of research and development undertaken in a company, relative to its size, and conventional signs of a company health, such as growth of net assets, productivity, profits and sales[8]. This correlation is not always strong, which is not surprising when one considers the many factors completely unrelated to research and development which can influence company growth However, even assuming that there is a correlation, this does not prove that research and development causes growth, and it has, in fact, been argued that growth either causes or enables companies to undertake more research and development This line of argument can, surely, safely be rejected in large part, since irrefutable evidence of the contribution of research and development to new and improved products is all around us

However, a more powerful line of argument is that both the volume of research and development expenditures and the various measures of company growth are results of something called 'good management' Common sense does in fact tell us that it requires good management to decide what sort of research and development to undertake, and to ensure that its results are effectively used In this context, it is worth noting that there is some evidence of a correlation between the growth of productivity of an industry or company, and the proportion of total employment in that industry or company which is made up of qualified scientists and engineers[9] One can conclude from this that good management increasingly requires some formal scientific or engineering training This has important implications for educational policy

To sum up, it can be said that those industries which are able to exploit scientific knowledge are growing rapidly, and that an adequate expenditure on research and development at the company-level is a necessary, though not sufficient, condition for growth.

Policies for Research, Innovation and Growth

From an economic point of view, research and development becomes of greater importance as a country becomes more industrialized With full or near-full employment and a small proportion of the working population in agriculture, increases in labour productivity will come from overall increases in labour productivity, together with the shifting of resources to the more productive industrial sectors of the economy From what has been shown here, both these conditions imply more research and development for economic purposes

Furthermore, if the economically advanced countries are to help the less-developed to produce more, to reduce unemployment and to find foreign exchange currency, one may regard it as their duty, as well as to their advantage, to move resources into the technologically advanced sectors, where they have a comparative advantage The less-developed countries need, and will continue to need for some time to come, markets in the advanced countries for simply manufactured products in such sectors as textiles and handicraft.

However, in the industrially advanced countries, the particularly long-term, risky and often costly nature of investment in research and development, and the often imperfect knowledge of industrialists as to its potential profitability, make it highly unlikely that an adequate level of expenditure on research and development for economic purposes will be achieved without governmental encouragement and help Some suggestions will therefore be made here about ways in which Governments can help to ensure an adequate level of research and development for the economy and rapid innovation

Scientific and Engineering Education. It is self-evident that the educational system should provide an adequate number of qualified scientists and engineers to undertake research and development for economic purposes. Such provision will require a considerable development of postgraduate investigations in science and engineering in many countries. Also scientists and engineers are wanted in increasing numbers in companies for related purposes From what has already been said, one can conclude that it is becoming increasingly difficult for managers without any scientific or engineering education to appreciate the economic value of some new scientific or technical discovery, or to give to the company's research and development workers the right problems to try to solve Scientists and engineers are therefore required to an increasing extent in general, production and sales management, in order to ensure that the results of research and development are fully and effectively used In this context we can include 'research and development' in such fields as market research, operational research and quality control

Adequate research and innovation in the economy therefore requires a generous supply of qualified scientists and engineers But it also requires, in many countries, that more persons of outstanding talent investigate applied science and engineering and that the content of this education be of high quality These requirements are shown clearly by the example of a country like Sweden, where industry is very responsive to the economic possibilities of technical change, and of the value of undertaking research and development This is largely because of the high academic and professional reputation of the Swedish institutes of technology, the entrance requirements of which are highly competitive, and the graduates of which are heavily represented among the leaders of Swedish industry

Economic and Fiscal Policies Research and development in a company is in the nature of an unusually long-term and risky investment, so that it presents problems even to enlightened management Uncertainty concerning the future prospects of the economy may make any long-term investment in research and development appear to be of little value, or may restrict capital investment programmes incorporating the results of past research and development Thus, general economic conditions and policies affect the amount of research and development undertaken in enterprises and the degree to which results of past research and development are used Scientific policy makers should bring these matters to the attention of their counterparts in ministries of finance, and should insist on the importance of an adequate overall level of demand in the economy, and of generous fiscal treatment with regard to company expenditures on research and development, and in certain cases to the writing off of past investments

Small or Backward Companies Many industries are made up of companies which are each too small to afford the men or money necessary to set up individual departments for research and development work In such industries, Governments can give aid and advice in setting up and running research and development establishments which care for the needs of a whole industry, and may be financed to some extent by companies in that industry Governmental research establishments already exist in most O E C D member countries to cover the needs of agriculture and building Industrial research associations similar to those in the United Kingdom exist in, for example, France, Holland, Norway, Sweden and Western Germany It is worth noting that industrial research associations can also be effective in industries made up of large firms when there is no technological competition

This form of governmental encouragement of research and development does not necessarily ensure that the results of the research and development are used, above all in small companies where management is often unable to appreciate their potential value Convincing management of the value of the results of research and development would appear to require not only an effective method of disseminating information from the research laboratory, but also a form of 'extension service', which is already widely used in agriculture Here, technical advisers are sent out to convince farmers of the value of recently developed techniques, and to assist them in putting these techniques into operation This is done to good effect in the industrial field by the T N O (Organization for Applied Scientific Research) in Holland, where about 20 per cent of industrial research and development is financed by firms with less than a thousand employees

Development Work Development work[4] represents the heaviest financial commitment of companies undertaking research and development programmes, accounting, on the average, for more than 75 per cent of the total cost of research and development work carried out in industry

In certain countries, development work in the aircraft and electronics industries is financed in large part by Governments as part of military, space or nuclear programmes The results of such development work can often be of use in the normal commercial operations of companies in these industries

However, in all countries, companies in the majority of industries rely on their own financial resources for the performance of development work, and it is clear that many are not able to undertake potentially profitable development programmes through lack of finance It can therefore be argued that Governments should make funds available for the performance of development work in the civil field in all industries, thus helping to restore a balance between those which benefit from governmental military, space and nuclear programmes and those which do not, and ensuring that no potentially profitable discovery is neglected through lack of funds for development work

Considerable efforts are already being made in this direction by the French Government, which is making funds available in increasingly large quantities Similar efforts, although on a smaller scale, are being made in Belgium and the United Kingdom In all the aforementioned countries, the Governments draw some financial benefit from development work which they help to finance and which proves to be commercially profitable

The Government as an Enlightened Buyer Even in the so-called capitalist countries, Government (central and local) is the largest single buyer of goods and services in the economy It is, therefore, in an excellent position to act as an enlightened buyer and to encourage, and even insist on, high technical standards from its suppliers This happens already to a large extent with governmental defence contracts in industry, because the fulfilment of the technical requirements specified by the Government in such contracts is considered to be of the highest national importance However, essentially the same thing happens in contracts between shirt manufacturers and Marks and Spencer

There is a growing awareness by Governments of the influence that they can have in this field In the United Kingdom, the Government is a customer for about 50 per cent of the output of the building industry and is making considerable efforts to improve building techniques[10] Similar developments are evident in the United States It is clear that the success which governmental departments have in encouraging innovation will depend largely on the degree to which they can call on technically competent staff

Obstacles to Innovation Some mention must be made here of factors which, although not directly related to research and development or to scientific policy, may nevertheless obstruct efforts to innovate and thus lead to a diminution of research and development activity Perhaps the most important obstacle to innovation can arise out of the restrictive attitude of a labour force which feels that innovation may upset established practices and, more specifically, lead to redundancy Governments can take effective action, in co-operation with industrial and Trade Union leaders, to overcome this obstacle by educating the labour force to the long-run advantages of innovation and, at the same time, by mitigating the worst short-run effects through an active programme for the retraining and redeployment of redundant workers Also Government rules and regulations may stifle innovation In the building industry, the use of new building methods and materials is often made impossible owing to statutory safety requirements, and mass-production and prefabrication often precluded through a lack of uniformity between locally required standards This is an obvious area where Government can act

Choice of Areas for Government Action The foregoing discussion shows that inadequate research and development and the inadequate use of scientific and technical knowledge for economic purposes can stem from a variety of causes, and that a variety of measures can be employed by Governments to try to put things right Some of the measures, such as the fiscal relief on research and development expenditures and those related to educational policy, are widespread in their effects Others, such as the setting up of industrial research associations and the awarding of development contracts, affect specific industries or firms.

It is very difficult in an article of this length, and knowing what we do at present, to assess the relative merits of these general and specific measures Such an assessment would, furthermore, be influenced by one's political leanings and by the circumstances existing in a particular country at a particular time However, it is possible to say a few words about the choice of priorities for governmental action in specific economic and technical sectors, since these choices are in fact being made In France, encouragement and aid to research and development are being given by the Government on the basis of the economic trends foreseen in the Fourth Economic Plan, and of the degree to which industries and companies 'import' their innovation from foreign countries in the form of patents and manufacturing licences. In the United States and the United Kingdom, efforts are being made to bring about innovation in the building industry, because present techniques are inadequate to fulfil expected future needs. In the United Kingdom, efforts have been made to improve the level of research and development in industries which, by comparison with other countries, were comparatively weak[11], and recommendations have been made that the allocation of funds to research and development should be influenced by the degree to which such an allocation might promote exports or save imports[12]

It is clear that, given that the financial and human capacity for research and development of a country is limited in the short run, choices of priorities will be made to an increasing extent, either explicitly or implicitly, in future It is to be hoped that economic criteria will be fully considered when these choices are being made

Scientific Discovery and Innovation Outside the Economy

So far, this paper has considered only research and development undertaken for economic purposes, and I have said that more of it is being done It must never be forgotten, however, that research and development is undertaken for other purposes, and that more of it is being done This other research and development accounts for more than half the national expenditures in certain countries In some cases it competes with the economy for research and development resources, and in some cases it complements the efforts of the economy We shall discuss here the two most important components of this other research and development namely, military and 'prestige' research and fundamental research

Military and Prestige Research National military strength is no longer primarily a function of the number of infantrymen nor even of industrial potential but rather of the quantity and quality of certain types of scientific and technological activity Similarly, prestige in foreign policy no longer depends on gunboats but on the technical virtuosity of space satellites. Certain Governments, therefore, spend a great deal of money, and employ a large number of scientists and engineers, on research and development as part of defence, nuclear and space programmes More than 70 per cent of the funds provided for research and development by the Governments of France, the United Kingdom and the United States are in these three fields

The main purpose of research and development work as part of national defence, nuclear[13] or space programmes is not to provide goods and services for the public, although knowledge of economic value may be produced by accident. However, a large part of the research and development work is undertaken in companies with civilian interests—mainly in the aircraft and electronics industries—so that some of the work may find civilian applications Nevertheless, some reservations are being expressed concerning the overall economic benefits of military and prestige research[14]. It is being argued that the nature of the problems being solved is becoming very different from those which concern the mainly earthbound economy, and that the possibilities of 'spin-off' from the military and prestige to the civilian field are diminishing

Furthermore, research and development projects for defence and space are competing with research and development projects in the economy and in the universities for the services of a comparatively restricted supply of people capable of undertaking research and development Large-scale programmes in defence and space can, if not carefully watched, lead to rapid increases in the salaries of research workers, or to unfilled vacancies in other research and development projects and in the teaching posts in the universities, without any real increase in the quantity of research and development activity actually undertaken

There is, in fact, some evidence that the three-fold increase in expenditure in the United States on defence and space research over the past ten years has led to a levelling off of research and development for economic purposes[14] By contrast, research and development for defence purposes over the past eight years in the United Kingdom has remained approximately constant as a proportion of gross national product, whereas research and development for economic purposes has seen a three-fold increase[15] Given, however, that many Governments will find it necessary to have large-scale research and development programmes in defence and space, scientific policy makers should ensure that these enormous programmes are well managed and that there is no unnecessary waste of money or—more important—men[16] At the same time they should ensure that, so far as the need for secrecy allows, the scientific and technical knowledge accumulated as a result of military and space programmes should be made readily available to industry and the universities

Fundamental Research Most fundamental research is undertaken in the universities or technical colleges, although a certain amount is carried out in governmental laboratories and in industry The increasing practical utility of science and technology has led to the increasing demand for scientists and technologists, so that the scientific and engineering faculties of the universities are expanding[17], and so are the facilities for research

An adequate level of fundamental research is, in the long run, an essential pre-requisite for innovation and economic growth Many modern industries are based on the discoveries of scientists who, at the outset, had no interest in the potential economic usefulness of their work Attempts to 'direct' fundamental research or to restrict the distribution of its results often meet with stiff opposition from the scientific community. This is right and proper on cultural and ethical grounds It is also right and proper on economic grounds, since most good scientists are more productive when following ideas of their own choosing, and their discoveries are more likely to find some practical application if the results of their work have wide and unrestricted distribution.

In most countries, the largest proportion of funds for fundamental research comes from Government It is, therefore, very important that the holders of the public purse take a generous and long-term view of expenditure on fundamental research, and that they evolve an effective method of providing adequate funds for fundamental research, above all in the universities

At the same time, both the nature and the role of fundamental research is changing It is becoming increasingly concerned with 'unnatural' states of, say, distance, speed, size, time, temperature and pressure[18] Heavy expenditure on equipment is often required to bring about these states, and increasingly refined techniques are required to measure and record them Moreover, there are now many remarkable aids to calculation, but they cost a great deal of money. This often means that special Government grants are now needed to set up research institutes which are used by more than one university—such as the National Institute for Research in the Nuclear Sciences at Harwell

Furthermore, fundamental research is becoming of practical importance, mainly as a result of the advance of techniques in the military, space and industrial fields 'Oriented' fundamental research is therefore being undertaken in industrial, governmental and university laboratories In the United States, the growth of oriented fundamental research mainly as a result of governmental and industrial contracts with the universities has been particularly rapid It is clear that Government should help ensure that industry and Government have fruitful contacts with the universities concerning oriented fundamental research. At the same time, however, it should be careful to ensure that the oriented research work does not swamp out the very necessary free fundamental research

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[1] Smith, Adam, *The Wealth of Nations*, Book I, Chap I

[2] Smith, Adam, *Op cit.*, Book I, Chap I

[3] Kuznets, Simon, *Six Lectures on Economic Growth*, Lecture II, Illinois (1959)

[4] The definitions of research and development used by the National Science Foundation for statistical surveys in the United States are the following

Research is systematic, intensive study directed towards fuller knowledge of the subject studied Research may be either basic (that is, fundamental) or applied

Basic (that is, fundamental) research is directed toward increase of knowledge, it is research where the primary aim of the investigator is a fuller knowledge or understanding of the subject under study rather than a practical application thereof

Applied research is directed toward practical applications of knowledge

Development is the systematic use of knowledge directed toward the design and production of useful prototypes, materials, devices, systems, methods or processes It does not include quality control or routine production testing

The fields of science covered by these surveys consist of the engineering, physical and life sciences

[5] For example, see the publications of the National Science Foundation, Washington, and of the Central Bureau of Statistics, the Netherlands, the report of the Advisory Council for Scientific Policy, 1961–62, London, U K , *Le Financement de la Recherche Scientifique et Technique en France* (Paris, 1961)

[6] For example, Solow, R , "Technical Change and the Aggregate Production Function" *Rev Economics and Statistics* (1957) Massell, B , *Determinants of Productivity Change in United States Manufacturing* (Rand Corporation, California)

[7] Freeman, C , "Research and Development A Comparison between British and American Industry" *Nat Inst Economic Rev* , No 20 (May, 1962)

[8] Federation of British Industries *Industrial Research in Manufacturing Industry, 1959–60* (London 1961) Some unpublished statistical work has also been done by Stanford Research Institute, California

[9] National Economic Development Council *Growth of the United Kingdom Economy to 1966*, 30 (London, 1963) Sveriges Industriforbund *Ingenjorsbehovet i Sverige* (*The demand for engineers in Sweden*) (Stockholm, Sweden 1957)

[10] *The Economist*, November 24, 1962

[11] *The Machine Tool Industry* Board of Trade, London, 1960, and, *Research and Development Requirements of the Shipbuilding and Marine Industries* D S I R (1960)

[12] Carter, C F , "The Distribution of Scientific Effort" in *Minerva*, 1, No 2 (1963) National Economic Development Council *Conditions Favourable to Faster Growth*, 32 (1963)

[13] In this context, one must exclude nuclear research concerned with the generation of energy

[14] Solo, Robert A , "Gearing Military Research and Development to Economic Growth", *Harvard Business Rev* (November–December, 1962)

[15] *Advisory Council for Scientific Policy Ann Rep , 1961–62* (London, 1963)

[16] In this context, see the recommendations of *The Management and Control of Research and Development* (H M S O , London, 1961) *Rep to the President on Government Contracting of Research and Development* (Government Printing Office, Washington, D C , 1962)

[17] O E C D *Third International Survey on the Demand and Supply of Scientific and Technical Personnel* (to be published)

[18] Sir John Cockcroft's Presidential Address to the British Association, 1962

PROBLEMS OF ECONOMIC GROWTH*

By Prof C. F. CARTER

Vice-Chancellor of the University of Lancaster and Joint Editor of the *Economic Journal*

THERE are two problems of growth one is how to begin and sustain the process in those countries which remain very poor, and the other is how to influence the rate of growth of developed countries In discussing these, it is important to keep a firm control of our time perspective A compound rate of growth of production per head at 5 per cent per annum is sufficient to give an increase of 11½ times in 50 years (which is enough to get the Indian standard of living within striking distance of that at present enjoyed by the United States), or of 132 times in a century Small differentials in growth-rates, operating over quite a short time in the history of the human race, are sufficient to produce large differences between rich and poor nations High *per capita* growth rates (of the kind, for example, which Britain hopes to achieve through the National Economic Development Council) must necessarily be a short-lived phenomenon when considered against the whole scale of history It is plausible that, with the substitution of new materials and sources of energy, the world can produce five times what it now produces, it is not plausible that it can produce a hundred times as much, either in total or per head, nor does it seem very likely that the human race would desire to create material wealth on this scale During the next century the increasing difficulty and real cost of winning extra supplies, and the cost of finding or making substitutes for easily won natural materials, may slow down the achievable rate of growth below the rate of rise of population, so that the world average income per head would necessarily fall

It follows that, so far from the future holding a promise of Cadillac motor-cars for Indian farmers and ever-larger television screens in the villages of Africa, our grandchildren or great-grandchildren might belong (on average) to the richest generation ever to dwell on this planet, at least until the human race learns to control its numbers, and the peak would be reached at a time when serious poverty was still the fate of a large part of the human race Beyond that time there would be a direct conflict between the relief of poverty and the desire of the rich to become still richer

The flow of wealth is created by the application of human effort, skill and ingenuity to the resources provided by Nature and to the stock of capital goods created in past times, within the framework of a particular method of organizing production and exchange It follows that economic growth requires one or more of the following more human effort, more skill and ingenuity (including here the use of a more advanced technology), the discovery of more natural resources, more capital goods (of an appropriate kind), more appropriate organization

Economists have sometimes given the impression that the key factor in economic growth is the size of the stock of capital In consequence, a country wishing to achieve rapid growth was to tighten its belt, to use less for its immediate needs, and to divert its effort to the creation of a capital stock to produce wealth in the future This, we were told, was the great secret of the growth of the Soviet economy It was an unhappy implication that those countries most in need of growth would find it hardest to achieve, because they would be too poor to divert resources from current consumption

Now, since economic growth usually involves the production of new kinds of goods, it is no doubt generally true that it involves the creation of new capital to make those goods But where several influences work together to create a result, it is dangerous to label one as a 'key factor'. It seems *a priori* more likely that the most important influences on growth will differ from one country to another and from one period to another Furthermore, the influence of a stock (whether of capital, natural resources, or knowledge) depends not only on its absolute size but also on the effectiveness of its exploitation Machines which work one shift can be made to work three, railway lines which carry trains once an hour can carry them every ten minutes It does not follow that, because the British rate of growth is low, therefore we immediately need a lot more capital Perhaps for a considerable period we need only make better use of what we have got already

(Mr Carter then illustrated the importance of alternative influences on growth by discussing a series of propositions, two of which were as follows)

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* Substance of an evening discourse delivered on August 30 to the British Association meeting in Aberdeen

(I) *In the short period, Britain could have a 5 per cent growth-rate if people worked harder and longer.* Such a proposition might not be true if applied to an Indian village, where people already work as hard as a diseased and under-nourished state allows. But few of us in Britain have reached the limit of our capacity to work; and we often, in common gossip, ascribe our lack of economic progress to our failure to work as hard as (say) the Germans or the Japanese. Why, then, do we not work harder? Or (to put it another way) why are there periods in which the energies of a community are easily stimulated and released, and other periods when men demand action of others which they will not give themselves?

The question is an obvious one; but it is not answered by sermons, political speeches, or leaders in *The Times*, calling for a 'new sense of national purpose'. This only states the problem: Why does the sense of national purpose vary? The social influences on intensity of effort remain, in fact, somewhat mysterious, despite the many millions of words which have been written about them—probably, again, because they are various, and operate in different combinations and to different degrees at different times and places. I select a few pointers:

(*a*) People will not work hard to create material wealth unless it is considered desirable and respectable to enjoy the fruits of their labour; in this sense economic growth requires a 'materialistic' society.

(*b*) They will not work hard unless they can imagine themselves attaining the desirable consequences. Energy is released by the spread of the use of money in an economy formerly producing for subsistence within closed village or family communities, for money carries the power to break out into a new kind of life, to buy the products of the towns or to emigrate. In a developed community, energy is stimulated by strong social pressure to keep up with the neighbours; the best achieved within our social group is the object of our own imaginings.

(*c*) It follows that a society with marked class or caste or racial barriers will offer an inferior stimulus; if a man knows or believes that he cannot rise far, why should he bother? Why work to get a Rolls-Royce motor-car if one is confined for life to a social group satisfied with a Mini-Minor car?

(*d*) The extent of people's effort depends on the weight which they put on the co-operative, as against the competitive, aspects of society. All economic life, in any society, involves elements both of co-operation and of conflict. But in some societies a man who works harder sees his efforts as benefiting 'Them'—the bosses, the shareholders, the Government—while in others he sees first the benefit to himself and to 'Us', the common interest in economic growth. These points of view bear no apparent relation to the actual division of the benefits, though they are perhaps related to the extent to which social divisions run along lines which separate economic functions.

(*e*) People will not work hard if they see the results of their efforts running away in waste and frustration. Three points are important here. First, the economic system must be organized with reasonable efficiency and honesty: lack of economic growth can sometimes be blamed on an inflexible and stupid administration, or on a prevalence of bribery and corruption. Secondly, the vigour of new ideas must not be suffocated by a dead weight of orthodoxy; and a problem here is that an orthodox community may not even realize that it is resisting new ideas. Thirdly, the energy of the young must not be frustrated by the power of the old. A system which closely relates influence to seniority, and which regards it as a positive advantage to be ruled by old men, is no place to expect rapid economic growth.

(II) *Economic growth depends on the level of education.* This is one of those remarks the apparently self-evident truth of which conceals the fact that it does not mean very much. There are several different ways in which lack of educational facilities may inhibit economic growth, and they call for different remedies, which are not accurately described by a general phrase about 'raising the level of education'. First of all, there may be specific shortages of technical skill—say, a lack of design engineers or of soil scientists. Such shortages, if they affect major sectors of the economy which in other respects are ready for vigorous growth, can properly be regarded as 'key factors', and they call for specific efforts of training or re-training applied to limited groups of people. Secondly, the spread between efficient and backward producers may be too great. This calls for an educational effort of a complex kind. It must predominantly be adult education if it is to take effect in a reasonable time. It must start by identifying why backward producers tolerate their own backwardness. Are they merely ignorant? Are they enjoying an easy protected life, without the trials of keeping in the front? Are the incentives to improve their ways too small? Are they held back by social pressures, or religious scruples, or faults of organization? To treat the disease by technical education assumes that ignorance is the main factor—that if the producer is told of a better way, he will follow it; and this is not necessarily true.

Thirdly, a generally higher level of education may contribute to economic growth by increasing adaptability to change. This idea involves, not instruction to meet a known need, but the development of ability and personality to meet unknown situations in the future. Instruction to meet a known need may, in fact, inhibit growth, if people become fixed in their habits of thought. We are, I think, in Britain in danger of getting too much instruction in technology and too little education in creative technological thinking; and though this may help to relieve certain specific shortages of skill, it does not sufficiently assist our adaptation to changing conditions of trade.

Fourthly, education may assist growth by altering the problem of communication. This is most obviously true of learning to read and write; the written instruction and message can replace the fallible memory of a verbal communication. Similarly, an improvement in what is now called 'numeracy' widens the range and precision of communication; a man who can interpret a graph, or read the message of a table of statistics, can acquire ideas more rapidly and accurately than he can from words alone. Again, if a country gives specialist scientific training to more people, it increases the number of those who are 'tuned to the same wave-length' as scientists in other countries—it provides itself with listening or observation posts, so that it becomes more likely than before to pick up new ideas quickly and effectively.

But more of the wrong kind of education may waste ability while doing nothing to promote economic growth; it may merely create a class of educated unemployables. More, even of the right kind of education, is achieved only at a cost—a shortening of working life, a diversion of ability into the teaching profession. The universities of Britain, for example, have a very large and increasing appetite for the services of their own most able graduates. First-rate ability is in desperately short supply, in industry, commerce, the professions and Government service. It is not entirely fanciful to suppose that in the short run our very necessary university expansion may be harmful to economic growth.

In my reading of past history I do not find, and I do not expect to find, any one cause of stagnation, or any one system of remedies for it. The way to economic growth is to be found through a profound understanding, not only of the economic influences bearing on a community, but also of its social structure and ways of thought. For this reason I am very doubtful of the value of dispatching economic experts to help underdeveloped countries to achieve development—except that an expert may provide outside authority for doing something already seen to be desirable but difficult to achieve.

For Britain, there is no technical or economic difficulty in achieving and maintaining for a considerable period a 4 or 5 per cent rate of growth. The resistant difficulties will, I think, be found to lie in our social structure and habits of thought: in the sharp class distinctions between master and men; in the traditions of trade union bargaining and cost-plus pricing; in the pressures of democracy to get benefits now at the expense of the future. There is no simple formula to dissolve these difficulties.

INCREASE IN RADIOCARBON FROM THE MOST RECENT SERIES OF THERMONUCLEAR TESTS

By REIDAR NYDAL

Radiological Dating Laboratory, Norwegian Institute of Technology, Trondheim, Norway

BEFORE development of the atomic bomb, the natural production of carbon-14 in the atmosphere was only effected by the cosmic ray neutrons (*n*), as nuclear reaction with nitrogen atoms (nitrogen-14) in the air produced the carbon-14 isotope according to the equation:

$$^{14}N + n = {}^{14}C + {}^{1}H$$

The natural equilibrium between production and disintegration of carbon-14 in the world reservoirs has been disturbed by atom bomb explosions, where liberated neutrons produce additional carbon-14 in the atmosphere. For explosions in the atmosphere the number of carbon-14 atoms produced is nearly equal to the number of liberated neutrons.

The first nuclear bombs operated by the fission of uranium or plutonium produced large quantities of fission isotopes such as strontium-90, cæsium-137 and iodine-131. In the hydrogen bomb, however, the main energy is released by a fusion reaction which does not result in liberation of fission products. The neutron yield per megaton in a fusion reaction may be greater than for fission. According to results obtained by Leipunsky[1], $2 \cdot 2 - 32 \times 10^{26}$ neutrons per megaton were released by various fusion reactions. For explosions prior to 1959 the total observed neutron yield (fission and fusion) was close to 2×10^{26} neutrons per megaton[2]. This was, however, a mean value derived from different types of nuclear weapons.

Radiocarbon in the atmosphere from nuclear tests has already been investigated by several authors[3]. The large series of thermonuclear tests carried out in 1961 (150 Mtons) and in 1962 (320 Mtons) have caused a more dramatic rise in artificial radiocarbon than before, and I have therefore considered it important to follow the recent increase in radiocarbon content of the troposphere and biosphere. The two main purposes of the present investigation are the following: (1) to obtain exact data on terrestrial carbon-14 activity for estimating the human hazard; (2) to examine transport processes in the atmosphere.

The recent international agreement on prohibition of nuclear tests in the atmosphere gives excellent opportunity to examine the distribution of carbon-14 from the later bomb series, which were mainly injected at the polar cap.

In Trondheim I began measurements of carbon-14 in the atmosphere in May 1962. In order to study meridional transport and increase in carbon-14 at various latitudes, several persons have collected weekly samples for the laboratory since March 1963, from Addis Abeba (9° N.) to Spitsbergen (78° N.). As seen in Fig. 1, the stations (dark rectangles) are mainly placed along the coastline of the continents and far away from large towns and industrial centres. Every precaution is taken to avoid carbon dioxide from local sources.

I used the method[4] of direct absorption by sodium hydroxide of carbon dioxide from the air. Generally, a solution amount of 2 l. 1/2 N sodium hydroxide in each of two dishes was used. Each dish has a cross-section of about 450 cm^2. For practical reasons, an absorption time of 7 days (from one Monday to the next) was generally used for each sample. The amount of carbon dioxide absorbed during this time varies widely (3–8 l.), depending on temperature and humidity of the air. During the

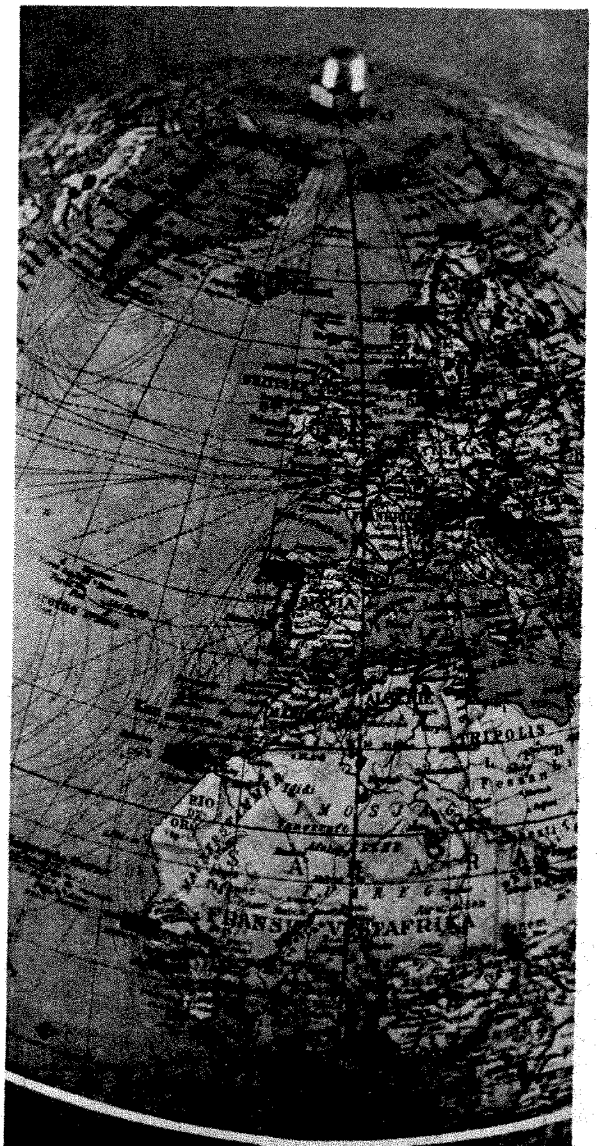

Fig. 1. Dark rectangles show the localities where weekly samples of carbon dioxide were collected from the air

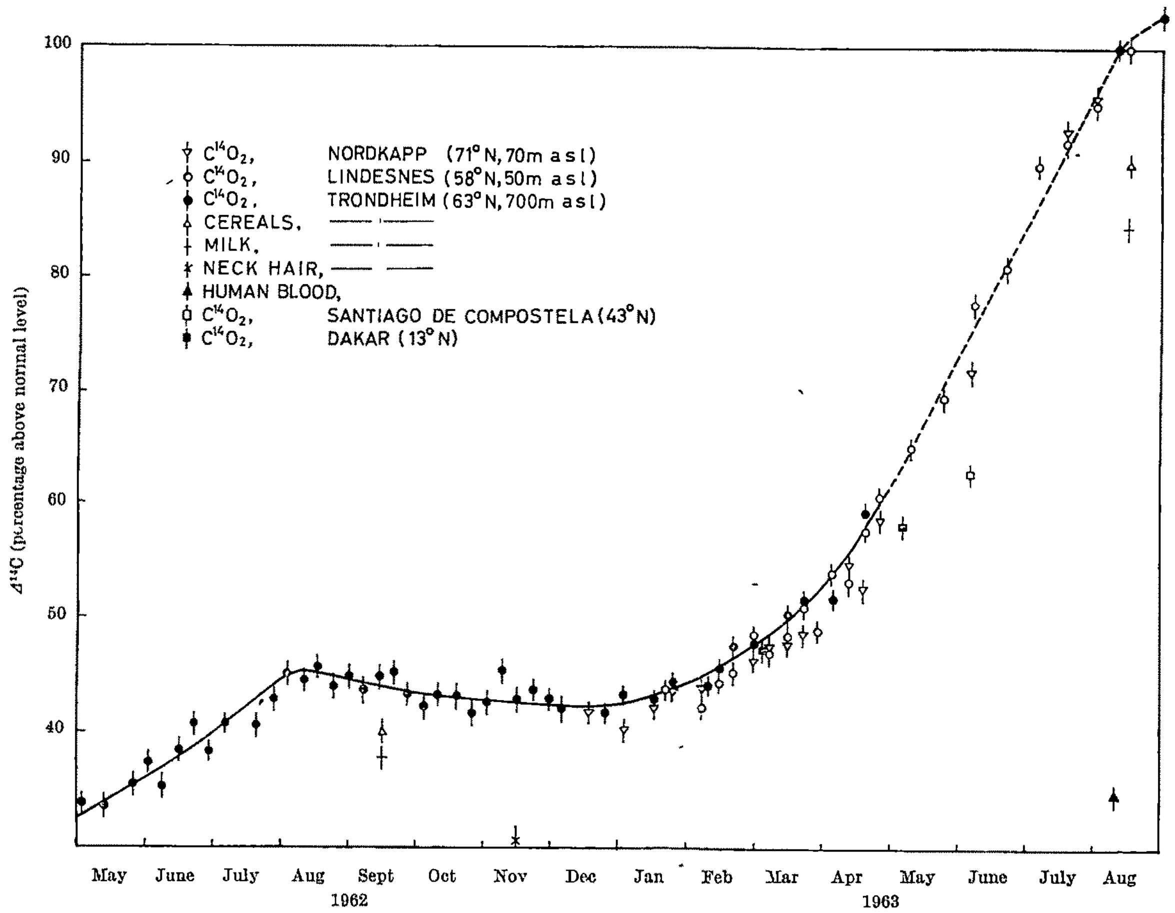

Fig 2 Increase in radiocarbon in the northern hemisphere

winter in northern latitudes, a thermostatically controlled heater element of 40 W in each dish served to keep the temperature slightly above zero °C

A fractionation between the carbon isotopes takes place during the absorption In order to make the measurements directly comparable with the oxalic acid standard[5] from the U S National Bureau of Standards, most of the samples are measured for fractionation error by mass-spectrometrical analysis of the $^{13}C/^{12}C$ ratio It turned out, however, that fractionation of the samples of carbon dioxide was very close to that found in vegetation Correction in the activity is only applied in a few single cases where the deviation is more than about 2 parts per thousand from the normal value

Generally, the percentage deviation in activity of carbon-14 ($\Delta^{14}C$) from the recent standard is calculated as

$$\Delta^{14}C = \frac{I - 0\ 95\ I^{\circ}}{0\ 95\ I^{\circ}} \times 100$$

where I represents the activity in the air sample, and 0 95 I° the normal activity in recent wood

The samples are partly measured in a carbon dioxide proportional counter, earlier described[6], and partly in a second counter with a background of 3 5 counts/min and a recent standard net count of 14 2 counts/min Generally, a counting time of more than 24 h is applied for each sample The limit of error for each measurement is one standard deviation

The percentage increase ($\Delta^{14}C$) in radiocarbon versus time given in Fig 2 shows a curve which is mainly the result of nuclear tests carried out in 1961 and 1962 The curve shows the typical seasonal variation, with increase in activity between January and August, and a decrease the rest of the year The variation is due to the transport mechanism from the stratosphere to the troposphere During large thermonuclear explosions, the radioactive elements are injected in the stratosphere The transfer back to the troposphere seems to some extent to be dependent on seasonal variation in the height of the tropopause[7,8], which shows larger heights in summer than in winter on northern latitudes In summer the turbulent motion in the troposphere is able to reach greater atmospheric heights, and parts of the lower stratosphere will then be incorporated in the troposphere This results in a much larger transport downwards in summer than in winter

In August 1963 a doubling of the normal carbon-14 level in the troposphere was observed on high northern latitudes Only a few samples are measured from Dakar (13° N) and Santiago de Compostela (43° N), but the measurements here indicate that the increase is about uniform in the northern hemisphere In the last four months of this year a decrease in activity up to 10 per cent is predicted because of the transfer of radiocarbon to the ocean and the southern hemisphere It is rather difficult to predict the prospective rise in tropospheric radiocarbon the following years, as we do not know exactly (1) the neutron yield of the bombs, (2) the altitude for the injection of radiocarbon

According to our observations, about 15 × 10^{27} carbon-14 atoms have until now been transferred to the troposphere from the 470 megaton thermonuclear explosions during 1961 and 1962 This amount constitutes only 15 per cent of the minimum amount of carbon-14 injected in the stratosphere in the period mentioned If this is correct, the tropospheric carbon-14 level will continue to rise for some years The period of existence of a carbon-14 atom in the troposphere is 6–7 years[3], before it is absorbed in the ocean This absorption will further determine the decrease in the troposphere until stabilization occurs in about 50 years After that time the remaining bomb carbon-14 will decay with a half-life of 5,700 years

By the process of photosynthesis in plants the radiocarbon is transferred to the biosphere Unfortunately, the growing season in northern latitudes coincides with the time of maximum increase in radiocarbon The samples of cereals measured in 1962 and 1963 show a specific activity not far from the maximum carbon-14 content in the troposphere in the respective years (Fig 2) This is also the case with the two samples of cows' milk from the local dairy at Trondheim A prediction of the carbon-14 rise in the human population is somewhat complicated because of varying diets This question is treated by Broecker *et al* [9], who found a time-lag in carbon-14 content between parts of the human organism and the troposphere of 1–2 years The authors state that the time-lag is due to (1) the time between photosynthesis of food and consumption by human beings, (2) the length of time the carbon remain in human tissue

Up to the present only two samples from human organisms have been measured in our laboratory The first sample was hair of the neck of a baby born on February 21, 1962 The hair which was cut for the first time on November 15, 1962, showed a carbon-14 activity of 30 70 ± 1 35 per cent above normal level The child's diet in the present case was perhaps somewhat unusual, because it consisted mainly of cows' milk for the past six months

The second sample was blood from a person twenty-four years old The sample, which was collected on September 9, 1963, showed an increase of 34 85 ± 1 10 per cent above normal. The activity corresponds to a time-lag behind the tropospheric level of 1 3 years, a result which fairly agrees with that found by Broecker (1·1 year) It is reasonable to assume that in future the amount of carbon-14 in the human population will rise to a maximum value, close to that of the final tropospheric level Even if no further nuclear tests are carried out, it is already obvious that this amount will at least be 2–3 times the normal activity within a few years Further measurements are needed, however, in order to obtain more exact information

I thank the following for collecting samples at various latitudes Mr Kåre Henriksen, Isfjord Radio, Spitsbergen, Mr Arild Klefstad, Fruholmen, Nordkap, Mr. Reimert Rasmussen, Lindesnes, Mr Enrique Lazo Alcola del Olmo, Santiago de Compostela, Mr Nicolás Miguel Zalote and collaborators, St Cruz, Tenerife, Dr C A Rouiller, jun , NASA, Project Mercury, Gran Canaria, Dr P Elouard, Université Dakar–Fann, Mr Oskar Nydal and Mr Hadle Hovda, Evangelical College, Addis Abeba

I also thank the following authorities, Dr José Antonio Barasoain and Dr Pio Pita Suarez de Cobián, of the Ministerio del Aire, Madrid, for assistance in arranging the sampling in Santiago and Tenerife

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OBITUARIES

Sir Geoffrey Evans, C.I E.

Sir Geoffrey Evans, who died on August 16 at the age of eighty, was a man of exceptional experience in tropical agriculture, and one who played an influential part in the organization and development of agriculture in Commonwealth countries His wide experience was gained in an active life of service abroad

From his home in a Lancashire vicarage and from school in Bury, Geoffrey Evans entered Downing College, Cambridge, in 1901, to read for the natural sciences tripos and then to take an agricultural diploma He remained in Cambridge on the staff of the School of Agriculture for a further year before taking an appointment in the Indian Agricultural Service He sailed for India in 1906 to become, in the words of an earlier biographer, "one of the early band of stalwarts who laid the foundations of the Indian Agricultural Service" During his time in India, which extended until 1923. his various posts included those of principal of the Agricultural College at Nagpur, deputy director of agriculture for a large district in the Central Provinces, and finally director of agriculture in Bengal

During the First World War, as an officer trained in the Indian Army Reserve, Evans saw active service in Iraq Use was there made of his technical knowledge, and he was appointed director of agriculture to the Mesopotamia Expeditionary Force with rank of colonel He also had an opportunity to visit Burma when chosen to advise on organization of its agricultural department Characteristically, in his report he stressed the importance of sound agricultural training, and one of his recommendations led to the founding of the Agricultural College at Mandalay

On leaving India in 1923, Evans took an appointment with the Empire Cotton Growing Corporation as director of cotton culture to the Queensland Government His three years in Australia he himself described as "somewhat strenuous, but most interesting" He arrived at a time when a boom in cotton-growing was collapsing and when the Government was introducing unpopular measures for regulating its planting He remained long enough to see some order return, to improve the seed-supply, to train a team of efficient field-officers, and to see started a well-equipped Cotton Research Station He found time to visit Fiji to advise on possibilities of extending cotton cultivation there, and wrote a typically helpful report outlining practical measures to be taken for establishing the industry on a sound basis A visit for the same purpose was later paid to Papua and New Guinea

Evans's experience was, therefore, already wide and varied when, in 1926, he was invited to join the staff of the Imperial College of Tropical Agriculture, Trinidad In the following year he was confirmed as principal, a post he was to fill for twelve years with outstanding ability The College, which had been formally opened in 1922, owed much to the inspiration of Viscount Milner, who envisaged it not only as a regional agricultural college for the West Indies but also as a place where men would be trained to serve as agriculturists throughout the British tropical possessions, and as a centre for fundamental research on tropical crops When Evans came, a good start had been made, but building had yet to be completed and land fully acquired finances remained insecure, the aims and courses of study had still to be fully worked out, and research policy planned It fell to Evans to make a working reality of the proposals of Milner, and the measure of his success may be gauged by the ability of the staff who were attracted to the College, by the high reputation for training which the College soon acquired, and by its fine record of research accomplished

While he has been described as helping to lay the foundations of the Indian Agricultural Service, it can be said with no less force that he did much to fashion the Colonial Agricultural Service Not the least important task of the College was the training of staff to man the Colonial departments of agriculture, and most of the officers recruited in that period could look back, in pleasurable memory, to the College under Evans

Evans left Trinidad in 1938 to become economic botanist at Kew, a position in which his wide knowledge of tropical crops could be well utilized In 1941, on the sudden death of Sir Arthur Hill, director of Kew, Evans was called on to act in his place, and he administered the affairs of Kew for two of the difficult war-years, leading its force of local defence volunteers, and afterwards commanding the Home Guard of the adjoining borough of Richmond

He remained as economic botanist until the end of 1953, taking special interest in the provision of useful plants to countries of the Commonwealth and their growing under conditions of quarantine at Kew He served as chairman of the Colonial Committee on Anti-locust Research, as a member of the Committee on Colonial Agricultural Research, and on various other committees and Government commissions During 1947–48 he was chairman of a commission appointed to examine the possibilities provided by British Guiana and British Honduras for settlement of people from over-populated West Indian islands and of people displaced from European countries Few commissions can have been as energetic as this one, as led by their chairman, ever an active man, they travelled by canoe and by jeep, on horseback and on foot, seeking areas fit for development in the interior of these countries Although little action followed publication of the report, it remains an admirable account of the resources of these territories and means for their development

On retiring to his home in Sussex from Kew and from his various committees, he yet retained one post in which he took a special interest, and, as chairman of the committee managing the National Pinetum at Bedgebury in Kent, he supervised with almost paternal care the progress of the coniferous plantings

Geoffrey Evans was honoured for his work in the First World War by the award of the C I.E , and was knighted in 1934 in recognition of his work in Trinidad He was honoured in a wider sense by those knowing him who respected his upright character and responded to the warmth of his genial and kindly friendliness The regard in which he was held extended equally to Lady Evans, who, after sharing his life for forty-nine years, survives him along with their two daughters T A RUSSELL

Dr. Francis M. Defandorf

DR FRANCIS MARION DEFANDORF, an authority in the field of high-voltage measurements at the U S National Bureau of Standards, died on August 18 He was born in Garrett Park, Maryland, in 1897 He received his bachelor's degree in electrical engineering from the Johns Hopkins University in 1920, a master's degree from Harvard in 1923, and a Ph D from Johns Hopkins in 1927

His professional career was at the National Bureau of Standards, where he first worked on a summer task in 1916 He rose to become chief of the Electrical Instruments Section for the last seventeen years of his life Here his broad knowledge, meticulous care and dedication to the task at hand served as inspiration to his associates The present 2-million-volt High Voltage Laboratory of the Bureau is in large measure the result of his initiative in starting it, his skill in planning it, and his wisdom in directing its development

He was very active in local civic and scientific matters His services to the Philosophical Society of Washington and to the Washington Academy of Sciences were recognized by his election to the presidency of each in 1946 and 1954, respectively He was a Fellow of the American Institute of Electrical Engineers and active in the work of its local section, of which he was chairman in 1941–42 He also devoted much effort to the national standardizing work of the American Institute of Electrical Engineers and of the American Standards Association, particularly in the field of lightning protection He was the author of a number of technical papers on lightning and on methods for measuring very high voltages

During the Second World War and the Korean conflict, he was engaged in highly classified applications of electricity

NEWS and VIEWS

The Sir William Dunn School of Pathology, University of Oxford. Sir Howard Florey, P R S

SIR HOWARD FLOREY, who became professor of pathology at Oxford in 1935, is now the Provost of the Queen's College and has relinquished the chair Sir Howard has made unrivalled contributions to the development of pathology as an experimental science in Britain His work has ranged from the study of inflammation and mucus secretion to the discovery, with Prof E B Chain and other colleagues, of the chemotherapeutic properties of penicillin and, more recently, to investigations with the electron microscope relating to occlusive diseases of the arteries His distinction has been recognized by the award, in 1945, of the Nobel Prize in Physiology and Medicine and of the Royal and Copley Medals of the Royal Society His outlook and leadership have made the Sir William Dunn School of Pathology a unique department of its kind, and fortunately he will be able to continue his work there At the same time, he will be well placed, as Provost of Queen's, to express his views effectively on matters relating to science in the University Sir Howard was elected a Fellow of the Royal Society in 1941, and first served on the Council during 1942–43 For the period 1951–53 he was a vice-president and in 1960 was elected president (see *Nature*, 188, 896, 1960)

Prof. H. Harris

SIR HOWARD has been succeeded in the chair and in a professorial fellowship at Lincoln College by Dr Henry Harris, who graduated at the University of Sydney, first in the Faculty of Arts and then in Medicine Dr Harris went to the Sir William Dunn School of Pathology, Oxford, in 1950 with a travelling fellowship from the Australian National University and continued to work there until 1959 After a year in the United States he became head of a new Department of Cell Biology at the John Innes Institute His first work at Oxford, which upset the prevailing view on the nature of chemotaxis in leucocytes, led to investigations on the physiology of other mammalian cells, and in recent years he has made significant and interesting contributions to our knowledge of the biological role of the ribonucleic acids in cell nuclei Dr Harris is a man with an unusual collection of abilities His appointment ensures that the Sir William Dunn School of Pathology will retain the character it acquired from his predecessor

Towards the Organization of Science by Scientists

THOSE concerned with the organization and teaching of science in Britain could well consider the possibility of introducing a compulsory one-year course on the administration and organization of science for all undergraduate students of science How many undergraduates are sufficiently well informed to understand the inosculations of the science effort, the workings of the Research Councils, the research associations, or science in industry or university? Such a course could include an appreciation of the interactions of science and the State—and where better could the student start than Prof R V Jones's address, on September 3, to Section X (General) of the British Association for the Advancement of Science meeting, in Aberdeen, on the life of Lyon Playfair (see *Nature*, **200**, 105, 197, 1963)? Few students (and many qualified scientists) really appreciate the interrelationships of science and Government, the workings of the University Grants Committee, or even what D S I R stands for, let alone have heard of Lyon Playfair, Colonel Strange, H E Armstrong, Sir Richard Gregory, etc , to mention a few The approach to the subject would best be historical and possibly subdivided as Playfair did his presidential address to the British Association in 1885, and which Prof Jones developed If such a course could be introduced by one compulsory lecture a week, all potential scientists would gain a better appreciation of the inherent difficulties of organizing the national science effort It would, in the long run, lead to the organization of science by scientists

Peaceful Uses of Atomic Energy

THE third International Conference on the Peaceful Uses of Atomic Energy will be held in Geneva during August 31–September 9, 1964 A provisional topical agenda constituting the general programme of the Conference has been prepared by the United Nations with the advice of the Scientific Advisory Committee and in co-operation with the International Atomic Energy Agency The main theme of the Conference will be new developments in power reactor technology, including descriptions of, experience with and prospects for reactor systems, economic aspects of nuclear power, nuclear fuel and other reactor materials, reactor physics and performance studies, safety implications and radioactive waste management Sessions are also proposed on new developments in controlled thermonuclear reactions, application of radioisotopes, research reactors and isotope separation and other new applications of atomic energy A scientific exhibition in connexion with the conference will be organized and participation will be limited to States taking part in the conference

Sir William Penney, deputy chairman of the United Kingdom Atomic Energy Authority, has been appointed chairman of a National Executive Committee with members representing the interests of the Atomic Energy Authority, Electricity Boards, the nuclear industry, Government Departments, universities and research organizations A committee has also been set up under the chairmanship of Sir William Penney to prepare papers for submission by the United Kingdom to the United Nations The Atomic Energy Authority is to provide the Secretariat, which will be responsible for all executive and administrative arrangements on behalf of the United Kingdom relating to papers, the exhibition and attendance at the conference, under the general guidance of the National Executive and Papers Committees For this purpose the Authority has established a Geneva Conference Secretariat at its London Office, 11 Charles II Street, London, S.W 1, under the direction of Dr J Gaunt

Scientific Research in France

THE *Rapport National de Conjoncture* for 1962–63 of the National Centre for Scientific Research, Paris, also entitled the "Report of the National Committee for Scientific Research", covers all the research proceeding under the Committee during the year, in which the number of research workers increased from 650 to 720 (Pp 264 Paris Centre National de la Recherche Scientifique, 1963) The information on current projects is arranged according to divisions of science and not institutions and the report is in two parts, the first dealing with the exact and natural sciences, and the second with the human sciences, including philosophy There is no index, but fairly full contents pages facilitate reference The whole volume is itself too condensed to permit further condensation without distortion

The Lister Institute of Preventive Medicine

THE report of the Governing Body of the Lister Institute of Preventive Medicine for the year 1962 includes a summary of the investigations made in 1962 (Pp 33 London Lister Institute of Preventive Medicine, 1963) In microbiology, the Guinness–Lister Unit continued to explore bacterial genetics, mainly of the *Salmonella* bacilli. The Trachoma Research Unit continued its epidemiological field study in the Gambia of trachoma in terms of virus and of bacilli of the *Haemophilus* group and is preparing to test the prophylactic value of a virus in the Gambia The immunological study of bacterial infections included an analysis of the action of two types of protective antibody in mice infected with the whooping cough bacillus, the laboratory assay of typhoid vaccines and the nature of the protective antigen in typhoid vaccines for human use, the immuno-pathology of actinomycetic dermatitis of sheep, and the process of refinement of antitoxin by proteolysis Work continued on the relation of serum permeability factors to plasma kininogenases and their respective roles in vascular reactions to injury Biochemical research work again concerns the blood group substances, including definition of the chemical heterogeneity of preparations of mucopolysaccharides and of the terminal sugar groups determining serological specificity, the cellular phospholipids and starches A list of publications is appended

The Sarawak Museum

VOLUME 10 of *The Sarawak Museum Journal* (Nos 19–20 N S July–December 1962) maintains the high standard of previous issues and, as before, owes much to the energy and enthusiasm of its editor, Mr Tom Harrisson, who is also Government ethnologist and curator of the Sarawak Museum The theme for the volume is given as "Sarawak in a Wider Context", and though the editor himself is the main contributor, he has inspired many others to write a total of thirty papers The first section deals chiefly with the classification and typology of local archæological material, and this is followed by articles concerned with neighbouring areas Investigations in the development of various resources comprise the third section, and the volume of nearly 300 pages closes with a few notes on natural history

The Institute of Applied Science of Victoria

THE report of activities for the year ended June 30, 1962, of the Institute of Applied Science of Victoria stresses the value to-day of the science museum and records substantial progress towards building a high-performance planetarium (Pp 16 Melbourne Institute of Applied Science of Victoria, 1963) Much concentrated work was carried out in the Radiocarbon Dating Laboratory, and the erection of a neutron reactor was completed The public demand for telescope demonstrations continued to increase Besides the opening of a new north-west wing in which the setting up of displays was a major preoccupation, considerable display reorganization was made in other galleries

A Computer-based Bibliographical Retrieval and Publications System

THE computer-based bibliographical retrieval and publications system now under development at the National Library of Medicine and known as 'Medlars' is described in an article by S I Taine, which has been reprinted from the *Bulletin of the Medical Library Association* (April 1963), and also more fully in a brochure, *The Medlars Story at the National Library of Medicine*, issued by the U S Department of Health, Education and Welfare (Pp vii+74 Bethesda, Md United States Department of Health, Education and Welfare, 1963) The system will become operational early in 1964 and it is designed to improve the quality of and enlarge *Index Medicus* as well as reduce the time of preparation of the monthly edition from twenty-two to five days It will increase the average depth of indexing five-fold and almost double the number of articles handled annually, as well as facilitate the inclusion of citations from other sources and from periodicals and the efficient servicing of requests With its installation the Library expects to add monographs to the list of source material to be indexed Use of a digital computer and special composing equipment capable of providing excellent typographical quality is involved

Preparations for an International Conference

MONOGRAPH No 1, issued by the Institute of Linguists, entitled *Preparations for an International Conference* and written by A G Readett, claims, with reason, to be the most comprehensive and detailed exposition so far published of the preparatory work required to ensure the success of an international conference (Pp 20 London Institute of Linguists, 1963 10*s* 6*d*) While it covers the papers and printing arrangements as well as the conference sessions, it deals more particularly with the interpreting and translating arrangements, including equipment and the conference office and its staff Some guidance is given as to costs

Present and Future of the Fundamentals of Natural Philosophy

IN the May issue of the *British Journal for the Philosophy of Science*, Mr L L Whyte contributes an article on "Some Thoughts on Certainty in Physical Science", in which he discusses the present and future of the fundamentals of natural philosophy He is particularly concerned with what is known absolutely and uniquely about some basic properties of the universe Such knowledge is clearly apodictic (that is, true of necessity) The theme is important in view of the tendency to concentrate on uncertainty principles in our mathematical thinking Without denying the possibility of surprises, the author gives sound reasons for believing that in certain regions "the scope for discovery may be finite". Organic chemistry provides an illustration of steady logical progress for about a century, geometrical concepts have proved remarkably stable By contrast, physics has gone ahead with ever-increasing abstraction, farther and farther removed apparently from any visual image in three-dimensional Euclidean space The perfect example of something established once and for all is the existence of the 230 space-groups, the 32 classes, and the 14 Bravais lattices of the crystallographer These facts are coercive on the human mind, and the agreement between 'pure' mathematics and observation is unmistakable The velocity of light *c* in empty space, as an assertion, is not wholly acceptable, since *c* may be found to vary in high external magnetic fields Our present comprehension refers largely to static configuration future work must take account of changes.

Pleistocene Stratigraphical Chronology of Ceylon

DR P E P DERANIYAGALA, director of the National Museums in Ceylon, has recently described certain gravel beds which will prove useful in determining some of the stages of Ceylon's Pleistocene cycle of erosion (*Spolia Zeylanica*, 30, Pt 1, 1963) Such a determination is, of course, vital if the early archæological developments are to be interlocked with the geological sequence The short paper is entitled "Some Indicators in the Pleistocene Stratigraphical Chronology of Ceylon" with a page of illustrations

Aboriginal Cultural Development in Latin America

A SYMPOSIUM entitled "Relaciones de las Culturas del Nuevo Mundo" was held at the thirty-fifth International Congress of Americanists in Mexico City in August 1962, and the recently published Proceedings gives, with commendable promptitude, the revised texts of the papers delivered at it (*Smithsonian Miscellaneous Collections*, 146, No 1 *Aboriginal Cultural Development in Latin America An Interpretative Review* Publication 4517 Pp vi+148. Washington, D C Smithsonian Institution, 1963) There were ten papers, each covering one of the regions into which the area was divided, and an eleventh with the title "An Interpretative Overview" Not all of them had much to say about the relationships between the areas, hence perhaps the alteration in the published version of the title, and for various reasons, some of them outside the control of authors and editors, some were more successful than others in summing up the present state of knowledge in their own areas, but all had useful things to say, and anyone who wants to keep abreast of developments in Latin American archæology ought to read the book Great credit is due to the editors, Drs B J Meggers and C Evans, for organizing the symposium and co-ordinating the work of scholars from nine countries, and we owe a further debt to Dr Meggers for her "Overview", which includes a statement of her controversial views on the extent and importance of trans-Pacific relationships, with its suggestion of contacts between Japan and Ecuador at the surprisingly early date of the first half of the third millennium B C

Variations in the Composition of Milk

THE milk produced in the United Kingdom every day of the year is worth considerably more than £1 million, but the real worth of that milk lies in its nutritive value, and that depends on the amounts of fat, carbohydrate, protein, minerals and vitamins it contains Fortunately, in recent years there has at last been an increasing awareness of the importance of the non-fatty solids of milk in nutrition and particularly of the protein content, with the result that schemes have been or are about to be introduced in England and Wales, Scotland and Northern Ireland whereby milk is, or will be, paid for not only according to its amount or its fat content but also according to its content of total solids This should do much to maintain the nutritive value of the United Kingdom's milk supplies at a satisfactorily high level If, however, this end is to be achieved, it is important that farmers with cows the milk of which does not attain a desirable standard should be given the help and advice they need to effect an improvement Leaflet No 131 prepared by the Ministry of Agriculture of Northern Ireland discusses briefly but clearly the main factors that are known to affect the composition of milk, such as feeding, the efficiency of milking and disease, to mention but three of them (*Variations in the Composition of Milk* Pp 6 Belfast Ministry of Agriculture, 1963) Helpful advice is given on how deficiencies in butter fat content and in the content of solids-not-fat can be prevented The pamphlet as a whole is well arranged and easy to read It should be a valuable guide to those concerned with milk production

Terminology for Exchange of Gas in the Lungs

ON September 6, a meeting was held at 20 Park Crescent, London, W 1, by permission of the Medical Research

Council, at which an international group of interested persons discussed terminology of some aspects of gas exchange in the lungs, in particular the term 'diffusing capacity' The participants represented Belgium, France, Germany, Great Britain. Holland, Scandinavia, Switzerland, and the United States Dr P Hugh-Jones, Department of Medicine, King's College Hospital Medical School, London, was chairman When the term 'diffusing capacity' was originally proposed, it was considered that diffusion was the only factor determining the rate of transfer of gas between alveolar capillary blood and alveolar gas It is now known that the rate of reaction between carbon monoxide, oxygen and intracellular hæmoglobin, as well as other factors, affect the transfer rate In addition the diffusing capacity as actually measured is dependent on additional variables such as the ventilation perfusion relationship in the lungs In the light of these considerations, the majority of those present agreed that the term 'transfer factor' be substituted for 'diffusing capacity', but no definite proposal was made that this be carried out immediately Persons interested in this field of research and wishing to submit their views should write to J E Cotes, Medical Research Council Pneumoconiosis Research Unit, Llandough Hospital, Penarth, Glamorgan

Causes and Treatment of Rheumatic Disease

DURING May 29–30, 1962, the Nuffield Foundation held a small two-day conference at Ashridge College, at which scientists from various biological disciplines discussed with rheumatologists the possible nature of fundamental rheumatic processes This exchange of ideas between clinical and non-clinical research workers proved stimulating, and the Foundation believes that the report of the papers and discussion may be of value to other people A copy of the report may be obtained (without charge) by writing to the Nuffield Foundation, Nuffield Lodge, Regent's Park, London, N W 1

International Commission on Zoological Nomenclature

NOTICE is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connexion with the following cases, full details of which will be found in *Bulletin of Zoological Nomenclature* (20, Part 5, to be published on October 21) (1) Designation of a type-species for *Crassispira* Swainson, 1840 (Gastropoda) *Z N* (*S*) 459 (2) Validation of *Alycus* Koch, 1842 (Acari) *Z N* (*S*) 465 (3) Validation of *Natrix barbouri* Taylor, 1922 (Reptilia) *Z N* (*S*) 1559 (4) Suppression of *Conus candidus* Born, 1778 (Gastropoda) *Z N* (*S*) 1567 (5) Suppression of *Pleuronectes grohmanni* Bonaparte, 1837 (Pisces) *Z N* (*S*) 1579 (6) Validation of, and designation of a type-species for, *Diplectrona* Westwood, 1839 (Insecta, Trichoptera) *Z N* (*S*) 1580 (7) Validation of *Sceloporus torquatus* Wiegmann, 1828 (Reptilia) *Z N* (*S*) 1582 (8) Validation of *Ortholitha* Hubner, [1825] (Insecta, Lepidoptera) *Z N* (*S*) 1585 (9) Validation of *Krohnia* Langerhans, 1880 (Chaetognatha) *Z N* (*S*) 1586 (10) Suppression of nine specific names of Holothurioidea *Z N* (*S*) 1587 (11) Suppression of *Eoscorpius inflatus* Peach, 1882 (Arachnida) *Z N* (*S*) 1588 (12) Validation of *Prochilodus argenteus* Agassiz, 1829 (Pisces) *Z N* (*S*) 1590 (13) Validation of *Tinodes pusillus* McLachlan, 1862 (Insecta, Trichoptera) *Z N* (*S*) 1592 (14) Rejection of the neotype and type-locality of *Thamnophis sirtalis* (Linnaeus) (Reptilia) *Z N* (*S*) 1600 Any zoologist who wishes to comment on any of the foregoing cases should do so in writing to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S W 7, before April 21, 1964

The International Academy of Astronautics

DR CHARLES S DRAPER (United States) has been appointed president of the International Academy of Astronautics in succession to Dr F J Malina Dr Draper is head of the Department of Aeronautics and Astronautics at the Massachusetts Institute of Technology, as well as director of the Institute's Instrumentation Laboratory Prof E A Brun (France), president of the International Astronautical Federation, and Prof U S von Euler (Sweden), secretary of the Nobel Prize Committee, have been elected vice-presidents The following Academicians have been elected to the Board of Trustees of the International Academy of Astronautics Section 1—Basic Sciences, Prof A Ehmert (German Federal Republic) and Dr D F Martyn (Australia), Section 2—Engineering Sciences, Prof J M J Kooy (Holland), Dr F J Malina (United States) and Prof R Pešek (Czechoslovakia), Section 3—Life Sciences, Dr W R Lovelace II (United States) and Air Commodore W K Stewart (United Kingdom)

Announcements

PROF LINUS PAULING, of the California Institute of Technology, has been awarded the Nobel Peace Prize for 1962 In 1954 Prof Pauling was awarded the Nobel Prize for Chemistry (see *Nature*, 174, 907, 1954) He is a foreign member of the Royal Society

THE Right Hon Lord Franks, Provost of Worcester College, Oxford, and former chairman of Lloyds Bank, and Prof R H S Thompson, professor of chemical pathology, Guy's Hospital Medical School, have been appointed additional trustees of the Wellcome Trust

A LEAFLET, *Another Kind of School*, has been issued by the National Association for Mental Health for parents of children who are not accepted for school, describing the Junior Training Centres and other openings and facilities available to enable mentally handicapped children to make the best use of their abilities (Pp 4 London Association for Mental Health, 1963 6*d*)

THE third Western National Meeting of the American Geophysical Union will be held in Boulder, Colorado, during December 26–28 Further information can be obtained from W W Kellogg, the Rand Corporation, 1700 Main Street, Santa Monica, California

THE eighth international conference on "Cosmic Rays", arranged under the joint auspices of the International Union of Pure and Applied Physics and the Department of Atomic Energy, Government of India, will be held at Jaipur during December 2–14 Further information can be obtained from Dr R R Daniel, Tata Institute of Fundamental Research, Colaba, Bombay 5

THE National Productivity Year Conference, "Productivity in the Next Five Years", will be held at Eastbourne during November 26–28 The conference is being organized by the Department of Scientific and Industrial Research and its aim is "to compare notes, to discuss future action and to provide a guide for firms and organizations in every sector of our working life" Further information can be obtained from the Secretary, British Productivity Council, Vintry House, Queen Street Place, London, E C 4.

A ONE-DAY course on "Aspects of Microbiology and Plant Protection" will be held by the Joint Biology Committee at the William Beveridge Hall, Senate House, London, W C 1, on November 16 The programme will include "Microbiology of the Air", by Dr P H Gregory, "The Migration and Dispersal of Aphids and the Dissemination of Plant Viruses", by Dr C G Johnson, "Some Chemical Aspects of Crop Protection", by Prof R L Wain, "Modern Methods of Plant Protection and the Conservation of Wildlife", by Dr N W Moore Further information and tickets can be obtained from the Joint Biology Committee, c/o the Association of Agriculture, 78 Buckingham Gate, London, S W 1

SCIENCE VERSUS TECHNOLOGY: THE BATTLE FOR BRAINS

By Prof P. V. DANCKWERTS
Department of Chemical Engineering, University of Cambridge

"THE general picture presented in this country, then, is of science sixth formers who divide the world only rather hazily into 'scientists' and 'technologists' and who attribute a quite exceptional position and prestige to doctors Scientists, they believe, are educated at universities and go on, if they are lucky, to 'do research'. research is glamorous, 'interesting', leading always to exciting new discoveries, is not really 'work', and may well make you famous Technologists, on the other hand, are educated at technical colleges, have low social prestige, are less intelligent and less well paid than scientists, and their work is often 'boring' The authors have been at some pains to identify what a boy means by saying that a job would be 'interesting' or 'boring' (the two main reasons for choice) and have come to the conclusion that in most cases the terms are simply 'grunts' of approval or disapproval and are probably related to the subject's degree of ignorance of the job concerned "

This paragraph is the crucial finding of a survey made by the Oxford University Department of Education[1] into the attitude of sixth-form scientists towards engineering and technology The survey was based on the analysis of Advanced Level of the General Certificate of Education qualifications of university entrants, questionnaires completed by 1,400 schoolboys, and interviews in schools

Those of us who are concerned with technological teaching already knew most of what the survey reveals, but there is great value in the publication of a certified, quantitative statement of the situation The evidence supports the assumption that boys in Britain tend to enter higher technological education only when they cannot get a place in faculties of pure science Applied science does not attract a fair proportion of the first-rate because of the 'public image' of the two careers current among schoolboys—an image based on a "quite unusual degree of ignorance about the nature of technology, of scientific research and of the courses open to science sixth-formers in higher education"

If we assume that more of the able boys should in fact go on to the study of technology, one of the first requirements must be to alleviate this ignorance Even factual information may not redress the balance, however, because of the hazy glamour with which science is invested in the boys' minds It seems as though it may be necessary to 'glamorize' engineering and technology in competition How far we have come since the days when Samuel Smiles' *Lives of the Engineers* was eagerly read by ambitious boys !

On the level of factual information, many attempts are being made to inform schoolboys and their masters about the true nature of the technologies as professions and subjects for higher education One hopes that the Oxford Education Department's survey itself will be widely read in schools, and that the Department's hand-book on engineering courses at the universities will be studied by headmasters, careers masters and science masters Engineering institutions and industrial concerns have taken pains to distribute literature to schools, to provide lectures, conferences and visits to university engineering departments and to industry The Duke of Edinburgh has made the point in an address to the Association for Science Education[2] on April 26 At least two films have been made in an attempt to glamorize engineering as a university and professional career There is a scheme afoot at Cambridge to invite science masters to spend a term at the University to learn about the work of the technological departments

In a recent issue of *Education*[3], Prof J T Allanson has trenchant comments to make on the general misconceptions among school-teachers and the general public about the nature of engineering Referring to its creative character, he says "Just as the painter is judged by his paintings and not by his writings on æsthetics, so the engineer is finally judged by what he makes The making involves him in the social sciences and in relations with other human beings It is not possible for a good engineer to be a 'back-room boy', nor can he believe in that curious battle between the 'two cultures' invented by Sir Charles Snow He must make an engineering unity of the physical and social sciences by the kind of intellectual and moral effort supposedly characteristic of the man who reads 'Greats' With this he must integrate imagination and personal drive, for he is not a scholar but a doer, and the more visionary and far-reaching his ideas the greater must be his struggle to achieve them against the conservatism of the orthodox"

Prof Allanson suggests that more engineers should become school-teachers He points out, too, that in the nature of things few science masters have an opportunity to practise science between graduating and beginning to teach, and that they are likely to be unaware of the type of work actually done by professional scientists—let alone that done by engineers

All these attempts to inform and to influence schoolboys and schoolmasters are no doubt having an effect, and yet they seem to make only a minor impression on the problem Schoolboys, schoolmasters, the public as a whole—even scientists—are all, in fact, under the powerful influence of the mythology of pure science Many pure scientists would share the schoolboys' views about science and technology, expressed in the first paragraph (except that they would have less respect for doctors and would know—perhaps regret—that technological subjects are taught in universities) The highest objective of university science departments is to turn out pure scientists, of whom the best will in turn get jobs in universities Pure research is held to be the supremely worth-while occupation The actual or potential applications of science do not play a perceptible part in undergraduate science courses, with the result that a school chemistry master, for example, will have learned next to nothing at his university about the chemical industry, and nothing at all about chemical engineering

The British system ensures not only that the ablest sixth-form scientists take up science rather than technology at the university, but that the ablest university science students want to take up university or pure research jobs The result is well known—too few of our best scientists going into industry, pressure for more money to be spent on pure scientific research than the country can afford, an exodus to the United States of a surplus of able young scientists who have been brought up in the tradition of pure science and are not assimilated into our economy As the Duke of Edinburgh remarked, the engineer is the means by which we are all able to enjoy the fruits of science We are not in fact fully enjoying the fruits of our science, and we shall not do so unless more of our able minds are turned towards technology during their formative period To leave it until after graduation (as sometimes suggested) is too late.

It is worth considering whether courses in 'engineering science' should form part of every undergraduate science syllabus Such courses (if well taught) might serve to light a spark of positive interest in the applications of science and to mitigate the excessively genteel attitude of graduate scientists towards technology At the same time much-increased opportunities and encouragement might be provided for changing over from science to technology in the course of a university career, this might come to be regarded as a normal route into technological studies The problem of channelling talent into technology would thereby be partly removed from the schools to the universities, where the case for technology can be properly put and the student may be expected to take a more mature and realistic view

However, both pure science departments and technological departments might find difficulties associated with changes of the kind suggested Indeed, radical changes of any kind are for many reasons difficult to introduce into established universities Perhaps there is an opportunity here for the new universities to give the rest of us a lead by breaking down the barriers between science and technology This seems to offer the best chance of balancing our scientific economy and restoring the status of the technologist to a healthy level.

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[1] *Technology and the Sixth Form Boy*, Oxf Univ Dep 'Education, 1963 See also *Nature*, 199, 958 (1963)

[2] Assoc Sci Educ , 52 Bateman Street, Cambridge, 1963

[3] *Education*, 1045 (May 24, 1963)

RADIO STAR AND SATELLITE SCINTILLATIONS

During August 19–20 a conference was held at the Physics Department of the University of Adelaide on the subject of "Radio Star and Satellite Scintillations" The conference was held under the auspices of the Australian Institute of Physics (South Australian Division)

The conference opened with a survey by Dr B H Briggs (University of Adelaide), who showed how irregularities in the ionosphere could be detected by various methods, all concerned with effects on radio waves In the case of the *F* region, these methods include radio star scintillations, fluctuations of radio waves received from satellites, spread echoes observed in ionosphere soundings (spread-*F*), and radar back-scatter using frequencies around 20 Mc/s These phenomena are found to be correlated, which suggests that they are all due to the same irregularities in the ionization of the *F* region There is good evidence that the irregularities are elongated, and 'field aligned', so that their longer dimension lies along the direction of the Earth's magnetic field They occur at heights from 200 km upwards, recent 'topside soundings' by the *Alouette* satellite (*Nature*, 197, 636, 1963) show that on some occasions they exist at heights up to at least 1,000 km

When radio stars are observed at low angles of elevation, irregularities lower down, in the *E* region, are found to be important This might be expected, since the increase in thickness along the line of sight is greater for a layer of irregularities at a low height than for a layer at a great height, so that irregularities low down become relatively more important Irregularities in the *E* region and below can also be detected by several other methods, including the fading of reflected waves, observations of 'sporadic *E*' echoes on ionograms, and the investigation of weak irregularities below the *E* region by the scattering of radio waves at oblique or vertical incidence A remarkable feature of the irregularities revealed by the latter technique is their tendency to occur in stratified layers with a vertical separation of 10 km

After discussing these experimental observations, Dr Briggs turned to the vexed question of the origin of the irregularities In the *E* region and below it is generally agreed that the irregularities are due to irregular air motions, though the relative importance of turbulence, random wave motions, and vertical shears in the horizontal wind is still uncertain Higher up, in the *F* region, these motions are damped out due to the high kinematic viscosity, and other mechanisms must be found The irregularities in the *F* region do, indeed, seem to be a separate phenomenon, as is shown by their large intensity (deviations of electron density of ± 50 per cent are common) and their tendency to occur mainly at night Two early theories, which accounted in a simple way for the occurrence at night, were the accretion theory and the atmospheric tail theory In the former the irregularities were thought to be produced by material falling into the Sun by gravitational attraction, and intercepted by the Earth. In the latter an atmospheric 'tail' was postulated, pointing away from the Sun and produced either by radiation pressure or the effect of the solar wind Later theories have attributed the irregularities to hydromagnetic waves entering the atmosphere, to particle streams associated with the Van Allen belts or the aurora, to instability of an ionospheric layer moving vertically under the action of an electrodynamic drift, and to irregular electric fields produced lower down in the *E* region Dr Briggs suggested that the observations should, where possible, be discussed in relation to these theories, in order to attempt to eliminate some of them

Dr J W Warwick (University of Colorado and High Altitude Observatory, Boulder, Colorado) then described observations of radio star scintillations obtained with a swept-frequency interferometer These observations showed that amplitude scintillations had a similar form over the range 7–41 Mc/s Also, fluctuations in position of the source could be determined and these often showed systematic trends as a function of frequency Dr Warwick showed that these could be explained by geometrical optics if a succession of 'lenses', focusing approximately at ground-level, were assumed to exist in the ionosphere On some occasions the observations could be explained in terms of a wave-like structure moving with a velocity of about 50 m/sec

Miss E M Matthew (University of Queensland) described some observations of radar echoes from field-aligned irregularities in the *F* region made at Brisbane The echoes were related to variations in the outer Van Allen belt and to various ionogram anomalies The northward speed of patches of irregularities could be observed, and a sudden increase of speed was interpreted as arising from some form of impact on the magnetosphere

Dr G G Bowman (University of Queensland) described investigations of the occurrence of spread-*F* echoes at mid-latitude stations, and these seem to show that spread-*F* is most prevalent when the air density in the upper *F* region is low Dr B H Briggs (University of Adelaide) described observations of radio star scintillations and spread-*F* echoes made in England At sunspot maximum the irregularities increase in intensity but occur mainly above the level of maximum ionization of the *F* region and do not, therefore, produce spread-*F* echoes on ionograms Thus the opposite trends of scintillation occurrence and spread-*F* occurrence with the solar cycle can be reconciled The results appear to conflict with the 'instability' theory of Dr D F Martyn, according to which irregularities would form mainly on the underside of the *F* region

Dr J D Whitehead (University of Queensland) then gave a critical survey of the theories of formation of irregularities in the F region He pointed out that, for hydromagnetic waves, the percentage fluctuation of electron density is equal to the percentage fluctuation of magnetic field, and this appears to demand unreasonably large amplitudes for the waves The instability theory was criticized on the grounds that the observed vertical motions of the F region were not those required by the theory Dr Whitehead suggested that the irregularities were produced either by air movements associated with internal gravity waves, or by irregular electric fields, and the theories of formation of irregularities by these mechanisms were considered Dr Whitehead stressed the need for more detailed measurements of the sizes and shapes of the irregularities so that the predictions of the theories could be tested

On the second day of the conference, satellite scintillations were discussed Unlike a radio star, a satellite sweeps rapidly across the sky from horizon to horizon, and so the method is particularly valuable for examining the geographical distribution of irregularities, especially if observations from several stations are combined Dr G H Munro (Radio Research Board, Sydney) described observations of this type, in which transmissions on 20 Mc/s from the satellites *Nora Alice I* and *II* were used The results showed that irregularities tend to occur in patches which become more frequent, and larger, at higher latitudes The patches appear to be elongated east–west and may stretch in this direction for 1,000 miles or more From measurements of the times of onset of scintillations at spaced stations the heights of the patches may also be determined

Mr. I A Parkin (University of Adelaide) discussed the theory of the diffraction process which links the irregularities in the ionosphere to the pattern of amplitude and phase which is observed over the ground He considered both a source at infinity and at a finite distance If detailed deductions about the sizes and shapes of the irregularities are to be made, it is necessary to use frequencies such that the fluctuation of phase across the emerging wavefront is less than one radian It is then possible to relate the auto-correlation function of amplitude measured on the ground to the auto-correlation function of the irregular medium Mr Parkin also pointed out that at a given distance from the irregularities certain Fourier components should be absent in the amplitude pattern, and so measurements of the power spectrum of the pattern may enable the height of the irregularities to be determined Some discussion of the relation between the diffraction approach and the method of geometrical optics followed Mr Parkin's paper, and it was agreed that in principle the diffraction method is always valid, but in practice it is most useful when the phase deviation is small, while the method of geometrical optics is useful when the phase deviation is large

Attention then turned to irregularities at lower heights Dr R G Roper (University of Adelaide) described measurements of winds and turbulence in the height range 70–110 km, and Dr J D Whitehead (University of Queensland) discussed the formation of sporadic E which, at temperate latitudes, he considered to be produced by vertical shears in the horizontal wind However, the irregular wind shears described by Dr Roper had the greatest magnitude at the equinoxes, and this does not agree with the occurrence of sporadic E, which is most prevalent in summer Dr Whitehead also discussed the formation of equatorial sporadic E, which he suggested was due to irregularities formed by sound waves

The conference concluded with two papers of a more technical nature Dr L H Heisler (Radio Research Board, Sydney) described equipment for the investigation of the variation with frequency of the amplitude of radio waves reflected from the ionosphere, and Dr J D Whitehead (University of Queensland) described a proposed method for measuring with increased precision the virtual height of reflexion

During the conference the opportunity was taken to discuss observations of the forthcoming *S66 Beacon* satellite which are being planned in Australia and New Zealand The general feeling was that much more work needs to be done before the mode of formation of ionospheric irregularities is properly understood, and that intensive observations using the *Beacon* satellite are likely to make a valuable contribution towards this end

B H BRIGGS

OXIDATIVE PHOSPHORYLATION AND RELATED PROCESSES

THE Medical Sciences Club of South Australia and the Australian Biochemical Society (South Australian Branch) held a symposium on "Oxidative Phosphorylation and Related Processes" in the Biochemistry Department, University of Adelaide, during June 14–15

Prof E C Slater (Amsterdam) opened the symposium with a review of present-day theories on the formation of adenosine triphosphate in mitochondria He contrasted Mitchell's theory that this process is the reversal of hydrolysis of adenosine triphosphate by an anisotropic adenosine triphosphatase to the more widely accepted view that adenosine triphosphate is produced by reactions involving a number of specific intermediates formed at several different 'sites' in the sequence of oxidation-reduction catalysts in mitochondria Much of the evidence for such intermediates has been obtained in experiments with oligomycin, which prevents formation of adenosine triphosphate but does not inhibit other reactions such as the reduction of nicotinamide-adenine dinucleotide by succinate, for thermodynamic reasons, this reduction must be coupled to exergonic processes such as the decomposition of compounds like the proposed intermediates However, these investigations do not allow a final choice between Mitchell's theory and that preferred by Slater Chance and others involving specific intermediates of the type often formulated as $A \sim C$ It was pointed out in discussion that the existence of adenosine triphosphate and adenosine diphosphate in a region of the mitochondrion not accessible to oligomycin would invalidate certain conclusions drawn from experiments with this inhibitor

Prof J S Sturtevant (Yale) described kinetic investigations of the interaction of cytochrome b_2 (the L(+)-lactate dehydrogenase of yeast) with lactate and ferricyanide Using a stopped-flow spectrophotometer at a number of wave-lengths, evidence was obtained which indicated a preliminary reduction of flavin mononucleotide, an intra-molecular transfer of electrons to protohæm, and interaction of both prosthetic groups of this flavohæmoprotein with ferricyanide The results support earlier suggestions by Morton, who found equimolar quantities of these prosthetic groups in the enzyme and proposed a mechanism involving intra-molecular transfer of electrons from flavin to hæm The need to find if cytochrome b_2 is a constituent of yeast mitochondria was considered in the general discussion following these papers Clearly a single flavohæmoprotein is a potential model for the more complex flavin-hæm systems of the whole mitochondrion

Dr M R Atkinson (Adelaide) reviewed recent evidence for phosphorylated and unphosphorylated intermediates of oxidative phosphorylation and described electrophoretic investigations (with urea-starch gels or on paper after tryptic hydrolysis) of the radioactive products obtained on exposure of mitochondria and mitochondrial extracts to radioactive orthophosphate He proposed a model for adenosine triphosphate synthesis in a concerted reaction with adenosine diphosphate, orthophosphate and an activated derivative of cytochrome *c* formed through acylation of the histidyl residue on the hæm iron by the adjacent glutamyl side-chain

Dr A M Snoswell (New South Wales) described experiments in which reduction of nicotinamide-adenine dinucleotide by succinate in mitochondria from rabbit heart was coupled to electron transfer from succinate or from tetramethylphenylenediamine to oxygen through cytochrome *c* and cytochrome oxidase The resistance of the coupled reductions to oligomycin was interpreted as evidence for activated intermediates other than adenosine triphosphate, which permit reduction of nicotinamide-adenine dinucleotide by compounds of higher oxidation-reduction potential Dr M L Birt (Melbourne) described related experiments with liver mitochondria prepared in a Krebs buffer in which reduction of the co-enzyme by succinate was inhibited by dinitrophenol but was not entirely inhibited by anaerobic conditions or by antimycin *A* and amytal The relative importance of reduction through enzymes such as malate dehydrogenase and reduction of co-enzyme through reversal of the respiratory chain was discussed

Prof R N Robertson (Adelaide) reviewed recent investigations of the relationship of oxidative phosphorylation to the active transport of ions against an electrochemical gradient Active transport need not be coupled to hydrolysis of adenosine triphosphate, but transport of ions or phosphorylation of adenosine diphosphate may be alternative consequences of the oxidative generation of compounds of the $A \sim B$ type or the oxidative separation of positive and negative charge, as envisaged by Mitchell Dr J Charnock (Adelaide) described experiments on an adenosine triphosphatase from the microsomal fraction of kidney, this enzyme requires sodium and potassium ions for full activity and is inhibited by ouabain Dr Charnock discussed the evidence that a phosphoprotein intermediate is involved in the action of the enzyme and that the phosphoprotein might carry cations across membranes Dr B Hetzel (Adelaide) discussed the uncoupling of oxidative phosphorylation by thyroxine and triiodothyronine and reviewed evidence that this process was involved in the physiological action of these compounds

In his concluding remarks, Prof R K Morton (Adelaide), who arranged the meeting, emphasized the need for caution in interpreting the results of investigations with phosphorylated compounds derived from mitochondria, these might differ from the true intermediates, just as the isolation of phosphorylated enzymes does not prove the involvement of these compounds in enzymatic phosphoryl transfer

The cryptic nature of many publications on oxidative phosphorylation caused much good-humoured but frustrated comment, and most participants looked forward to the publication of experimental details which will permit more detailed examination of many recent claims in this remarkably active field of scientific investigation

M R Atkinson
[The late] R K Morton

ORGANIC MATTER AND SOIL PRODUCTIVITY

IT has commonly been accepted that farmyard manure is desirable or even essential to maintain or improve soil fertility, because it exerts a beneficial effect on the physical condition of practically all mineral soils and steadily supplies plant nutrients as it decomposes Market gardeners have relied on stable and byre manures from the nearest towns and farmers in arable areas have kept cattle over winter to provide manure for the potato and root crops in the rotation Most growers in fact have been so convinced of the value of regular additions of organic waste material to the soil that, in face of declining supplies of farmyard manure, they have adopted various methods of green manuring or of composting or even of bringing in peat In effect, a normal dressing of 10–15 tons of farmyard manure amounts to some 2 tons of organic matter per acre or 0 2 per cent in the top 9 in of soil This amount is barely measurable by sampling and analysis It is substantially the same as the annual leaf litter in woodland, it is not much more than the amount in the stubble and roots of a cereal crop, it is much less than what is incorporated in the soil by ploughing in grass turf Nevertheless, there remains the conviction that on many types of soil fresh organic matter confers a benefit that is not obtained from plant nutrients alone

It is practically impossible to make a strict comparison of organic manures with inorganic fertilizers since the former contain nutrients that become available to the plant over a more or less prolonged period and, at the same time, modify the soil in a manner that affects all the factors like æration, water movement and biological activity that play a part in crop development There is undoubtedly evidence that the crop responses to the total nutrients in farmyard manure are very much less than those obtained from the same amounts of nutrients applied as soluble fertilizers There have also been recent assessments from field experiments which indicate that the merit of farmyard manure is to be attributed almost solely to its nutrient content But the fact remains that on some soils it is not possible to produce maximum crops, with or without fertilizers, unless farmyard manure has been used It is not surprising, therefore, that investigators have been searching for more precise information on the effects of additions of organic matter to the soil Is it possible, for example, that organic matter may affect the availability of nutrients to the plant, or may produce derivatives that influence plant growth either directly, through absorption by the roots, or indirectly, by controlling the incidence of disease?

These matters are the subject of a well-documented review by D C Whitehead entitled "Some Aspects of the Influence of Organic Matter on Soil Fertility" in a recent issue of *Soils and Fertilizers* (26, No 4, Commonwealth Bureau of Soils, 1963) He discusses the question of soil nitrogen released from organic residues or fixed by symbiotic or free-living organisms, of the ability of organic acids to release insoluble forms of phosphorus and potassium, and certain trace elements, from the soil particles He also directs attention to the absorption by the roots of organic molecules that could influence plant metabolism—this covers such substances as amino-acids, vitamins, phenolic compounds and auxins—and to the ability of organic matter to favour saprophytic organisms rather than parasitic, there are also various decomposition products that are toxic to fungi There is much contradictory evidence and obviously a need for further work to establish the significance of these compounds They are known to be produced, but the quantities concerned and the probable interactions are still largely speculative

Closely related to this review is an article on "The Biological Nature of Soil Productivity", by G V Jacks (26, No 3, 1963) This is designed to show how fertility is dependent on bio-physical rather than on physico-chemical changes in the soil It is the action of plants that converts radiant energy into energy for animals and micro-organisms, which in turn produce a structure out of structureless material. The cementing together of individual soil particles to form aggregates is essentially a physico-chemical process, but the building of the aggregates into a stable granular structure is dependent on the activity of living organisms. The agencies are varied and include organic acids and polysaccharides, bacterial gums and fungal hyphæ and mycelia, all stages in the decomposition of organic residues. There is also the action of plant roots in binding soil particles and giving rise to a porous crumb, and of earthworms in not only producing casts of mineral-organic aggregates but also of making channels for æration and water movement. Indeed, much of the organic matter in the soil consists of excrement from soil fauna.

The soil medium becomes, therefore, a climax association of plant and fauna with a definite level of productivity. This productivity can be enhanced by man, through the physical and chemical modifications brought about by cultivation and manuring; but it can also be reduced by destroying the environment built by natural agencies. It is possible to grow healthy plants without soil, but that is a specialized business; so long as soil is used the grower must endeavour to preserve or improve its structure.

A. M. Smith

RESOURCES OF PETROLEUM: HOW MUCH OIL?

EVER since petroleum became a world wide commercial proposition and the industry thus firmly established, the phenomenal and rapid developments of the world's oilfields with concomitant expansion of production, especially in the United States since the turn of the century, have periodically posed these questions: How long will oil resources last, is production outstripping supply? Economists, perhaps even more vociferous than technologists, have from time to time uttered dire warnings that the day may come when oil will become scarce; that other sources of fuel and energy must be harnessed to make up any deficiences in supply. For example, there was a memorable occasion in 1923 when it was thought that the United States had passed peak production; thenceforward a downward trend was predicted, with some 15–20 years ahead before calamity overtook the industry. In 1925 I myself stated: "Petroleum production has hitherto been, still is, and for some time is likely to be entirely dominated by the output from the United States. With the average figure of 15 per cent as representative of the rest of the world's contribution to total production, we have probably not got a real assessment of the potentialities of other countries, since their economic activities must largely be subservient to the greater influence. In other words, it is quite conceivable that in the event of material decline in United States output, more could be made good by external oil production than appears at first glance. Factors tending to this end include increased geological exploration, increased production from shut-in reserves, and a higher technique in petroleum engineering". "The possibilities of the world's 'peak' of production of oil having been passed in 1923 have been shown to be open to considerable doubt" (*Mining Magazine*, November 1925).

Subsequent events have more than justified that opinion and the present position is adequate answer to pessimistic prophecies of the years between. It is a fact that one-third of the world's proved reserves of petroleum to-day are in oilfields that were literally unknown, probably still less imagined, before 1950. This fact, its significance and the theme "How Much Oil" are the substance of an article by Mr. M. J. Rathbone, chairman of the Standard Oil Co. (New Jersey), in a recent issue of the house-journal, *The Lamp* (45, No. 2; 1963). Therein he speaks with authority and makes some cogent comments on the present world oil situation. A nice distinction is rightly drawn between 'reserves' and 'resources', two words often loosely applied and economically misleading out of proper context. He comments, "If you read that the world's proved oil reserves are estimated at 300 billion barrels, that oil is being consumed at the rate of nearly 9 billion barrels a year, and that this rate of consumption will probably double by 1980—you may well wonder how soon we will reach the bottom of the barrel . . . that figure of 300 billion barrels of 'proved reserves' . . . is one of our most misunderstood statistics. It does not measure the world's true oil resources at all. It is no more than an estimate of the oil we can obtain from fields already discovered, using present recovery techniques. Despite rising world consumption rates, 'proved reserve' figures go up every year". The fact is that petroleum to-day is being discovered faster than it can be used. More oil is being obtained from old fields, new fields are being proved simultaneously. Submarine oilfields, often in extension of known continental developments, have to be reckoned with. One example is the shelf region off the Louisiana coast, opened to exploration only last year and, according to Mr. Rathbone, now proved to contain billions of barrels of oil. Another case he cites is that of Libya, where six years ago there were no known oil reserves, but to-day considered to be a major source of supply.

Thus, reserves are proved on a very large scale. Resources, that is, extensive land-areas of the world potentially oil-bearing but as yet untouched by the drill, remain to be explored quite apart from under-water researches. In view of the drastic readjustment of our ideas concerning geographical distribution of favourable oil-pool structures, occasioned by the discoveries of the past decade, the location of oil and gas-fields in regions where twenty or thirty years ago no hopes whatever were entertained, then we may agree with Mr. Rathbone's contention that ". . . we have solid grounds for reassurance that petroleum resources are abundant relative to foreseeable demand".

H. B. Milner

MONOMER – DIMER FORMS OF BENCE JONES PROTEINS

By Dr. GEORGE M. BERNIER and Prof. FRANK W. PUTNAM

Department of Biochemistry, College of Medicine, University of Florida, Gainesville

BENCE JONES proteins, the abnormal biosynthetic products excreted in the urine of myeloma patients, are defined as low-molecular-weight proteins, antigenically and chemically related to γ-globulin[1,2], which precipitate when heated to temperatures near 50° C, dissolve on boiling, and reprecipitate on cooling[3]. Isotopic investigations have shown that this protein probably represents an aberrant synthetic portion of γ-globulin[4]. Edelman and

Gally[5] have presented evidence that L-chains obtained by reductive cleavage of 7 *S* myeloma globulins are closely related to, if not identical with, the Bence Jones protein from the same individual. These L-chains have a molecular weight of approximately 20,000, and these authors postulate that the usual 3·5 *S* Bence Jones protein exists as a dimer of L-chains. Although most Bence Jones proteins have sedimentation coefficients of 3·5 *S*, some do occur naturally as 1·8–2·5 *S* units[6], and earlier work by Putnam and Stelos showed that 3·5 *S* Bence Jones proteins dissociate into smaller components at *p*H 4 (ref. 7). In the present investigation, the existence of polymerism in several Bence Jones proteins has been demonstrated. The results are illustrated by a description of chemically and antigenically identical monomer and dimer forms of one such protein.

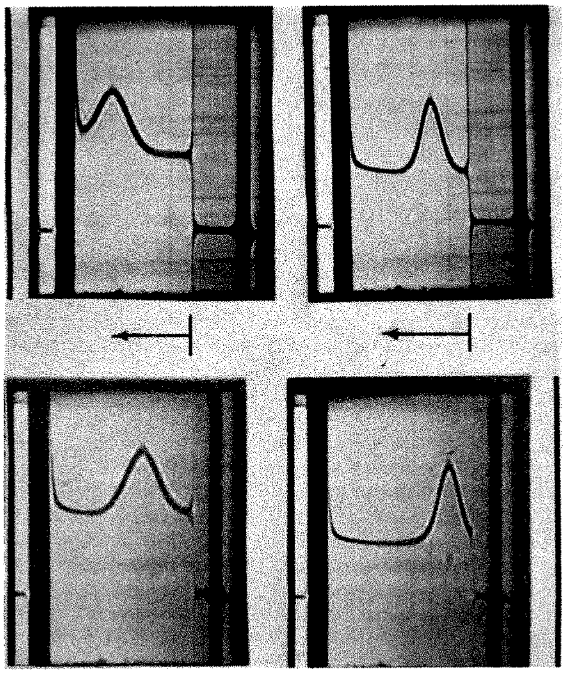

Fig. 1. Ultracentrifuge diagrams of monomer and dimer in 0·15 M sodium chloride, 20° C, 59,780 r.p.m. Above, dimer; below, monomer. In each case the pictures were taken 64 and 128 min after start of run. The arrow shows the direction of migration; the tail of the arrow indicates the meniscus

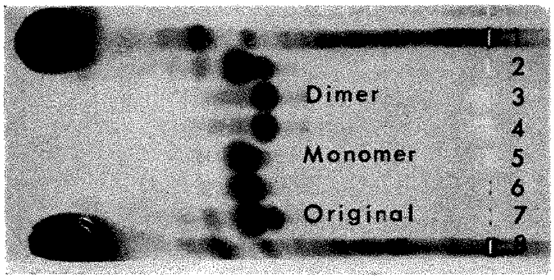

Fig. 2. Starch gel electrophoresis at *p*H 8·65, discontinuous buffer system, of normal human serum (1 + 8), original Bence Jones (*Ag*) (2 + 7), dimer (3 + 4), and monomer (5 + 6)

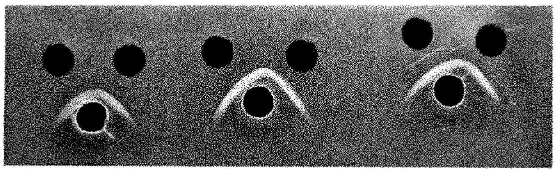

Fig. 3. Ouchterlony double diffusion in agar. The top left well in each triangle is monomer, and the top right well is dimer. The rabbit antisera are anti-normal human γ-globulin (left), anti-type I Bence Jones protein (centre), and anti-type I macroglobulin (right). The reactions are those of identity except that the monomer has precipitated closer to the antiserum well in each case. The same reaction was given by specific antisera prepared against the monomer

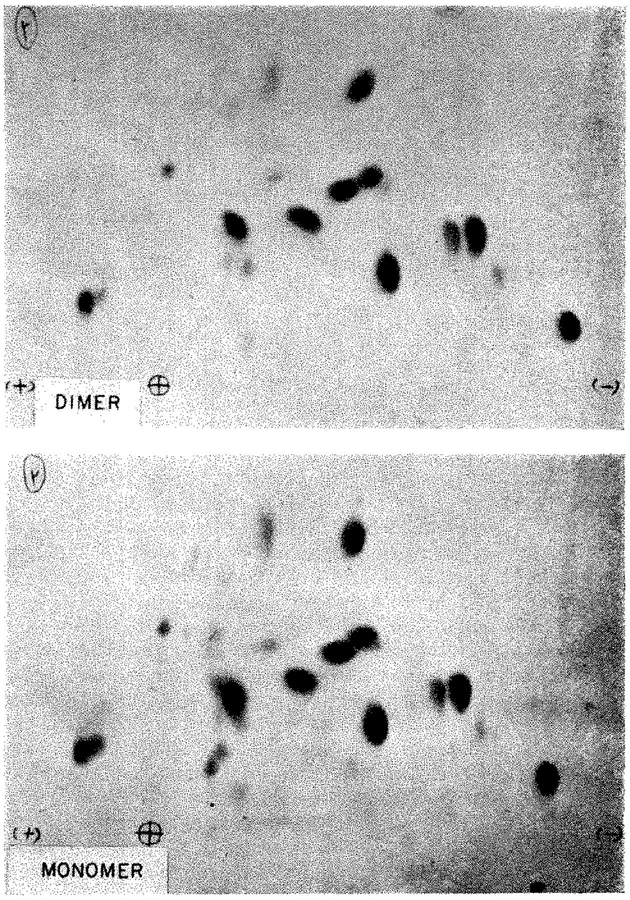

Fig. 4. Maps of tryptic peptides of dimer (above) and monomer (below). The samples were chromatographed for 20 h in butanol/acetic acid/water (4 : 1 : 5) and electrophoresed at *p*H 3·7, pyridine/acetic acid/water (1 : 10 : 289)

This Bence Jones protein was obtained by ammonium sulphate precipitation from the urine of a myeloma patient (*Ag*). Although more than 90 per cent homogeneous in Tiselius electrophoresis, this protein showed characteristic multiple banding in vertical starch-gel electrophoresis with a discontinuous buffer system of *p*H 8·65. It was chromatographed on the Beckman Model 130 Spectrochrom, using DEAE 'Sephadex (*A*-50)' with starting buffer *p*H 8·0, 0·02 M potassium phosphate and limiting buffer *p*H 8·0, 0·02 M potassium phosphate–1·0 M sodium chloride. Fractions so obtained were further chromatographed on 'Sephadex *G*-100'. A number of components were isolated, each of which migrated largely as a single band in starch-gel electrophoresis.

Two of the isolated fractions which were obtained in appreciable amounts had sedimentation coefficients in saline of 3·6 *S* and 2·4 *S* respectively (Fig. 1). The two fractions differed in starch-gel mobility (Fig. 2), but were identical by immunological testing (Fig. 3), peptide mapping (Fig. 4), and thermal solubility curves. Total amino-acid analyses (Table 1) were virtually identical. Significantly, methionine, an amino-acid often absent in Bence Jones proteins, was present as one mole per 21,900 g of the smaller protein, and as two moles per 42,400 of the larger. Half-cystine was present as four residues per monomer. There are 12 lysine and 6 arginine residues present in the monomer. From the known specificity of

Table 1. AMINO-ACID COMPOSITION OF MONOMER AND DIMER

Amino-acid	g Amino-acid residue/100 g		No. residues/22,000 g	
	Monomer	Dimer	Monomer	Dimer
Lysine	7·00	7·58	12·0	13·0
Histidine	1·69	1·90	2·71	3·05
Arginine	4·04	4·38	5·69	6·17
Aspartic acid	9·58	9·51	18·3	18·2
Threonine	7·82	7·70	17·0	16·8
Serine	9·00	8·96	22·7	22·6
Glutamic acid	14·65	14·48	25·0	24·7
Proline	4·74	4·57	10·7	10·4
Glycine	3·18	3·10	12·2	12·0
Alanine	3·58	3·60	11·1	11·1
Half-cystine	1·99	1·74	4·3	3·75
Valine	5·56	5·28	12·3	11·7
Methionine	0·60	0·62	1·00	1·04
Isoleucine	4·08	4·17	7·93	8·10
Leucine	7·06	7·12	13·7	13·8
Tyrosine	6·84	6·74	9·2	9·09
Phenylalanine	5·59	5·52	8·36	8·25
Tryptophan	not determined			

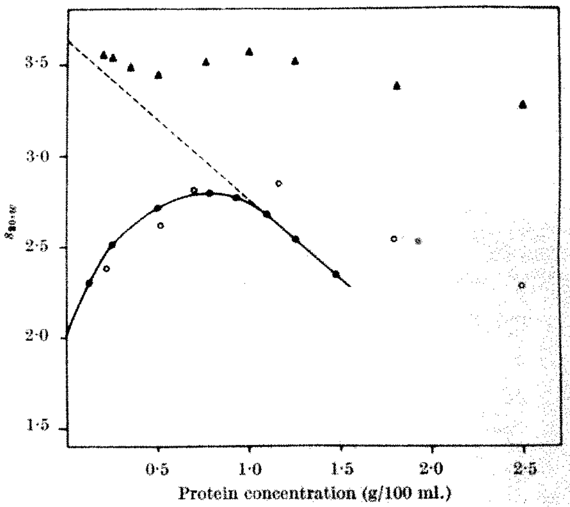

Fig. 5. Concentration dependence of $s_{20,w}$ of monomer at *p*H 6·8 and *p*H 8·6, and of another Bence Jones (*Sh*) at *p*H 6·8. All buffers were 0·1 M. ▲, Bence Jones (*Sh*) *p*H 6·8; ○, Bence Jones (*Ag* monomer) *p*H 8·6; ●, Bence Jones (*Ag* monomer) *p*H 6·8

trypsin, 19 tryptic peptides would be anticipated. The peptide maps shown here each contain some 20 peptides. Twelve of these have been isolated and their composition determined. The presence of slightly less than 2 moles of *N*-terminal aspartic acid per 44,000 g of this protein has earlier been reported[8].

Further proof of dimerization was established by the curvilinear concentration dependence of $s_{20,w}$ exhibited by the monomer, analogous to the behaviour of α-chymotrypsinogen[9]. Fig. 5 contrasts the concentration dependence of the *Ag* monomer (antigenic type I) and that of another Bence Jones protein (*Sh*) of antigenic type II. In our experience all Bence Jones proteins having an $s_{20,w}$ of 2·5–3 *S* at neutral *p*H have been of antigenic type I, but it is premature to conclude that this type dissociates more readily than type II.

These findings give further evidence that the usual 3·5 *S* Bence Jones proteins exist as dimers of two identical polypeptide chains which have molecular weights of approximately 22,000. It makes more plausible the presence of a 22,000 molecular weight Bence Jones-like polypeptide chain in the papain-produced 3·5 *S* 'slow' (*S*) fragment of myeloma globulin, about half of which appears to consist of Bence Jones protein by peptide mapping. At least two analogous (Bence Jones-like) polypeptide chains must occur in normal human γ-globulin though, to be sure, these may vary in structure among different antibodies just as the Bence Jones proteins of individual patients differ[10].

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ORIGIN OF OSTEOCLASTS FROM THE FUSION OF PHAGOCYTES

An investigation of the sequelæ of partial ischæmia by the intra-arterial injection of particulate suspensions of bone charcoal in the nutrient arteries of femurs in growing rabbits reveals evidence in support of the view that osteoclasts arise by fusion of mononuclear wandering cells (histiocytes, macrophages). Our findings support the idea of Hancox[1] that "it is in the histiocytic state alone that cells (whose origin is then irrelevant) are capable of fusing to form osteoclasts".

Our experiment involves the injection of 8–15 c.c. of a 2 per cent suspension of dextran sulphate and bone charcoal in physiological saline into the nutrient artery of the right femur in 43 8-week-old New Zealand white rabbits. The contralateral femur was injected with isotonic saline and served as the control. The treated animals were killed immediately after the injection and serially up to 240 days. Both femurs were fixed in 10 per cent neutral formalin, decalcified, embedded in celloidin, sectioned, and stained in hæmatoxylin and eosin.

Up to 4 days post-injection, the bulk of the charcoal was found to be localized in the vascular channels (arterioles and capillaries) of the femoral metaphysis. Some isolated macrophages in the vicinity of the vessels laden with charcoal contained a few finely dispersed charcoal particles less than a micron in size. At 5 days post-injection, a larger population of macrophages as well as some spindle-shaped cells contained the charcoal (Fig. 1). It was difficult to differentiate histologically some of the spindle cells from a reticular cell. These cells could best be described as phagocytic reticular cells. Although the free macrophages surrounding osteoclasts were heavily burdened with charcoal, the osteoclasts were free of particulate material. This led us to conclude that the osteoclasts were incapable of phagocytizing the charcoal.

At 12 days post-injection, the particulate material was found in macrophages, spindle cells (phagocytic reticular cells), and a few osteoclasts. At this time only a few finely dispersed charcoal particles were found in occasional osteoclasts, while between 15 and 35 days there was a progressive increase in the number of osteoclasts and spindle cells laden with charcoal (Figs. 1 and 2). The

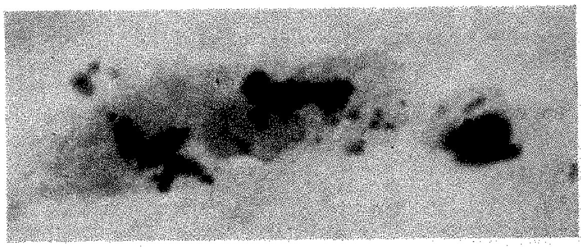

Fig. 1. Spindle-shaped cell (probably a phagocytic reticular cell) with particulate charcoal within its cytoplasm. (× 900)

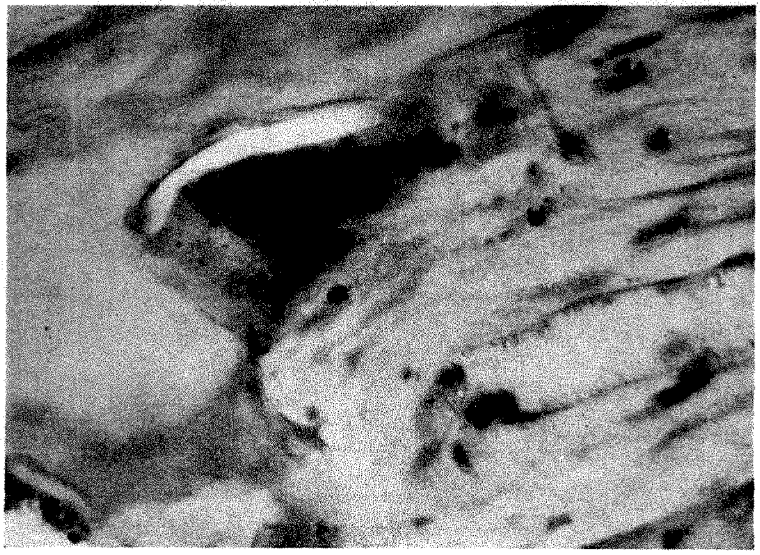

Fig. 2. Examples of osteoclast formed by the fusion of macrophages, containing charcoal in part of its cytoplasm. (× 800)

distribution of the charcoal in the osteoclasts was uneven (Figs. 2 and 3). These charcoal-laden osteoclasts were observed to arise from the coalescence of macrophages (histiocytes) containing the particulates. It should be emphasized again that when the charcoal was phagocytized by the macrophage, the particulate was absent in osteoclasts. At no time did we observe any charcoal in osteoblasts.

It was difficult to determine whether the macrophages transformed into spindle (reticular) cells. The difficulty arose because they both contained charcoal at the same time. It is generally agreed that the phagocytic reticular cell (free macrophage[2]) is capable of phagocytosis. A huge population of charcoal-laden osteoclasts and spindle cells were observed between 15 and 30 days. The appearance of these cells was related to massive osteoclasis of the femoral metaphysis as a consequence of the partial ischæmia. A more detailed description of these dynamic events will be reported elsewhere.

Recent contributions to the question concerning the origin of the osteoclast have been reported in the investigations of the inter-relationships of the cells of bone using the technique of tritiated-thymidine autoradiography. These investigations have re-affirmed that osteoclasts are formed by coalescing uninucleated precursors. However, the same investigations have led to different conclusions that the precursors are osteoblasts[3] or osteoprogenitor (mesenchymal, spindle, reticular) cells[4,5]. Our findings support the view that the osteoblasts are not the prerequisite cells for the formation of osteoclasts. Many workers believed that the osteoclast arose by the fusion of mononuclear wandering cells (histiocytes, macrophage). Hancox cited these investigators and their contributions in his excellent review on osteoclasts[1].

To sum up, we have evidence to support Hancox's hypothesis[1] that the cells in the phagocytic (histiocytic) state do fuse to form osteoclasts. Therefore, the cells of bone marrow possessing the ability to phagocytize and mobility are capable of coalescing to form osteoclasts.

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W. S. S. JEE
P. D. NOLAN

Division of Radiobiology,
Department of Anatomy,
University of Utah College of Medicine,
Salt Lake City 12.

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IN 1873 Kölliker[1] proposed that osteoclasts were formed by the fusion of osteoblasts. Since then many alternative claims have been made. These have included the origin of osteoclasts by fusion of fibroblasts, reticulum cells, bone cells with cartilage cells liberated from skeletal matrix, histiocytes, macrophages, monocytes, lymphocytes and liberated chondrocytes. Unfortunately these investigations have been generally conducted at the metaphyseal regions of long bones which contain numerous cell lines. This is a serious drawback in that many cells cannot be properly distinguished, for example, spindle cells, reticular cells, pre-osteoblasts, etc.

In order to avoid this difficulty and to increase the specificity of the observations, investigations were conducted at the periosteum using tritiated-thymidine (^{3}HTDR) autoradiography[2-4]. At the periosteum the origin of the osteoclast can only be attributed to pre-osteoblasts and/or osteoblasts, since these cells exclusively make up the osteogenic layer. The specific results which are achieved with the use of ^{3}HTDR have led us to the unquestionable evidence that osteoclasts are formed by fusion of osteoblasts and possibly pre-osteoblasts as well[4]. Young's ^{3}HTDR investigations[5] on ribs and tibiæ which followed presented good evidence that pre-osteoblasts were equally involved. Recently, excellent ^{3}HTDR evidence has been presented for the origin of osteoclasts from fusion of mononuclear leucocytes in newt limbs[6].

It appears that not all osteoclasts are produced by the same precursors. In periosteum, they are formed from osteo-

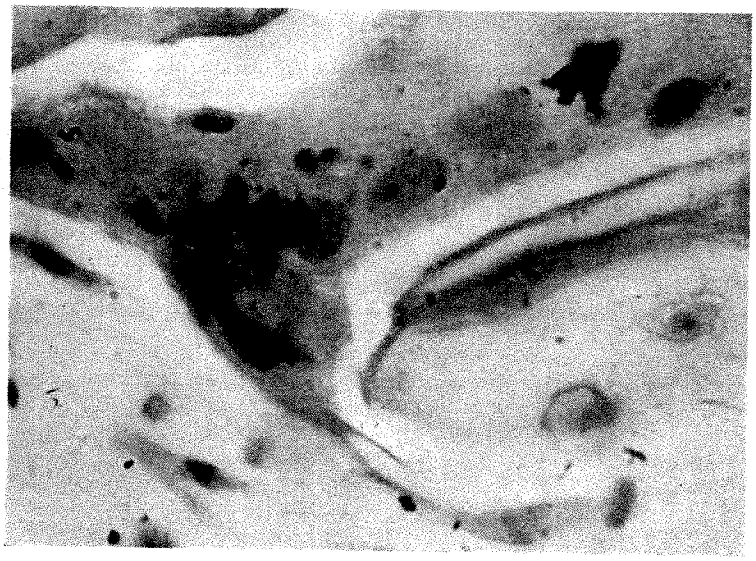

Fig. 3. An osteoclast surrounding a bone spicule with uneven distribution of charcoal particles within the cell. (× 800)

blasts and pre-osteoblasts, at the metaphyseal region other cells claimed to be precursors may well be. However, interpretations at metaphyseal sites are extremely difficult to make

Jee and Nolan have claimed that histiocytes are the precursors of osteoclasts on the basis of observations made on tissues of animals previously injected with charcoal particles Within the first 4 days charcoal was seen almost exclusively in vascular channels Not until the 12th day was there evidence of an occasional osteoclast containing charcoal, whereas, from the 5th day on, macrophages in the vicinity of osteoclasts were heavily burdened with charcoal

In ^{3}HTDR investigations, Young[5] saw labelled osteoclasts within 9 h Kember[7] observed osteoclasts at the metaphysis by 24 h We[4] have observed them at the metaphysis in 18 h and 36 h at the periosteum If histiocytes were the precursors of osteoclasts, Why was charcoal not observed in osteoclasts earlier? Why did it take a long period of 12 days for them to contain charcoal? Osteoblasts do not phagocytize charcoal and if they were to participate in osteoclast formation at the metaphysis, the results would not be different On the other hand, it is well-known that osteoclasts themselves are phagocytic However, this does not mean that the phagocytic property is unlimited or unspecific Jee and Nolan's work shows clearly that osteoclasts do not normally phagocytize charcoal particles, but does not discount the possibility of active phagocytosis by osteoclasts of cells which may have lost their ability to cope with life after gorging themselves with charcoal particles This could easily lead to the conclusion that histiocytes are the precursors of osteoclasts. However, this seems highly unlikely since 12 days were required before several charcoal containing osteoclasts were observed.

At present it appears that several cellular types are capable of fusion to form osteoclasts, perhaps also histiocytes In view of the extremely long period which was required to find charcoal containing osteoclasts in adversity to several and repeated ^{3}HTDR investigations, where labelled osteoclasts were found after 9 or more hours, it is believed that, contrary to the conclusions of Jee and Nolan, their investigation shows that histiocytes are not the major contributors to osteoclast formation and may well not be involved at all.

E A TONNA

Medical Research Centre,
Brookhaven National Laboratory,
Upton, New York

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SITE OF SYNTHESIS OF CYTOPLASMIC RIBONUCLEIC ACID

By PROF HENRY HARRIS and L F. LaCOUR

Sir William Dunn School of Pathology, Oxford, and Department of Cell Biology, John Innes Institute, Bayfordbury, Hertford

ALTHOUGH the question has been investigated extensively over the past decade, in both animal and plant cells, it has not yet been decided whether RNA is synthesized in the cytoplasm of the cell or whether synthesis of RNA occurs only in the nucleus Numerous chemical[1,2] and autoradiographic[3–8] experiments have demonstrated that when cells are exposed to a radioactive RNA precursor the RNA in the nucleus becomes labelled more rapidly than the RNA in the cytoplasm When labelled cells are transferred to non-radioactive medium the amount of radioactivity in the nuclear RNA falls and the amount in the cytoplasmic RNA rises It has been claimed that the disappearance of radioactivity from the nuclear RNA and its appearance in the cytoplasmic RNA represent the passage of RNA from the nucleus to the cytoplasm, and experiments of this sort have been quoted as evidence that all the RNA in the cell is synthesized in the nucleus[6,8]

However, these observations are open to more than one interpretation, and the kinetics of labelling of the nuclear and cytoplasmic ribonucleic acids can be explained equally well by assuming that the rapidly labelled nuclear RNA has a short life in the cell and that the cytoplasmic RNA is synthesized independently The work of Harris and Watts[9–13] indicates that much of the rapidly labelled nuclear RNA does undergo breakdown within the cell to acid-soluble compounds, and an increasing body of experiment[12–17] suggests that this breakdown occurs, for the most part, within the nucleus These experiments make it clear that redistribution of radioactivity between nuclear and cytoplasmic RNA does not necessarily represent transfer of RNA from nucleus to cytoplasm, but they do not demonstrate where the cytoplasmic RNA is synthesized

A more direct approach to the problem has been made by examining the ability of enucleated cells to synthesize RNA It has been shown that under certain conditions enucleate amoebae[18,19] and enucleate fragments of HeLa cells[20] do not incorporate radioactive nucleosides into RNA although they do incorporate radioactive aminoacids into protein These experiments do indeed suggest that the cytoplasm does not have the ability to synthesize RNA, but the objection has been raised that the process of enucleation is itself exceedingly traumatic and that the synthetic capacities of enucleate cytoplasmic fragments may not be a true measure of the synthetic capacities of the cytoplasm in the intact cell The force of this objection is enhanced by the experiments of Schweiger and Bremer[21] on *Acetabularia* These authors have shown that under the correct conditions of growth, but only under these conditions, a clear-cut net synthesis of RNA may occur in the cytoplasm of these cells after removal of the nucleus On the other hand, it may be argued that *Acetabularia* is a rather special case and that results obtained with it cannot be extrapolated to other organisms

Some recent experiments with tissue culture cells have shown that the antibiotic, actinomycin *D*, can, at certain concentrations, abolish all RNA synthesis in the cell except the terminal or sub-terminal incorporation of nucleotides into the soluble RNA[22–25] Since actinomycin *D* has been shown to form specific complexes with DNA[26] and to inhibit the DNA-primed synthesis of RNA *in vitro*[27,28], the fact that it can inhibit all RNA synthesis in the intact cell has been interpreted as evidence that all cellular RNA is made in the nucleus[22] However, it has now been found that in *L* cells there is some synthesis of both nuclear and cytoplasmic RNA which is resistant to very high concentrations of actinomycin D[17]. Moreover, when the synthesis of RNA in animal cells is stopped by actinomycin D the rapidly labelled RNA in the cell nucleus breaks down to acid-soluble compounds, but no transfer of RNA from the nucleus to the cytoplasm can be detected[13-17].

It is clear from the foregoing observations that the most convincing demonstration of cytoplasmic RNA synthesis would be one in which a substantial synthesis of RNA could be demonstrated in the cytoplasm of a typical animal or plant cell either after enucleation or under conditions in which synthesis of RNA on the chromosomes has been suspended. Such conditions are found in all animal and higher plant cells during the period of mitosis. It has been shown that incorporation of radioactive precursors into the nuclear RNA ceases completely by mid-prophase and does not resume until mid-telophase[29-31]. Prescott and Bender[30] and Feinendegen and Bond[31], using autoradiographic techniques, have examined the incorporation of radioactive precursors into RNA during mitosis by exposing cultures of HeLa and Chinese hamster cells to tritiated uridine and cytidine for periods of 5–30 min. These authors found that cells in metaphase and anaphase were not labelled and concluded that no synthesis of RNA occurs in the cell during these stages. However, under the conditions of labelling used by these authors there may be little or no incorporation of radioactivity into the cytoplasmic RNA of interphase cells in a 30 min period. It is therefore scarcely surprising that cells in metaphase and anaphase were not labelled. Indeed, under such conditions of labelling, it is doubtful whether these cells are a suitable material for the examination of the problem, since their mitotic time is probably not much more than 30 min.

In the present investigation the synthesis of RNA during the mitotic period has been re-examined with two important modifications in the experimental design: (1) cells with a longer mitotic time were chosen, (2) the conditions of labelling were such as to permit a rapid and virtually linear incorporation of precursor into cytoplasmic RNA in the cells in interphase.

It has been shown that, under standard conditions of culture, the meristematic cells in the root tips of the broad bean *Vicia faba* have a mean mitotic time of 3.9 ± 0.2 h (ref. 32). Four hours seemed long enough, since, even under the usual conditions of labelling, radioactive RNA precursors are incorporated into cytoplasmic RNA in interphase cells within this period. This material was therefore chosen for the experiments recorded here.

Since radioactive adenosine, cytidine and uridine can be incorporated terminally or sub-terminally into soluble RNA, the choice of guanosine as a precursor was indicated. No terminal incorporation of guanine nucleotides into soluble RNA has been reported[33]. A further indication that guanosine might be a suitable precursor arises from a consideration of the metabolic pathways by which adenylic and guanylic acids are synthesized[34]. It appears that the conversion of guanosine into adenine nucleotides is in general a much slower process than the conversion of adenosine into guanine nucleotides[35]. This would make it probable that, during a short period of incubation, radioactive guanosine would be incorporated mainly into RNA guanine residues.

The concentration of the radioactive precursor is also of importance. In most autoradiographic experiments tritiated precursors of very high specific activity are used and the actual concentration of the precursor in the medium is trivial. Under such conditions there is usually a lag before any appreciable radioactivity can be detected in the cytoplasm. The duration of this lag is variable, but in some cells it may be as long as an hour[36]. It has, however, recently been shown by Watts[37] that this lag may be greatly reduced, and in some cases virtually eliminated, if high concentrations of precursor are used. This effect is probably due to the repression of endogenous synthesis of purines and pyrimidines by high concentrations of related exogenous compounds[38].

Seedlings of *Vicia faba* were grown in aerated tap water until secondary roots were produced. The leaves were then removed and the roots of the plant were immersed at room temperature (approximately 20° C) in half-strength Knop's solution containing tritiated guanosine at a concentration of 5×10^{-4} M (Radiochemical Centre, Amersham, Bucks, specific activity 481 mc./mM). Samples of roots were removed at 30, 60 and 90 min, washed in water and fixed in acetic alcohol for 4 h. The fixed roots were hardened overnight in 4 per cent formalin, then washed in water and extracted for 30 min in 5 per cent trichloroacetic acid at 4° C. They were then washed in water, dehydrated through an alcohol series and embedded in paraffin wax. Sections were cut at 2μ. The mounted sections were extracted again with 5 per cent trichloroacetic acid at 4° C for 30 min and then washed in distilled water. Some of the sections were treated for 4 h at 37° C with ribonuclease at a concentration of 1 mg/ml. (Worthington Biochemical Corp., Freehold, New Jersey). Kodak 'V1042' stripping film was applied to the sections and the autoradiographs were developed after 80 days. The sections were stained, after the autoradiographs had been processed, with Ehrlich's hæmatoxylin and counter-stained with eosin.

Cells in full metaphase or anaphase were sought in the sections and the number of grains over each of 50 such cells was counted. Counts were made at the same time of the number of grains over the cytoplasm of two interphase cells adjacent to each cell in anaphase or metaphase.

The work of Gray and Scholes[39] on the duration of the stages of mitosis in the root cells of *Vicia faba* permits one to calculate that, in a total mitotic time of 4 h, prophase would last about 2.5 h and metaphase about 50 min. It is thus very unlikely that any of the cells in full metaphase or anaphase would have been in the first half of prophase 30 or 60 min previously, although a few might have been in this stage 90 min previously. Since synthesis of RNA on the chromosomes ceases by mid-prophase, any labelling of RNA in cells in full metaphase or anaphase must therefore represent labelling of cytoplasmic RNA, except, perhaps, in a few of the cells from roots exposed to the precursor for 90 min. In fact, none of the cells in metaphase or anaphase showed any evidence of chromosomal labelling even in the 90 min sample. This would be detected in an experiment of this sort by a greater concentration of grains over the chromosomes than elsewhere in the cell.

In order to determine whether all the acid-insoluble radioactivity was in nucleic acid, some roots were extracted with 5 per cent trichloroacetic acid at 90° C for 30 min and the amounts of radioactivity in the extract and residue determined. Some roots were subjected to overnight hydrolysis in 0.3 N potassium hydroxide, and the amount of radioactivity in the RNA purines was determined after these had been isolated and separated by paper chromatography[11].

Table 1 shows, for each period of exposure to the radioactive precursors, the mean number of grains over 50 cells in metaphase or anaphase and the mean number of grains over the cytoplasm of 50 pairs of adjacent cells in interphase.

Table 1. MEAN NUMBER OF GRAINS PER CELL

Period of incubation with labelled precursor (min)	30	60	90
Cytoplasm of cells in interphase	8.13 ± 0.6	17.28 ± 1.0	25.59 ± 1.0
Cells in metaphase and anaphase	16.08 ± 0.7	36.82 ± 0.9	65.24 ± 2.1

It will be seen: (1) The number of grains over the cytoplasm of the interphase cells increases in a linear fashion from zero time. This finding illustrates that the lag in cytoplasmic labelling can be eliminated if the right precursor is used under the right conditions. (2) The number of grains over cells in metaphase and anaphase increases progressively with time and is at all times at least twice as large as the number over the adjacent interphase cells This indicates that substantial incorporation of tritiated guanosine into acid-insoluble material occurs in the cytoplasm at a time when there is no synthesis of RNA on the chromosomes The large difference between the counts over cells in mitosis and adjacent cells in interphase may be due, at least in part, to the fact that for cells in mitosis the number of grains over the whole cell was scored, whereas for cells in interphase the area over the nucleus was excluded It is conceivable that, in sections, about half the radioactivity in the cytoplasm may be lost if the area overlying the nucleus is omitted from the count, although it is difficult to calculate the magnitude of this factor Another possibility is that in cells in interphase the rapid synthesis of RNA in the nucleus competes with the cytoplasm for the labelled precursor, whereas in cells in mitosis this competition is suspended In any event, it is clear that the amount of precursor incorporated in the cytoplasm of the cells in mitosis cannot be much less than the amount incorporated in the cytoplasm of the cells in interphase (3) While the increase in the number of grains over the cytoplasm of cells in interphase is linear for the whole of the 90-min period, the number of grains over cells in metaphase and anaphase is greater in the 90 min sample than the value obtained by a linear extrapolation through zero time and the 30 and 60 min samples The reason for this high value in the 90 min sample is obscure It could be explained by the fact that some of these cells might have been in the first half of prophase 90 min previously and might thus at metaphase contain some labelled chromosomal RNA If this were the case one would expect a proportion of the metaphase cells in the 90 min sample to show unusually high grain counts, and these would not be accompanied by correspondingly high values in the adjacent interphase cells But a statistical analysis of the grain counts revealed no evidence of this type of labelling Moreover, if the chromosomal RNA were labelled in some of the cells one would expect that the mean number of grains over cells in metaphase would be greater than over cells in anaphase But no significant difference was found between metaphase and anaphase in this respect

Treatment with ribonuclease almost completely removed the radioactivity from the cells in mitosis and from the cytoplasm of the cells in interphase This indicates that the cytoplasmic acid-insoluble material into which the precursor was incorporated was RNA All the acid-insoluble radioactivity in the roots was extracted by treatment with 5 per cent trichloroacetic acid at 90° C for 30 min Isolation of the RNA purines after alkali hydrolysis of the roots revealed that the radioactivity was almost entirely in guanine residues, the amount of radioactivity in RNA adenine residues was scarcely greater than background

Tritiated guanosine is incorporated into cytoplasmic RNA during those stages of mitosis in which synthesis of RNA on the chromosomes is suspended The amount of precursor incorporated into the cytoplasmic RNA of cells in mitosis is at least comparable with the amount incorporated in the same time into the cytoplasmic RNA of cells in interphase The tritiated guanosine is incorporated almost entirely into RNA guanine residues These findings do not support the view that all the RNA of the cell is synthesized on the chromosomes; they indicate that substantial synthesis of RNA takes place in the cytoplasm

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STUDIES OF RADIATION-INDUCED CHANGES IN CELLULAR PROLIFERATION, USING A DOUBLE LABELLING AUTORADIOGRAPHIC TECHNIQUE

By Dr D. R. DAVIES* and Dr D E WIMBER†

Biology Department, Brookhaven National Laboratory, Upton, New York

RADIATION-INDUCED perturbations in proliferating cell populations can be due to the death or premature differentiation of a portion of the cell population, or to retardation of movement of certain phases of the mitotic cycle The last phenomenon is one that has long been recognized as one of the earliest consequences of radiation exposure Initially, the interruptions of the orderly progression of proliferating cells through the mitotic cycle were recognized through changes in the mitotic index[1], but critical analyses of the phenomenon became possible

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* Present address Wantage Research Laboratory, Wantage, Berks
† Present address Department of Biology, University of Oregon, Eugene

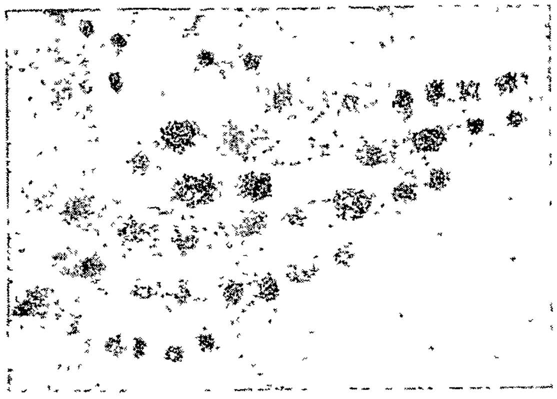


Fig 1 Autoradiographs of staminal hair cells tagged with tritium-labelled thymidine

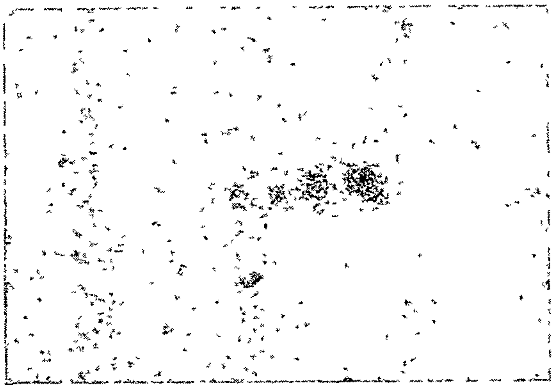


Fig 2 Autoradiographs of staminal hair cells that have been doubly labelled with ^{3}H- and ^{14}C-thymidine Cells with the silver grains directly over the nuclei are labelled only with tritium, those with a halo of grains around the nuclei are labelled with carbon-14

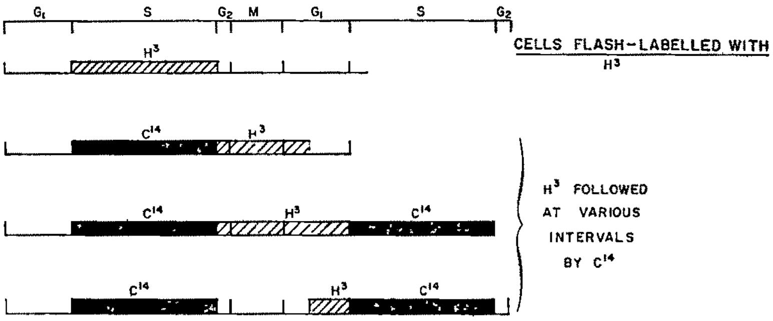
only after the advent of high-resolution autoradiographic techniques These have indicated that both the nature and extent of the changes induced are dose-, species- and probably tissue-dependent A few examples will suffice to indicate the diversity of responses observed G_2 (post-DNA-synthesis) blocks have been observed in HeLa[2], *Vicia*[3], *Pisum*[4], and mouse *S* (ref 5) cells, G_1 (pre-DNA-synthesis) blocks in *Tradescantia*[6–8], *Vicia*[9] and human cells[2,10] and blocks or delays in *S* (DNA synthesis) in mouse[11] and human[10] cells The doses used in the induction of these blocks have varied greatly if measured in terms of roentgens or rads, but until the nature and size of the relevant target or targets are known, comparisons of doses in these terms are not likely to be very meaningful[12]. The consequences of these delays or blocks, and the pattern of recovery with time, will probably also be varied and complex

In the experiments recorded here, an analysis was made (over an extended period of time) of the changes in the kinetics of an asynchronous cell population following a low dose of X-rays The double-labelling autoradiographic technique, and the particular tissues used, allowed a more critical evaluation of these changes than has previously been possible Moreover, from previous experience[13] it was known that the dose of X-rays used was sufficiently low that virtually no death or premature differentiation of the cells occurred, and the changes could be solely attributed to phase changes

The tissues used were staminal hairs produced within the flowers of a diploid clone (Sparrow's 02) of a variety of *Tradescantia occidentalis* Each flower has six filaments, each of which bears about 50 hairs (under the environmental conditions of the experiment—24 h light, 950 ft candles 21 ± 1° C) Each hair is a chain of single cells and its pattern of growth is as follows a cell in a filament divides in such a way that one of the daughter cells protrudes obliquely out of the filament This protruding cell is meristematic and becomes the terminal cell of the hair, continuing to divide until the mature hair (20–30 cells) is formed behind it The sub-terminal cell always divides once after its formation and occasionally an intermediate cell also divides Thus at any given time it is usually only the terminal and subterminal cells that are meristematic These terminal hair cells were superior to many tissues used in similar experiments because they were all known to be meristematic and because their unique position on the staminal hair eliminated complications introduced into most analyses by cell division In addition, cell lineages could to some extent be followed

The double-labelling autoradiographic technique utilized was similar to that described by Wimber and Quastler[8] The rationale of the technique is that a cell population is pulse labelled with tritiated thymidine and at a later time with thymidine labelled with carbon-14, or vice versa, and fixed immediately after administration of the second label Autoradiographs then reveal three groups of cells (*a*) those labelled with tritium alone, (*b*) those with carbon-14 (with or without tritium), (*c*) those with no label (Figs 1 and 2) If the tritium were given first, theoretically one would expect the number of terminal and subterminal cells labelled with tritium alone to increase as the interval between tritium and carbon-14 administration was increased (Fig 3) This increase would occur over the period of time it would take for the cells labelled with tritium (that is, those in *S* at the time of labelling) to move through G_2, *M* and G_1 If ^{14}C-thymidine were available at the time this block of cells commenced to synthesize DNA again, they would take up carbon-14 and so the number of tritium-labelled cells would decrease

Inflorescences were used which were still attached to the parent plant Each inflorescence contained a large number of buds The tips of buds with actively dividing hair cells were removed and the inflorescences immersed for 1 h in a water solution containing 1 μc /ml tritium-labelled thymidine (3 1 c /mM, New England Nuclear) One drop of 'Tween 80' per 20 c c solution was added to facilitate penetration of the thymidine into the bud As a further aid to penetration the whole plant with its inflorescences immersed in thymidine was placed in a vacuum for about 5 min at the beginning of the labelling period Preliminary experiments had shown that an adequate replacement of the air trapped within the buds, by thymidine solution, was difficult to achieve without this A low (1 μc /ml) concentration of tritium-labelled thymidine was used, as the work of Wimber and Quastler[8]



G₁
S
G₂
M
G₁
S
G₂
H³
CELLS FLASH-LABELLED WITH H³
C¹⁴
H³
C¹⁴
H³
C¹⁴
C¹⁴
H³
C¹⁴
H³ FOLLOWED AT VARIOUS INTERVALS BY C¹⁴

Fig 3 Diagrammatic representation of the ^{3}H-,^{14}C-thymidine double-labelling method used in these experiments Cells are visualized as passing through the nuclear cycle from left to right The cell populations are initially labelled with ^{3}H-thymidine and then at various later times are treated with ^{14}C-thymidine Fixation follows the second labelling

had indicated that the endogenous radiation from tritium could in itself affect the patterns of cellular proliferation. At the end of the labelling period the buds were thoroughly washed in water, the control series returned to the growth chamber and the irradiated series exposed in air to 54 r. of X-rays (6 m.amp, 250 kV, 1 mm aluminium filter, dose rate 108 r./min) prior to its also being replaced in the growth chamber. The inflorescences were wrapped in polyethylene 'gloves' after treatment to prevent undue drying of the buds following excision of their tips. At various intervals of time of 2–37 h after exposure to the tritium-labelled thymidine, the buds were immersed in a water solution of 2 μc./ml. ^{14}C-2-thymidine (25 mc./mM, New England Nuclear) for 1 h, under the same conditions as for the tritium-labelled thymidine exposure. Immediately after the treatment with carbon-14 the buds were washed and fixed in 3 parts absolute alcohol: 1 part glacial acetic acid. Later they were hydrolysed in 1 N hydrochloric acid for 6 min at 60° C, stained by the Feulgen method, the filaments dissected out, and squash preparations made of the filaments and hairs. Autoradiographs were prepared using two dips of Kodak *NTB* liquid emulsion, the slides were exposed for 3·5 months and were developed using standard procedures. After developing, the slides were counterstained in 1 per cent fast green to aid in the recognition of the terminal cells of the hairs. These terminal and subterminal cells were then scored for the presence and type of label and division stage. A differentiation of tritium-labelled cells into those which were in G_2, that is, those which had not divided, and those in G_1, that is, those which had divided, was also possible at the earlier fixation times (Fig. 4). Initially it was only the two meristematic end cells that took up label—after division the cells which were not going to divide again remained in G_1 for a considerable time. Ultimately these cells synthesized more DNA—this time without any further division, and accumulated up to 8C and possibly higher quantities of DNA (Davies, unpublished observations). Between 400 and 700 terminal cells were scored at each of the fixation times for control and irradiated series, and data on a similar number of subterminal cells also collected for the earlier fixation times. There was no significant difference between the pattern of behaviour of terminal and subterminal cells.

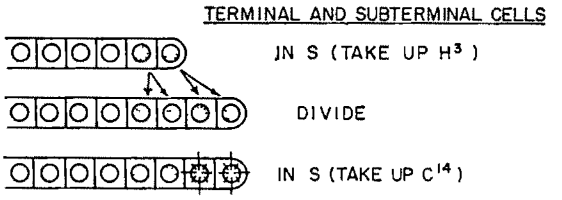


Fig. 4. Labelling patterns observed in the meristematic cells of the staminal hairs. Only the first two cells are meristematic at any one time. By observing the labelling distribution it is possible to determine whether cells are in G_2 or G_1 after double labelling. Those having two tritium-labelled cells distally are in G_2, while those having four tritium-labelled cells distally are in G_1

Control series. The percentage of cells in S, that is, those labelled with carbon-14, remained fairly constant throughout the cycle (Fig. 5)—the mean value for all fixation times being 52·0 per cent. There may have been a slight reduction in the number in the period 2–10 h after administration of the tritium label, probably due to a reduced flow of cells from G_1 to S (Fig. 5). A similar effect of the endogenous radiation from an incorporated radioactive label has been observed by Wimber and Quastler[8]. The rate of movement of cells in the mitotic cycle was given by the slope of the line denoting the increase in tritium-labelled cells, 2–8 h after labelling. This increase was due to cells leaving S and moving into G_2, M and G_1, the time at which they started decreasing again therefore represented the sum of G_2, M and G_1.

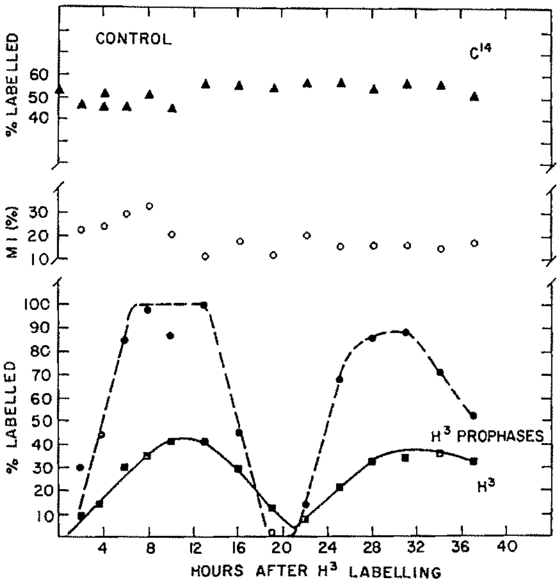


Fig. 5. Control series. Data collected from apical cells only. Top, carbon-14 labelling indices; middle, mitotic indices; bottom, labelled prophase curves and tritium-labelling indices. See text for interpretations

In other words, those cells which were in S at the time of tritium labelling were starting to enter S again—this time they would take up carbon-14, and the tritium label would not be recognized. The slope of the increase in number of tritium cells from 2–8 h was 4·77 per cent/h (Fig. 7). Since the mean number of cells in S was 52·0 per cent, the total time of S must have been approximately 10·9 h minus 1 h labelling time—9·9 h (1*a*)

S could also be estimated, but not as accurately as in (1*a*), from the distance between the 50 per cent points on the upward and downward slopes of the labelled prophase curve—12 h minus 1 h labelling time—11 h (1*b*)

The total cycle time (T) could similarly be calculated from the rate of movement of cells and was 21·0 h (2*a*)

Another estimate of T, from the distance between the two 50 per cent labelled prophases points on the upward slopes of the successive cycles, was 20 h (2*b*)

Yet another was 20·5 h—the distance between the 20 per cent tritium-labelled cells on the upward slope for the two cycles (2*c*)

The mitotic index, being based on many fewer cells, was somewhat variable but had a mean value of 19·3 per cent, indicating a time of 4·0 h for mitosis (3)

The G_2+M+G_1 time could be obtained from the time taken to reach the peak of tritium labelling (Fig. 5), and was approximately 11 h. Similarly the difference between T and S from earlier estimates (1) and (2) was also approximately 11 h. The time of G_2+M was given by the distance between the lines representing the increase in tritium-labelled cells, and increase in divided tritium-labelled cells in Fig. 7. This interval G_2+M was 5 h, and knowing M to be 4 h (estimate 3) G_2 must have been 1 h (4)

The usual method of estimating G_2 is to determine the time between labelling and the 50 per cent labelled prophases point on the upward slope. One-half of the duration of the prophase and labelling time should be subtracted from this figure to give the value for G_2 approximately 3·5 h—not 1 h as calculated earlier. However, the slope of increase in number of labelled prophases was, in this experiment, very much shallower than the theoretically expected vertical line. This in part was due to the length of prophase (2 h), to the variation in the length of G_2 and to the length of the labelling period. Hence the best estimate of G_2 must be 1 h (4) and by difference $T-(M+S+G_2)$, an estimate of G_1 was $21·0-(4·0+9·9+1)=6·1$ h (5)

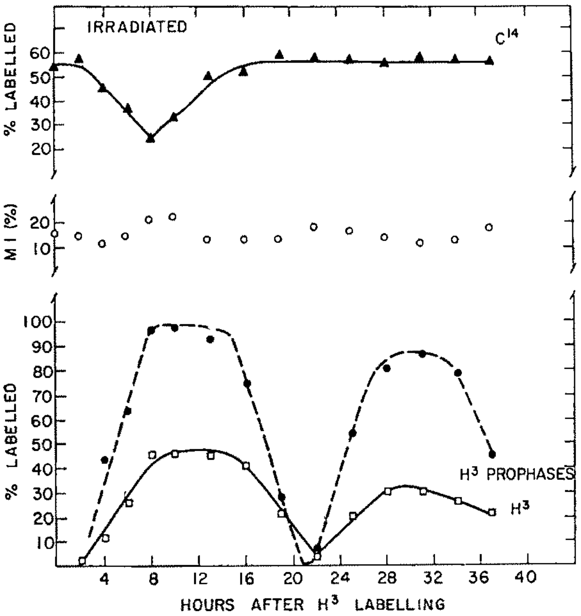


Fig. 6. Irradiated series. Data collected from apical cells only. Top, carbon-14 labelling indices; middle, mitotic indices; bottom, labelled prophase curves and tritium-labelling indices. See text for interpretations.

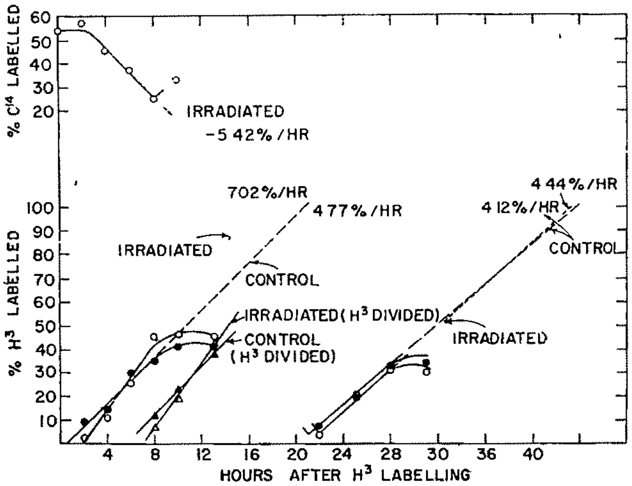


Fig. 7. Irradiated and control series compared. Top, decreasing carbon-14 labelling indices for the irradiated material, indicating a block in G_1; bottom, tritium-labelling indices for both control and irradiated material with slopes plotted. In addition, the increase in divided tritium cells is plotted for both irradiated and control populations. These are determined by the method illustrated in Fig. 4.

Irradiated series. The time for the first post-irradiation mitotic cycle appeared little changed from that seen in the control (Figs. 5 and 6). However, within the cycles there were considerable differences. Emergence of cells from S was slightly delayed (compare the time of emergence of tritium-labelled cells after labelling in Figs. 5 and 6). However, after this initial delay, the cells moved through G_2, M and into G_1 at a faster than normal rate—7.0 per cent as opposed to the control value of 4.8 per cent/h (Fig. 7). The maximum proportion of cells labelled with tritium was similar in both the irradiated series and control, but the maximum level was maintained for a longer period of time. This indicated an increase in the time of G_2+M+G_1. Since the distance between the tritium-labelled line and the divided tritium-labelled line, that is, G_2+M, was similar for control and irradiated (Fig. 7), it must have meant a radiation-induced increase in G_1. That this was the case was shown conclusively by the carbon-14 labelling index, that is, the number of cells in S at any given time. Up to 2 h after tritium labelling and irradiation there was no change, but this may well have been due to a block both in cells leaving S, as considered earlier, and in the entry of cells into S from G_1. However, after 2 h the carbon-14 index dropped to a low value of 23 per cent at 8 h (Fig. 6). This decrease could be interpreted as being due to an almost complete blockage of cells in G_1—the theoretical line predicted for a complete block would drop to 14 per cent at 8 h, if the rate of cells leaving S were 7.0 per cent/h. Movement of cells from S to G_2, M, and G_1 (that is, increase in tritium-labelled cells in Fig. 6), it must be remembered, was not retarded. From 8 h onwards the number of cells taking up carbon-14 increased rapidly. If the flow into and out of S had been restored to its normal value, S would have remained at its low level (23 per cent). The rapid increase in the number of labelled cells back to the normal value indicated a more rapid than normal flow from 'G_1' into S. The definition of S in the particular context of this experiment now becomes ambiguous. It is conveniently equated to that stage in which precursors are incorporated into DNA. However, the cells which were prevented from incorporating thymidine (2–8 h post-irradiation) were possibly 'competent' in every respect other than their DNA content, and, once the block to thymidine incorporation was removed, surged through 'S' at a faster than normal rate. The radiation-induced lesion—the possible nature of which has been summarized by Stocken[14]—was temporary in nature and its influence transient, having no effect on the subsequent mitotic cycle. In the second cycle, rates of movement of cells, the number of cells in S, etc., all appeared similar to that seen in the control (Figs. 5, 6, 7).

The experimental material utilized here appeared similar, in its type of initial response, to *Tradescantia* roots[7,8], but not in its pattern of recovery. Even with the low doses of radiation used on the root systems (1 and 10 rads), there was no marked recovery within 20-h post-irradiation. The extent of the 'G_1 effect'—leading to a 40–50 per cent depression in the number of cells in 'S'—was similar for both, and similar to that observed in human bone marrow cells[10]. In the case of the latter tissue it was suggested that either the G_1 population was heterogeneous in terms of radiosensitivity, or that its rate of movement was halved. In contrast to these results, the present experiments showed that most of the G_1 to S cells were for a period of time impaired with respect to their ability to take up tritium-labelled thymidine. They then recovered at a rapid rate. If there had been a blockage and an accumulation of a large number of cells in a normal G_1 state, then a partial synchronization of cells would have resulted, and changes in the mitotic index observed. There was no direct correlation between the effects on DNA synthesis and on the actual process of cell division (the mitotic index was essentially unchanged throughout) and in contrast to most experiments the blockage in G_1 was induced at a lower dose than the latter. Furthermore, the long-term consequences of this effect on DNA synthesis must be negligible, at least in terms of survival of the cell, since 54 r. has little effect on this parameter[13].

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TREATMENT OF CANINE NEOPLASMS WITH AUTOGENOUS VACCINIAL PREPARATIONS

By Dr. C. W. RIGGS
University of California Medical Center, Vivarium, San Francisco

IN a previous report[1] a procedure was described wherein it was demonstrated that an immune response was invoked in *A* strain mice against sarcoma 37 when the mice received implants of their own sarcoma 37 which had been grown in a strain of mice normally resistant to this tumour. Twenty of one hundred experimental mice were afforded complete protection from a single implant of the altered tumour. The average life-span of the eighty which eventually died of sarcoma was twice that of control animals. In another similar experiment (not reported) involving the mouse mammary carcinoma *E*0771 in 100 mice, 25 per cent survived and the life span was again double the controls in those which developed tumours and died.

The procedure was based on the premise that a significant change might occur in a neoplasm if the neoplasm could survive and receive its nutrients in a genetically foreign host. Such a change could render the tumour antigenic when returned to the original host so that the host might reject the altered transplant. The antigens produced against the altered tumour might also be effective against the closely related original tumour. If the growth rate of spontaneous neoplasms is dependent on the host's ability to produce a limited amount of tumour antibodies, it is possible that the addition of even a weak antigen would tip the balance of tumour antibodies enough to bring about tumour regression.

An attempt was made to utilize the same technique on an aged female beagle hound which had a scirrhous mammary carcinoma with nineteen palpable breast lesions ranging from pea size to two lesions approximately 1,000 c.c. in volume. There was radiological evidence of lung metastases and the bitch evidenced severe dyspnœa, anorexia, loss of weight, and lethargy. The abdominal skin was darkly pigmented in large patches.

The largest lesion on the beagle was removed surgically, a fragment was minced and one piece implanted subcutaneously into each of ten 3-week-old Swiss strain mice. The mice were each injected with 1 mg of prednisolone daily, and at the end of one week palpable tumours were growing in five of the ten mice. A section of the mouse-grown tumour is illustrated in Fig. 2. A biopsy of the beagle's second large lesion was taken for histological study and comparison of the tumour before (Fig. 1) and after treatment (Fig. 3).

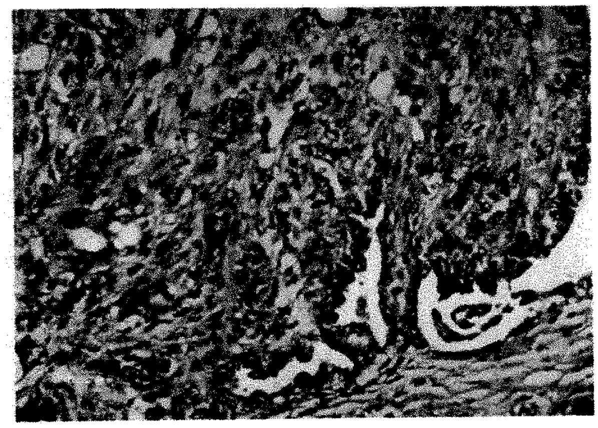

Fig. 1. Scirrhous mammary carcinoma of beagle, before treatment

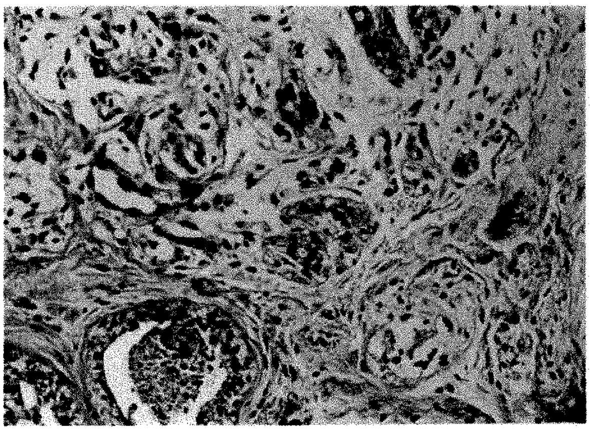

Fig. 2. Beagle tumour after growing in mouse 7 days

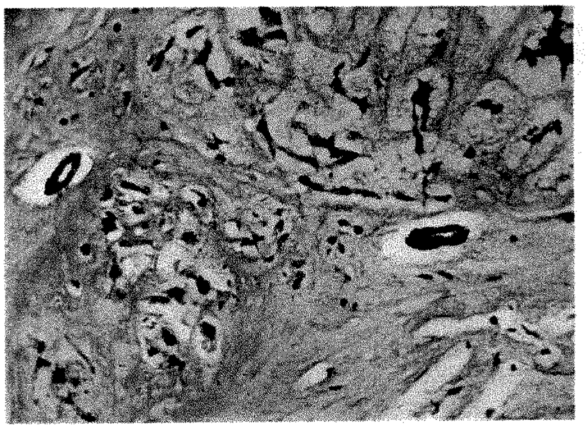

Fig. 3. Section of tumour shown in Fig. 1, 45 days after treatment

One mouse bearing the canine tumour was killed on each of the seventh, eighth, ninth and tenth days after implant, and the tumour tissue from each was returned to the beagle within 1 min after collecting. Implants were made subcutaneously by means of a 19*ga* trocar and cannula in the dorsal cervical region on the four successive days. Induration occurred at each implant site and persisted for approximately four days, but no growth of the mouse tumours occurred after their return to the beagle. The beagle received no other form of therapy.

Two of the eighteen breast tumours were measured on the day of the first mouse-grown implant. One, almost cuboid in shape, had a volume of 930 c.c., and the second, a shallow lesion, had an area of 7·5 cm². Measurements one week later were 600 c.c. and 3·75 cm², respectively. The tumour dimensions continued to decrease slowly, and three months after treatment the largest lesion volume was 345 c.c., while the smaller had completely disappeared. Eight other small lesions were no longer evident. The bitch weighed 6·2 kg before treatment and 9 kg three months later. Dyspnœa and anorexia subsided and normal vigour was restored. Pigmentation of the skin was no longer evident. The gross appearance of the largest lesion changed throughout the course of observation from a soft lumpy mass to a hard nodular mass. Biopsy sections taken 45 days after treatment with the mouse-grown tumours showed marked fibrosis, no mitotic figures, crenated and pyknotic nuclei. The animal lived 1·5 years after treatment with no apparent recurrence of her carcinoma, and died of causes incident to old age.

Another case treated in a similar manner, as an 'out-patient', deserves mention although it was not followed long enough to draw any definite conclusion. This was a ten-year-old male Afghan hound with generalized lymphoblastic sarcoma involving gross enlargement of all palpable lymph nodes. The animal was treated in September 1959 with a single dose of nitrogen mustard. A slight regression of size of node was observed for a 2-week period, followed by rapid growth of nodes and development of diarrhœa, anorexia, vomiting and weakness.

A 1 mm cube of an enlarged cervical lymph node from the Afghan hound was implanted into each of ten prednisolone-treated mice. Rapid growth of the tumour occurred in seven of the ten mice. On the fifth, sixth and seventh days of growth in mice, the tumour was returned to the Afghan.

Seven days after the first treatment with mouse-grown tumour the dog was brought to the laboratory, by its owner, for examination. All palpable glands were half or less the size they had been at the onset of treatment. The appetite had improved, vomiting stopped, the animal was stronger, but the diarrhœa was more severe. No other therapy was given.

A week later the owner called to report the dog had appeared to improve, except for the diarrhœa, had been struck by a car three days prior to his call, died in its sleep that night, and had been buried. No post-treatment sections were available.

The regression of lesions in the case of the beagle hound, the Afghan hound, and the inhibition of growth of a sarcoma and a carcinoma in mice similarly treated, suggest the possibility of producing what might be termed an autogenous vaccinal preparation for treatment of neoplasms. Prolonged growth of a neoplasm in a foreign host would not be necessary for production of such a preparation. Any growth at all should be sufficient to produce some alteration of molecular structure such as transposition of elements in nucleic acids, substitution of some side-chains, etc. Such preparations might be highly host specific if the structure of ætiological agent of any given neoplasm is closely related to the genetic make-up of its host, so that an autogenous preparation, effective in one animal, would be ineffective in another with a similar-appearing tumour.

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BIOMETRICS OF THE KARYOTYPE OF *PROTEMNODON BICOLOR*, WITH REFERENCE TO THE LIMITATIONS IN ACCURACY OF IDENTIFYING HUMAN CHROMOSOMES

By RUTH C. MOORE and Dr. G. GREGORY
Cancer Institute Board, Melbourne, Australia, and Department of Statistics, University of Melbourne

THE identification of human chromosomes depends mainly on the measured differences in their relative lengths and arm ratios at metaphase[1]. Both these parameters may vary in standard preparations owing to distortion or stretching caused by fixation and flattening of cells.

Identity can also be based on the appearance of characteristic constrictions and heteropyknotic regions. At the present time the significance of satellites on some human chromosomes is still *sub judice*[2-4]. The presence of constrictions on others has been noted[5,6] which, with further refinements of technique, may be sufficiently uniform to serve identification.

The magnitude of the variation in length and arm ratio is such that only the three largest human chromosomes and number 16 can be identified unequivocally; and this in turn means that it is impossible to assess variation and errors of measurement. However, the chromosomes of the Black-tailed Wallaby, *Protemnodon bicolor*, can be identified with ease. This species has a diploid number of 10 female, 11 male[7], the male having 2 Y chromosomes, perhaps due to centric fusion between the true X and a large acrocentric autosome which is the homologue of Y_2 in the male[8].

Two male wallabies were obtained with the co-operation of the Victorian Fisheries and Wild Life Department. The technique of Moorhead *et al.*[9] was used to examine chromosomes from peripheral blood.

A typical idiogram is shown in Fig. 1. Each of the four pairs of chromosomes is distinguishable. The X is similar in size to autosome 2, but can be identified by a constriction at the base of the short arm.

42 cells (21 from each specimen) were photographed on quarter-plate Kodak orthochromatic high-contrast film, and printed at a final magnification of 3,750. A piece of fine wire was laid along the midline of each photographed chromatid from the end to the estimated position of the centromere and the straightened wire measured against a ruler graduated

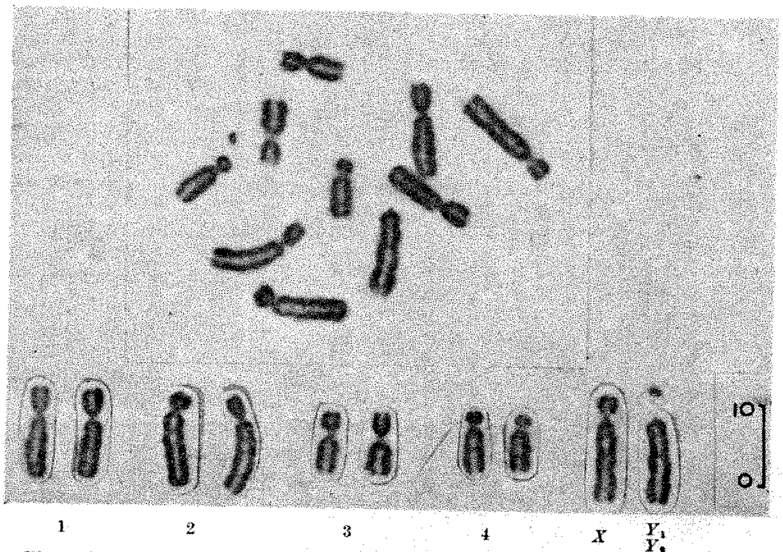

Fig. 1. Karyotype of a male black-tailed wallaby (*Protemnodon bicolor*) (scale in μ)

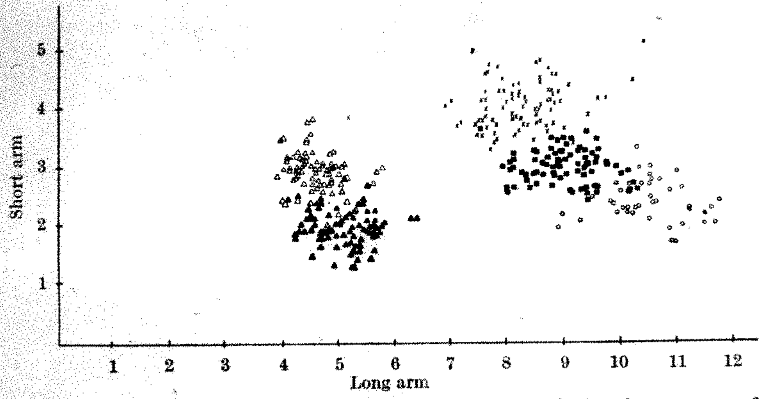

Fig. 2. Plot of short-arm versus long-arm measurements of the chromosomes of *Protemnodon bicolor*. Arm-length is expressed as a percentage of the total chromosome length in the cell. Crosses, chromosome 1; squares, chromosome 2; open triangles, chromosome 3; closed triangles, chromosome 4; circles, *X* chromosome

to 0·5 mm. Arm-length was taken as the sum of the chromatid lengths.

The Y_1 chromosome entering metaphase showed marked positive heteropyknosis and was, therefore, excluded.

The data were examined to find some criteria of identification the errors of which had a recognizable pattern. Initially, observed shorter versus longer arm-lengths were plotted, length being expressed as a percentage of total chromosome length per cell (Fig. 2).

The tendency for the results from each chromosome to cluster round some mean value is very noticeable. Identification with a high degree of confidence is still possible even in regions of overlap by considering the relative position of points corresponding to other chromosomes in the cell.

The larger chromosomes appear more variable than the smaller ones, and there is a tendency for the observations to be scattered in the shape of a sector of a circular annulus. That is, the two arm-lengths did not vary independently, but showed a weak negative correlation, presumably due to errors in determining the position of the centromere.

There appear to be two sources of variability-location of the centromere and total length of the chromosome. This suggests consideration of two parameters—the angle subtended at the origin by the plotted points (which, unlike the arm ratio, has the advantage of symmetry in the two arm measurements) and total chromosome length. These two variables were uncorrelated for each chromosome, indicating that the sources of variability are independent.

For any chromosome the following variables are defined: y = length of short arm as a percentage of total chromosome length in the cell; x = length of long arm as a percentage of total chromosome length in the cell; $\theta = \text{artan}\ y/x$, expressed in radians.

The data on the wallaby chromosomes are summarized in Table 1.

The wallabies are numbered in the order in which the measurements were made. The measurements on the second wallaby tend to be less variable, due, it is felt, to improvement in technique and greater discrimination in selection of material, and consequently rather more emphasis should be placed on the reliability of observations on the second wallaby.

The decrease in the variability of $x+y$ as the average value of $x+y$ decreases suggests a logarithmic transformation[10] to stabilize the variance.

If $z = \log\ (x+y)$, the standard deviations of z show greater stability and there is no relationship between mean and standard deviations.

The relationship between z, θ, x and y is shown in Fig. 3. The assumption is now made that the variability in the measurements on human chromosomes is governed by the same laws and can be analysed by the same procedures and hence that in a sample of sufficient size the variances of z and θ will be constant for all chromosomes.

Analysis of human chromosomes. 36 and 22 metaphase plates respectively from the peripheral blood of 2 male subjects were photographed under a Leitz 90 times objective and printed at a final magnification of 5,300. Measurements were made as before on pairs (1) and (2) and the largest and smallest pairs, arranged in order of length irrespective of arm ratio, of the 6–12 + X group (Denver classification).

Short-arm versus long-arm plots are shown in Fig. 4.

One immediate conclusion was that in the largest chromosome the shorter measurement does not always correspond to the same arm of the chromosome, hence the truncation of the region of variability along the 45° line, suggesting that the true value of the arm ratio is nearer 50 per cent than has previously been claimed. This has the effect of biasing the estimate of θ towards a lower value, although the variability of z is not affected.

Table 2 summarizes the data on human chromosomes. Chromosomes 1 and 2 exhibit stability in the means and standard deviations of their z values, and in the means of the θ values, but the standard error of θ for chromosome 1 is significantly lower due to the truncation mentioned

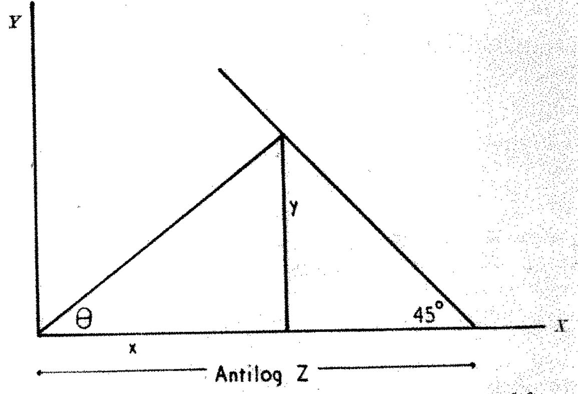

Fig. 3. Relationship between the parameters x, y, z and θ

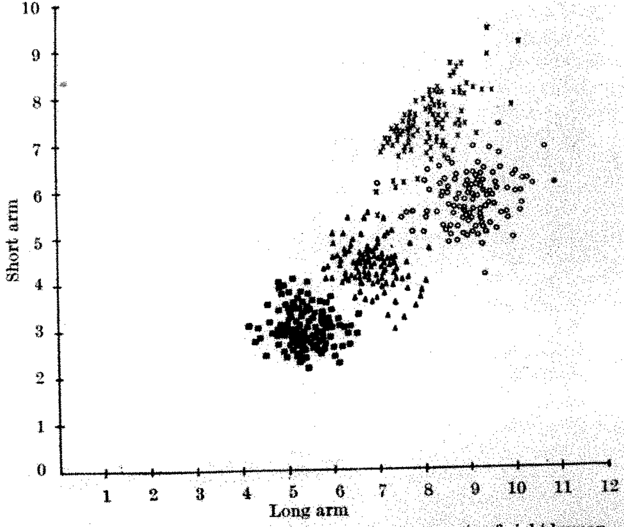

Fig. 4. Plot of short-arm versus long-arm measurements of eight human chromosomes. Arm-length is expressed as a percentage of the total length of the eight chromosomes measured. Crosses, chromosome 1; circles, chromosome 2; triangles, the largest two chromosomes of the 6–12 + X group; squares, the smallest two chromosomes of the 6–12 + X group

Table 1

Chromosome	1		2		3		4		x	
Wallaby	1	2	1	2	1	2	1	2	1	2
Average $x + y$	12·19	12·41	11·96	12·04	7·41	7·36	6·90	6·98	12·97	12·43
Standard deviation of $x + y$	1·02	0·71	0·65	0·53	0·45	0·44	0·59	0·41	0·61	0·69
Average z	1·084	1·093	1·077	1·080	0·869	0·866	0·836	0·842	1·112	1·094
Standard deviation of z	0·037	0·024	0·024	0·019	0·026	0·026	0·038	0·026	0·021	0·024
Average θ	0·451	0·435	0·307	0·301	0·537	0·544	0·344	0·315	0·214	0·201
Standard deviation of θ	0·049	0·040	0·039	0·036	0·071	0·054	0·058	0·055	0·041	0·033

Table 2. CHROMOSOME NUMBER

	(1) Subject			(2) Subject			max. (6)–(12) + X Subject			min. (6)–(12) + X Subject		
	A	*B*	*A* and *B*	*A*	*B*	*A* and *B*	*A*	*B*	*A* and *B*	*A*	*B*	*A* and *B*
Sample size	72	44	116	72	44	116	72	44	116	72	44	116
Average z	1·195	1·186	1·191	1·171	1·171	1·171	1·041	1·054	1·046	0·925	0·922	0·924
Standard deviation of z	0·032	0·033	0·032	0·029	0·031	0·030	0·026	0·022	0·025	0·038	0·029	0·035
Average θ	0·738	0·744	0·740	0·566	0·569	0·567	0·568	0·558	0·564	0·521	0·519	0·520
Standard deviation of θ	0·033	0·033	0·033	0·054	0·043	0·050	0·068	0·059	0·065	0·082	0·077	0·080
Correlation (z,θ)	—	—	—	0·098	− 0·245	—	0·082	0·191	—	0·170	0·275	—

here. The observations on the largest of the 6–12+X group indicate that the same chromosome has not been measured throughout. The criterion of selection was length, and the decreased standard deviation of z is consistent with the statistical theory of the extreme value of a sample[11]. The same conclusion can be drawn for the smallest pair in the group. In both cases, the increased standard derivation of θ indicates a mixed population. The two smallest chromosomes of the group have distinct arm ratios but similar lengths[12] (Fig. 5). The standard deviation of z in the smaller sample is approximately the same as for chromosome 2, but that of θ is larger.

Correlations between z and θ were not significant at the 5 per cent level for each subject and chromosome measured. This computation was not carried out for chromosome 1 owing to the unreliability of the estimate of θ.

In computing z values for Fig. 5, the lengths relative to the total lengths of the eight chromosomes measured have been taken. The more customary measure is the length relative to the total length of the 22 autosomes plus the X chromosome. The effect of the restricted measure is to add a constant to the mean, and to change the standard deviation. The size of this change depends on the relative size of the chromosome under consideration and the chromosomes measured. In the present case it can be calculated that the standard deviation of z for chromosome 2 relative to the complete haploid set is underestimated by approximately 6 per cent. Values of θ are unaffected by the scale of measurement of length. Correcting by the data published by Levan and Hsu gives relative lengths of 8·1 and 7·8 per cent for the first and second chromosomes respectively.

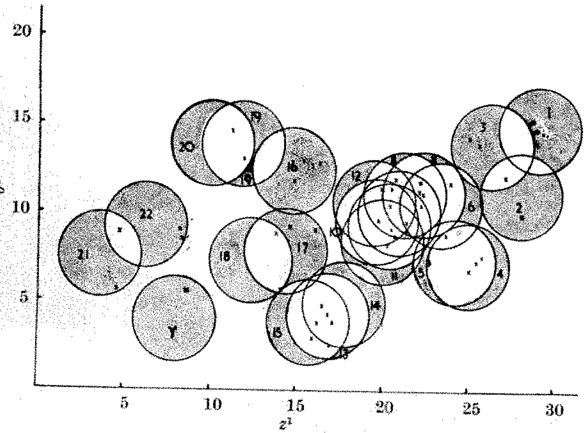

Fig. 5. Plot of z^1 versus θ^1 for the 22 human autosomes and the X and Y chromosomes, using the mean values of Levan and Hsu (ref. 12). The circles represent the boundaries of the 95 per cent tolerance regions. Shaded areas are those regions in which a value can be identified unequivocally. The crosses represent values of z^1 and θ^1 calculated from measurements of a single male metaphase cell

As chromosome 1 (and, to a certain extent, chromosome 3) will give a spuriously low standard deviation of θ, the standard deviation of chromosome 2 (0·50) is taken. The value of 0·032 for the standard deviation of z is found by taking the value from Table 2 for both subjects and correcting for using a limited sample. The magnitude of this standard deviation is a measure of the efficiency of one of us in taking the observations, and relates only to that individual. The smaller the value, the more accurate will be the identification procedure, but over-scrupulous selection of material to reduce errors in those chromosomes which can be identified is no assurance that the standard deviation of other groups will be similarly reduced.

The assumption of the normality of errors in z and θ permits the definition of tolerance regions for observed values about some assumed mean value for each chromosome. If the scales are corrected so that $z^1 = z/0{\cdot}32$ and $\theta^1 = \theta/0{\cdot}050$, z^1 and θ^1 are independent normal values with unit variance and the tolerance regions are circles centred at the mean value. For 95 per cent and 99 per cent confidence the circles will have radii of 2·45 and 3·03 respectively. These are the square roots of the corresponding percentage points of a chi-square distribution with 2 degrees of freedom.

Taking the mean values of Levan and Hsu, this gives the 95 per cent tolerance regions depicted in Fig. 5. In fact, as these authors have pointed out, individual means within a group are probably displaced outwards from their true value.

The difficulties of identification are depicted in Fig. 5. The shaded regions denote areas of the (z^1 θ^1) plane which are contained in one tolerance region only, and there would be cause to allocate a chromosome the point of which lay in such a region to the corresponding number. The remaining interior points would lie in the overlap of 2 or more regions and if possible could be allocated by default. But with present techniques there seems no possibility of allocating within particular groups.

An interesting feature of the diagram is that there are regions within the general regions of dispersion where points are unlikely to be found. An observation in one of these regions suggests an abnormal chromosome.

Examination of further cells from the same individual could confirm the deduction. If an abnormality had the effect of transforming the appearance from one group to another, this again would be discovered from the diagram.

It would obviously be advantageous if measurements could be reckoned on the basis of comparison with the autosome set only, as this would mean that male and female could be referred to a common plot As the X chromosome cannot be identified this is impossible, but the sexes can be compared if a correction factor is applied to measurements from one sex to reduce the estimated z values for chromosomes 1, 2 and 3 to a common value Similarly a correction value could be calculated for any abnormal idiogram with a gross change in total chromosome length, for comparison with normal chromosomes In the case of many abnormalities this correction would be small and could be ignored

It is suggested (1) That before any claim that an aberrant chromosome has been found is substantiated, the chromosome complement should be measured and the measurements specified, as the presentation of individual idiograms can be misleading owing to normal variations

(2) That the exercise undertaken by many authors of pairing off chromosomes within a group is unnecessary and can be misleading and that the recommendation by the Denver study group that the X chromosome be separated from the 6–12 group is inadvisable

(3) That the presence of any sporadic and unique chromosome aberrations (for example, after irradiation) can be confirmed only by a system of allocation like that described here Admittedly many aberrations will not be detected, but the practice of identifying them only by eye, although in fact depending on the same method, has no value, as different workers may vary considerably in acuity of visual perception and powers of self-persuasion

Note added in proof Since submitting this paper for publication, we have examined the chromosomes of some other marsupial species, and find that, for short chromosomes, both z and θ errors are increased In the human chromosomes 19–22 and the Y chromosome will have larger errors for z and θ than chromosomes $1\text{–}18+X$ This difference will be discussed in greater detail elsewhere[12]

We thank the Victorian Department of Fisheries and Wild Life, which provided the animals for this investigation, and Mrs J Uren, Misses B Lady, M Byrne and V Ferguson for assistance

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EFFECT OF POSTERIOR PITUITARY EXTRACTS ON THE LIFE-SPAN OF OLD RATS

By Prof SYDNEY M FRIEDMAN and Prof CONSTANCE L. FRIEDMAN
Department of Anatomy, University of British Columbia, Canada

INVESTIGATIONS in the rat and in man have consistently shown a disturbance in salt and water metabolism with age Detailed analysis of electrolyte partition in muscle in the old rat has shown that there is a gain of sodium both in cells and in their fluid environment, a corresponding loss of potassium from cells, and a shift of water from cells to environment[1] This type of salt and water distribution is also characteristic of a preponderance of adrenal mineralo-cortical activity in relation to the concurrent level of neurohypophysial function[2] In this relation, adrenal cortical activity may be subnormal and still dominant if neurohypophysial function is ever lower The failure of an intracarotid injection of hypertonic sodium chloride to elicit an appropriate antidiuretic effect in the old rat indicates that a decline in neurohypophysial responsiveness[3] or effectiveness[4] does, in fact, obtain More recently, we have found that in such animals vasopressin tends to restore salt and water distribution towards normal, and, in association with this, improves their ability to perform work[5] The effectiveness of vasopressin is enhanced by the concurrent administration of a trace of aldosterone, suggesting that adrenal cortical function is also sub-normal in age

Since the metabolism of salt and water plays so critical a part in the body economy, an improvement in the old rat sufficient to affect work performance might well be expected to produce a demonstrable effect on life-span The experiments recorded here were designed to test this Because the experiments were necessarily long and our colony of aged rats limited, these first investigations were confined to two posterior pituitary extracts only Pitressin tannate in oil was selected as one test extract because of our previous experience of its effects and because of its sustained action Whole posterior pituitary powder (U S P , Armour) suspended in saline by sonic agitation (3 mg/ml) was selected as the second test extract to provide more complete neurohypophysial replacement This latter extract was freshly prepared each week, and its potency assessed in terms of its pressor activity in the rat

Male rats of an inbred Wistar strain 24 months old at the start of the experiment were used In this colony, rats of this age are clinically old and usually few survive more than another 6–8 months Two experiments were carried out In the first, 3 matched groups of 15 old rats were set out in individual cages One group remained untreated, a second received pitressin tannate and the third received posterior pituitary powder in suspension The treated rats were given 100 mU/100 g three times weekly in either case This dose corresponds to 50 mU/100 g/day which is the approximate replacement requirement for the rat with diabetes insipidus[2] In the second experiment, 2 matched groups of 16 were set out, the first to remain untreated and the second to receive 75 mU/100 g of pitressin tannate plus the same amount of posterior pituitary powder thrice weekly The experiments were terminated arbitrarily, the first after 7 months, the second after 6 months In both, a control group of 12 young rats, 4 months of age at the start, were maintained alongside the old groups Since nothing untoward occurred in these young groups and all survived the experiments in good condition they will not be considered further No special or individual care was given to any animal in these experiments

Although obviously not objective, the weekly technician's reports, especially during the first 3 months of treatment, directed attention to the healthier appearance of animals receiving the powder suspension The actual findings for the old animals are presented in Fig 1 Objectively, posterior pituitary powder decreased the mortality rate during an effective period of approximately 4 months By contrast, only one mortality rate operated for both the untreated and pitressin-tannate-treated groups, and there is even a small suggestion that the

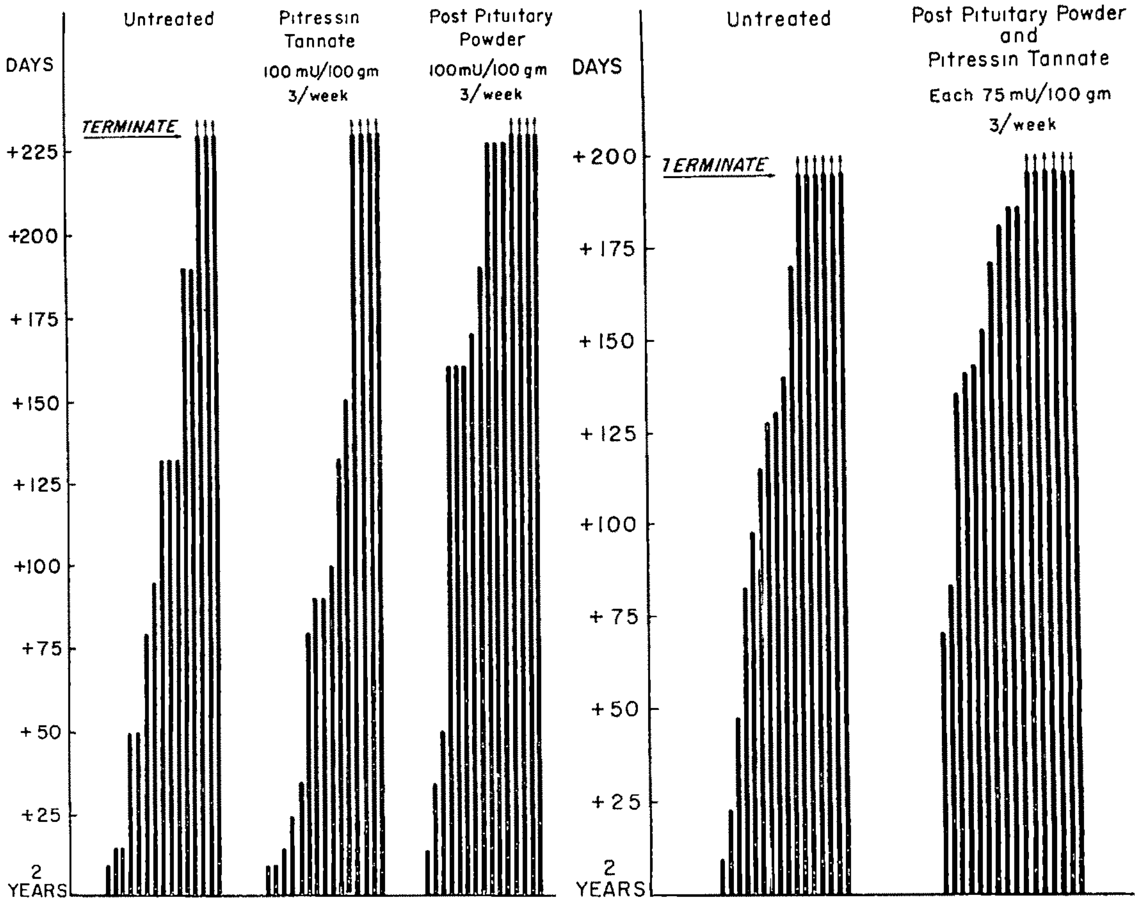

Fig. 1. Life-span in old rats treated with pitressin tannate and/or with posterior pituitary powder compared with untreated controls. Each animal is indicated by a bar and survivors at the time of termination by arrows. Experiments begin with 2-year-old animals

prolonged use of pitressin tannate may have been harmful. The beneficial effect was sharpest in the first experiment in which the larger dose of posterior pituitary powder and no pitressin tannate was used. Here the number of deaths in the untreated group 1 exceeded ($P < 0.012$) those in the treated group 3 by the 135th day of the experiment and this difference was maintained for almost one month. In the second experiment, a similar absolute significance, that is, survivors/deaths, was reached at the 130th day ($P < 0.026$) but was quickly lost. The concordance of the two experiments, of course, heightens the overall significance of the observations. Although a definite logic dictated the use of whole posterior pituitary powder, we must bear in mind that the results obtained with such a crude material might be due wholly to unrelated causes.

Since treatment with a suspension of whole posterior pituitary powder, imprecise as it was, temporarily reduced the death rate almost to zero over a span of approximately 3 months, more precise treatment should narrow the gap between average and longest individual life-span still further. The addition of a trace of aldosterone, for example, is indicated and an investigation of this has now begun. Certainly these, as well as previous beneficial results of therapy directed at the neurohypophysial–adrenal cortical integration, show that at least some of the phenomena associated with senescence can be delayed. The question of how long such a delay can be maintained remains open.

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AGE RESISTANCE BY TURKEYS TO *EIMERIA MELEAGRIMITIS* TYZZER, 1929

By E. W. WARREN, S. J. BALL and JOAN R. FAGG
Veterinary Pesearch Station, May and Baker, Ltd., Ongar, Essex

ACQUIRED immunity to *Eimeria* spp. is dependent on previous infection in both chickens and turkeys. However, turkeys also show an innate immunity, age resistance, to infections of *Eimeria meleagrimitis*[1] and *E. adenoeides*[2,3]. This phenomenon is not shown by chickens[4-6]. So far, age resistance in turkeys has been demonstrated by showing that infections which are pathogenic to young poults are not pathogenic to older birds.

On inspection of recent results obtained in this laboratory in an attempt to obtain a clearer picture of this host–parasite relationship, the correlation between the

Table 1. Showing the Mean Number of Oocysts given and passed for Each Sub-group

Group*	Age at infection (days)	Sub-group	Mean weight (g)	Mean absolute No. of oocysts given	Mean No. of oocysts given/g body-weight	Mean No. of oocysts passed as millions per bird
A	8	1	79·2	7,920	100	152·87
		2	82·2	23,280	288	145·30
B	22	1	232·8	23,280	100	305·12
		2	238·8	60,280	255	192·78
C	36	1	602·8	60,280	100	337·97
		2	589·5	119,440	203	362·07
D	50	1	1,194·4	119,440	100	220·16
		2	1,208·4	205,100	171	24[illegible]·75
E	64	1	2,051	205,100	100	344·85

* Uninfected control groups not included.

numbers of oocysts passed per oocyst given (reproductive index[7]) and age seemed to offer a useful criterion of resistance particularly when the number of oocysts given per gram body-weight was fairly constant.

To examine this hypothesis the following experiment was performed. At 14-day intervals day-old poults of either sex were obtained from a commercial hatchery and maintained free of extraneous infection as evinced by fæcal examination[8] twice a week, until 8 days (group *A*), 22 days (group *B*), 36 days (group *C*), 50 days (group *D*) and 64 days (group *E*). All the birds were then weighed and selected into groups by the method of Gardiner and Wehr[9], the main groups *A*–*D* having three sub-groups and main group *E* having two sub-groups. There were 4–5 poults in each sub-group.

All birds of sub-groups *A*1–*E*1, the experimental series, were given, orally, 100 sporulated oocysts of *E. meleagrimitis* (the same strain as used previously by Ball and Warren[10]) per gram body-weight according to the scheme shown in Table 1. Control sub-groups *A*2–*D*2 received the average number of oocysts given to the sub-group in the experimental series, which was 14 days older than itself. The oocyst dose of 100 per gram was selected in an attempt to minimize any 'crowding effects'[11,12]. The control groups, sub-groups *A*3–*D*3 and *E*2 were the uninfected controls.

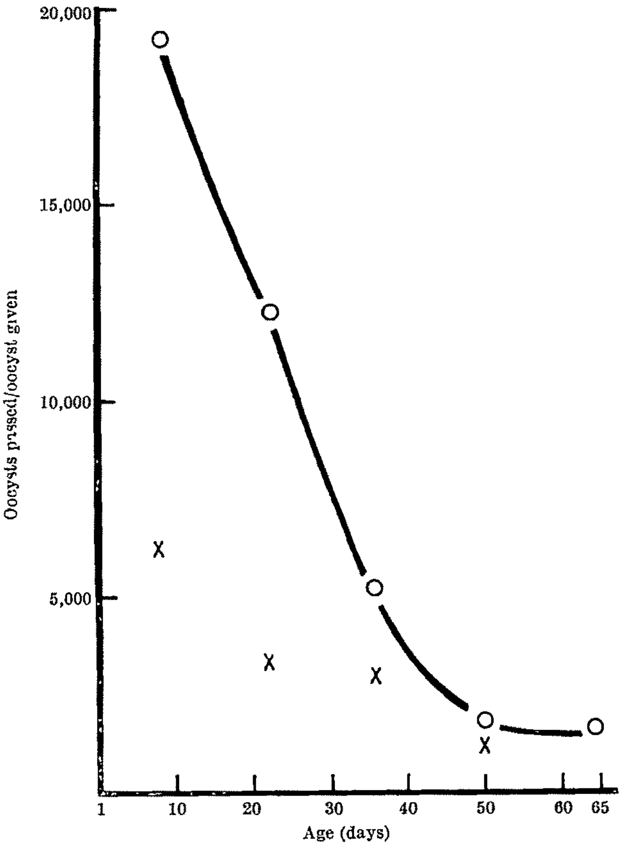

Fig. 1. Relationship between the numbers of oocysts passed per oocyst given and the age of turkey poults infected with *E. meleagrimitis*. O, Means for sub-groups 1; ×, means for sub-groups 2.

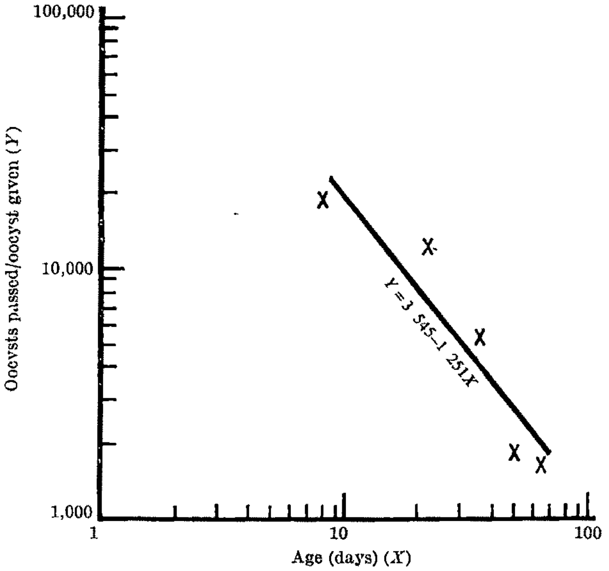

Fig. 2. The relationship (plotted on a log scale) between the numbers of oocysts passed per oocyst given and the age of turkey poults given 100 sporulated oocysts of *E. meleagrimitis*/g body-weight.

The poults were housed individually in wire-floored cages, in one of two rooms with continuous artificial lighting and heated according to their requirements. Unmedicated food and water were given *ad lib*. From four days after infection total oocyst counts[8] were determined at 24-h intervals for each individual poult until thirteen days after infection, when oocyst production ceased. No oocysts were seen in the fæces of any of the uninfected control groups throughout the test. The results are shown in Table 1 and are plotted in Fig. 1, as age against oocysts passed per oocyst given. Sub-groups 1 are comparable on the basis of a constant number of oocysts given per gram. Sub-groups *A*2 and *B*1, *B*2 and *C*1, *C*2 and *D*1, and *D*2 and *E*1 are comparable on the basis of total numbers of oocysts given.

Age resistance is shown in sub-groups 1 by the fact that the reproductive index decreases with age. If there were no age resistance, a horizontal line would be expected in Fig. 1, and this was obtained with a similar type of experiment using *E. acervulina* infections in chickens, for which age resistance has not been postulated.

The graph for sub-groups 1 (Fig. 1), showing the mean number of oocysts passed per oocyst given plotted against age in days suggests a curved relationship of the type

$$y = ax^{-b}$$

where y represents the mean number of oocysts passed per oocyst given and x represents age in days.

Transformed using logarithms (Fig. 2), this gives a straight line of

$$Y = 3{\cdot}545 - 1{\cdot}251X$$

the slope of which is significantly different from zero at $P < 0{\cdot}02$.

We conclude from this that given a constant number of oocysts per gram, the log number of oocysts passed per oocyst given is inversely related to log age.

No relationship could be found within the sub-groups for either the reproductive index or the total number of oocysts passed when plotted against weight.

Despite the older birds receiving fewer oocysts per gram than the younger birds, a similar relationship (Fig 1) was found to hold for sub-groups 2 as for sub-groups 1 If age resistance did not exist then it would be expected that the reproductive index for sub-group *A*2 would be the same as for sub-group *B*1 as they both received the same absolute number of oocysts, and similarly for sub-groups *B*2 and *C*1, *C*2 and *D*1, and *D*2 and *E*1 However, in each case except for sub-group *C*2, the sub-groups 1 are above their corresponding sub-groups 2 receiving the same number of oocysts If age resistance were the only factor affecting the results of these comparable groups, then the reproductive index for sub-group 1 would be less than that of their sub-group 2, but this is not so This could be due to a 'crowding effect'[11,12] within the gut This possibility is further supported by the fact that, although the falling off of the reproductive index with age of the sub-groups 2 is similar to that for sub-groups 1, it is less marked, which is probably due to the decreasing numbers of oocysts given per gram with age, so that the effects of 'crowding' become less in the older birds Presumably an arbitrary inoculum of 100 oocysts per gram is too many to avoid overcrowding using this experimental design and fewer oocysts per gram might avoid this

Inspection of previous results with *E adenoeides* suggests that the above relationship may also hold for this species, but a test designed to examine this has not been attempted

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ROLE OF NON-COVALENT BONDS FOR THE HOLDING, ACTIVATION AND RELEASE OF MITOCHONDRIAL ENZYMES

By E EGGER and Prof. S RAPOPORT

Physiologisch-Chemisches Institut, Humbolt-Universitat, Berlin

WORK by us on latency, activation and release of mitochondrial enzymes has led us to a general conception of the relationship between their holding and activity The main points are as follows

(1) The enzymes are 'held' in the mitochondrion, that is, fixed by various bonds in the mitochondrial structure

(2) Release of an enzyme is brought about by the breaking of non-covalent bonds If such bonds mask the active centre of an enzyme, the phenomenon of latency is observed; activation is the breaking of the masking bonds

(3) Two types of mitochondrial enzymes may be distinguished (*a*) Non-sessile enzymes, held by weak bonds, which may be broken by freezing and thawing Thereby such enzymes are released and, if previously latent, activated (*b*) Sessile enzymes, which are held by additional types of bonds Activation of such enzymes is caused by breaking masking bonds of the first type

(4) Release and activation of enzymes are independent of mitochondrial swelling

Examples of activation and release of some mitochondrial enzymes are shown in Tables 1*a* and *b*

It would appear that all types of non-covalent bonds, which occur in the protein molecule, may also play a part in the holding and masking of mitochondrial proteins This is supported by our observations that the same conditions which affect the predominant breaking of such bonds also affect the mitochondrial enzymes

(*a*) *Role of imidazole bonding* The release of any $NADH_2$-cytochrome-*c*-oxydo-reductase from the mitochondria of rat liver and the conversion of the enzyme in a soluble diaphorase depend on the breaking of two imidazole bonds[1,2], which are responsible for the holding of the enzyme in the mitochondrion The breakage is caused by the ionization of the imidazole group This conclusion was based on *p*H-dependence and heat of reaction inactivation of the enzyme The *p*H-dependence of the activation of mitochondrial malic dehydrogenase follows an analogous course (Fig 1*A*) Liberation of mitochondrial proteins shows likewise similar *p*H-dependence[3] (Fig 1*B*)

The remarkable correspondence in the *p*H-dependence in all three types of processes would suggest that the imidazole bonds, which may be hydrogen bonds, are of general importance for holding and masking mitochondrial enzymes.

Table 1*a* ACTIVATION AND RELEASE OF ENZYMES BY FREEZING AND THAWING RAT LIVER MITOCHONDRIA

Enzyme	No of exp	Activity*		Final activity†	Activity in the final supernatant‡
		Fresh mitochondria	After freezing and thawing		
Malic dehydrog	2	1092 ± 60	4356 ± 108	400 ± 12	90 6 ± 4 3
Glutamic dehydr	3	38 9 ± 13 7	95 5 ± 28 1	253 ± 32	86 2 ± 7 4
Isocitric deh (NADP-dependent)	3	8 6 ± 3 1	181 ± 63	2126 ± 440	74 7 ± 9 6
β-Hydroxybutyrate-dehydrogenase	2	83 5 ± 13 0	227 ± 28	274 ± 9	0 7 ± 0 5

* In μmol/g liver/h

† In per cent of the initial activity

‡ Per cent of total final activity

The mitochondria were suspended in 0 16 M sodium chloride and frozen at − 40° C

Table 1*b* ACTIVATION AND RELEASE OF ENZYMES BY FREEZING AND THAWING RAT LIVER MITOCHONDRIA IN SUCROSE AND SALINE

Enzyme	Mitochondria suspended in	Activity/mg protein		Final activity*	Activity in the final supernatant†	
		Fresh mitochondria	After freezing and thawing			
Catalase‡	0 30 M sucr	427	641	150	79	*P* < 0 05
	0 16 M NaCl	521	741	142	105	*P* < 0 05
Rhodanese§	0 30 M sucr	614	586	96	19	*P* > 0 25
	0 16 M NaCl	550	800	145	92	*P* < 0 01

* In per cent of the initial activity

† In per cent of the total final activity

‡ Activity in arbitrary units, following the method of Adams (ref 6)

§ Activity in 10^{-3} μmol SCN^-/min

Mitochondria were frozen at − 40° C Statistical significance of activation was proved by variance analysis, probabilities of error are given in the last column

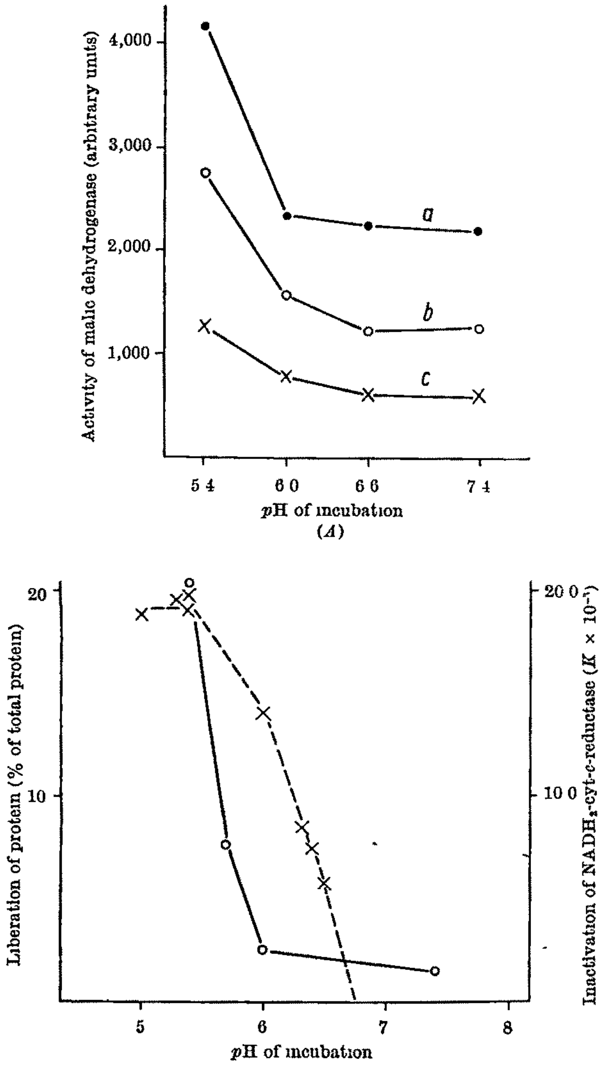

Fig 1 (A) pH-dependence of activation and liberation of mitochondrial malic dehydrogenase Incubation of mitochondria suspensions (a) 180 min at 25°, (b) 48 h at 0°, (c) activity in the supernatant of (b) (B) pH-dependence of liberation of mitochondrial proteins (ref 10) (full line, left scale) Mitochondria were incubated at 37° in 0 1 M phosphate buffer Values are the percentage of total mitochondrial proteins, released between 30 and 270 min of incubation For comparison rate of inactivation of the mitochondrial NADH$_2$-cytochrome c-oxydo-reductase at 0° is also shown (broken line right scale, the values are rate constants)

(b) *Peptide-hydrogen bonds* The importance of such bonds is suggested by the influence of urea In Fig 2a it is shown that activation and liberation of NADP-dependent isocitrate dehydrogenase of rat liver mitochondria by freezing and thawing may be enhanced by urea in low concentrations[4] In other work it has been shown that the latent NADP-dependent isocitric dehydrogenase of rabbit reticulocytes may be activated and liberated by incubation with urea[5] (Fig 2b) Liberation of mitochondrial proteins by freezing and thawing is likewise greatly increased by dilute urea solutions[4] (Fig 2c).

(c) *Electromagnetic interactions and hydrophobic bonds* The importance of these types of bonds is based on several observations Liberation of proteins, as well as activation and release of enzymes, is much greater in an ionic, as compared with a non-ionic, medium The effect of freezing and thawing depends on both the temperature and the duration of storage in the frozen state (Fig 3) Here also are differences between ionic and non-ionic media In sodium chloride the liberation of protein increases with lower temperatures, with sucrose the reverse is the case

It may be assumed that this effect is caused by the interaction of polar and hydrophobic bonds The stability of hydrophobic bonds, which are stabilized by entropy effects[7,8], decreases with lower temperatures, which may be the explanation for the effect of freezing and thawing

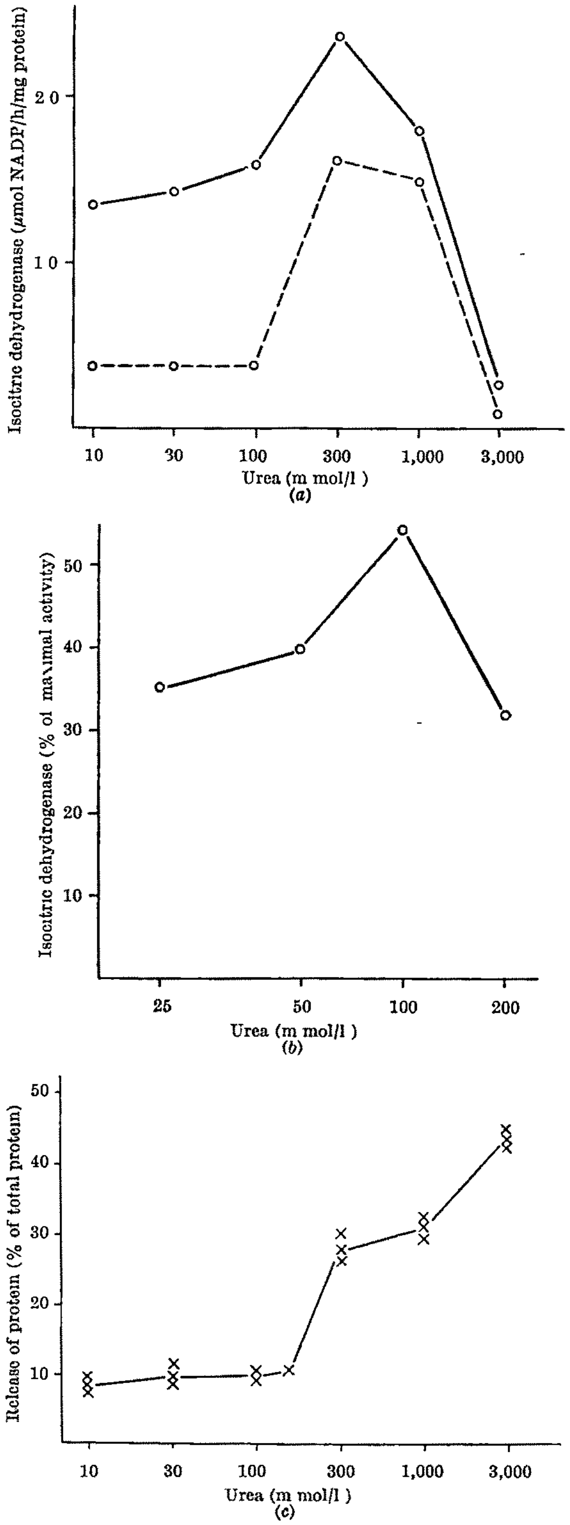

Fig 2 (a) Effect of urea on activation and liberation of NADP-dependent isocitric dehydrogenase from rat liver mitochondria by freezing and thawing at −36° Upper curve, activity of the suspension, lower curve, activity of the supernatant
(b) Effect of urea on the activation of NADP-dependent isocitric dehydrogenase in osmotic hæmolysates of rabbit reticulocytes by short-time incubation at 25°
(c) Effect of urea on the release of proteins from rat liver mitochondria by freezing and thawing at −36°

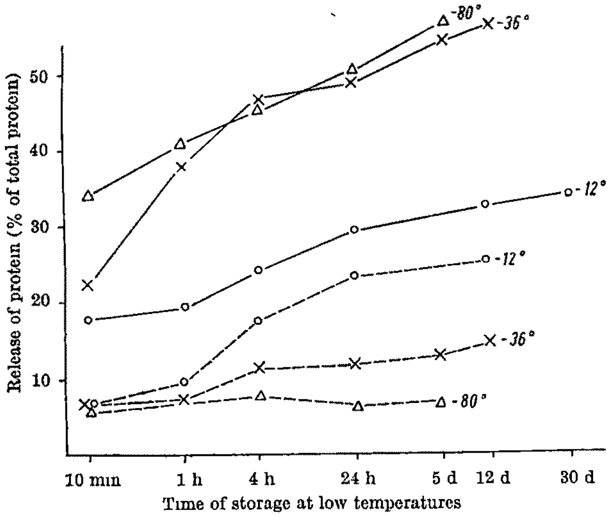

Fig 3 Effect of temperature and duration of storage in frozen state on the release of proteins from rat liver mitochondria in saline and in 0 25 M sucrose (ref 11)
——, 0 16 M sodium chloride, – – –, 0 25 M sucrose

The greater release in salt media is presumably caused by the breaking of both polar and hydrophobic bonds The fact that maximal liberation in salt media requires extremely low temperatures suggests a certain stabilization of the hydrophobic bonds by the ionic medium However, kinetic effects and modifications of the structure of ice may play a part An explanation on the basis of mechanical disruption of the mitochondrial membrane by ice crystals is difficult to imagine

Breakage of each type of bond may cause activation and release of a mitochondrial enzyme, greater liberation of proteins is observed when several effects are combined (for example, urea plus freezing and thawing, salt plus freezing and thawing) This speaks in favour of the co-operation of various bonds in masking and holding of the mitochondrial enzymes Such bonds may be of importance for the sessile, as well as the non-sessile, enzymes in the membrane as well as in the matrix of mitochondria

Mitochondrial swelling, although sometimes concurrent with activation or release of enzymes, may be shown to be an independent process (Fig 4) Usually it has a lower time constant[9]

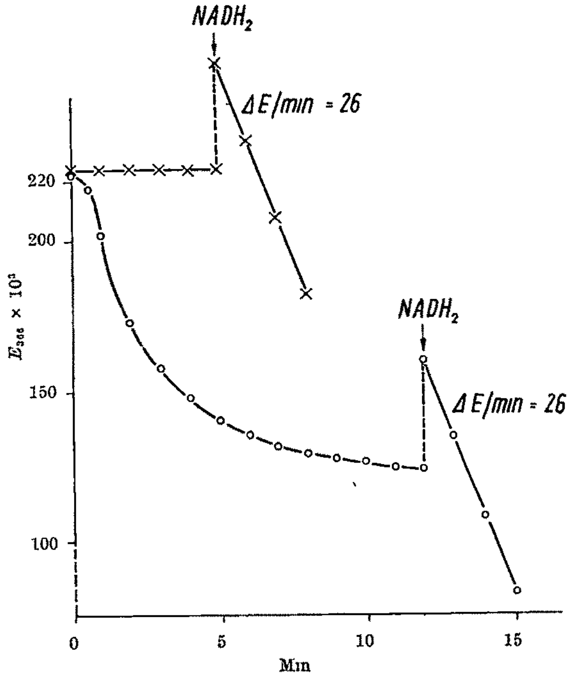

Fig 4 Activity of malic dehydrogenase in swollen and unswollen rat liver mitochondria Swelling is demonstrated by decrease of optical density before addition of NADH₂ Strong swelling results with phosphate, no swelling with salicylate (ref 12) Salicylate medium, sucrose 250, *tris* buffer *p*H 7 4 25, sodium salicylate 5, oxaloacetate 1, phosphate medium, sucrose 250, *tris* buffer *p*H 7 4 25, phosphate buffer *p*H 7 4 25, oxaloacetate 1 (m mol/l in the final test mixture)
×, Salicylate medium, O, phosphate medium

The question of permeability of the mitochondrial membrane will be considered elsewhere

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FLUORESCENCE OF STYRENE HOMOPOLYMERS AND COPOLYMERS

By Dr S. S YANARI and Dr F A. BOVEY*

Central Research Department, Minnesota Mining and Manufacturing Co , St Paul, Minnesota

AND

Prof. R. LUMRY

Department of Chemistry, University of Minnesota, Minneapolis

THERE have been no fundamental investigations of the fluorescence of synthetic high polymers in solution, although polymer fluorescence has received some attention as a complicating factor in light-scattering measurements[1] The fluorescence of synthetic polymers, particularly polystyrene, in the solid state has been examined chiefly in connexion with their performance in scintillation devices, in which the polymer serves as solvent and energy donor[2] We have undertaken an investigation of the fluorescence of styrene homopolymers (both isotactic and 'atactic') and copolymers in dilute solution in the hope of obtaining new information concerning both the transfer of energy in large molecules and the conformations of synthetic polymers We have also carried out a very limited investigation of the fluorescence of films of these polymers The results of the first part of this work are reported here

In order to observe primarily those effects due to energy transfer within, rather than between, polymer molecules, we used dilute polymer solutions, ranging from 0 002 per cent for polystyrene (about 2 × 10^{-4} M in styrene units) to 0 015 per cent for the copolymer containing the lowest proportion (16 mole per cent) of styrene These dilute solutions also have the decided advantage of having

* Present address Bell Telephone Laboratories, Inc , Murray Hill, New Jersey

optical densities no higher than about 0.05 at the absorption maximum, thereby assuring that the exciting beam has a nearly uniform intensity within the sample cell. Commercially available fluorescence spectrometers are unsuitable for the measurement of dilute solutions of simple benzenoid compounds, and therefore, with the assistance of Mr Dale Burling (University of Minnesota), we have constructed a highly sensitive apparatus suitable for the examination of substances which absorb below 270 mμ and have very low fluorescence quantum yields. Briefly, it consists of a high-pressure xenon arc, the light from which enters an excitation monochromator after being chopped mechanically at 150 c/s. Monochromatic light is focused on a 1-cm square quartz sample cell held in a rotatable hollowed turret through which water is circulated to maintain a temperature of 20.0 ± 0.1°. The fluorescence is analysed at right angles to the exciting beam by an exit monochromator, the output of which is focused on a 1*P*28 photomultiplier. The signal from the photomultiplier is fed to a two-stage a.c. amplifier peaked for 150 c/s. Apparent excitation and fluorescence spectra are recorded by synchronized wave-length and recorded chart drives. Spectra were corrected for the combined effects of the wave-length response of the photomultiplier and attenuation of light by the analysing monochromator. Quantum yields were calculated from the areas of the corrected fluorescence spectra, tryptophan in aqueous solution being employed as a quantum standard[3].

The choice of suitable solvents is severely limited by the solubility of the polymers and by the necessity of being essentially non-absorbing and non-fluorescent from 250 to 350 mμ. *Cyclo*hexane (Eastman Kodak 'Spectro' grade) and 1,2-dichloroethane (Matheson 'Spectroquality') were found to be satisfactory. Most of our work was done in the latter.

Polystyrene, assumed to be 'atactic' or nearly so, was prepared in carbon tetrachloride, using stannic chloride as catalyst[4]. The molecular weight was low (*Mv* approximately 2 × 10^3) (ref. 4), facilitating solution in a variety of solvents. Isotactic polystyrene was obtained from Montecatini. The preparation of polymethyl methacrylate and the styrene methyl methacrylate copolymers has been described[5]. The polymethyl methacrylate showed only an extremely weak fluorescence, such as might be expected from aromatic end groups arising from the benzoyl peroxide initiator. It is probable that the fluorescence behaviour of our polymers was not significantly influenced by the terminal groups due to initiator. Although these groups might quench benzene fluorescence, they are present in very low concentration.

Typical fluorescence-emission spectra are shown in Fig. 1 which also contains the near-ultra-violet absorption spectra of solutions of 'atactic' polystyrene and isotactic polystyrene. Toluene, the two forms of pure polystyrene polymer and the copolymers were found to give substantially identical absorption spectra. The emission spectra of toluene and the 'atactic' polymer were identical within error. The most striking feature of the emission spectra is the appearance of a new band at 324 mμ in the isotactic polystyrene spectrum. This same band, but peaked at 320 mμ, is found in concentrated solutions of toluene or benzene and in the pure liquids, but not in *p*-xylene[6]. These substances show concentration-independent absorption spectra and the new band in toluene and benzene has been attributed to a dimeric species formed between an excited phenyl ring and one in the ground-state[7]. This species is called an excimer. It does not appear, however, that proof of the existence of this species has been given, at least for benzene and toluene. Since our polymers have identical absorption spectra, the new band might be due to excimer formation. For convenience, we shall henceforth call the dimeric or polymeric species responsible for the new band an excimer, realizing that this species may ultimately prove to be of a more complicated type.

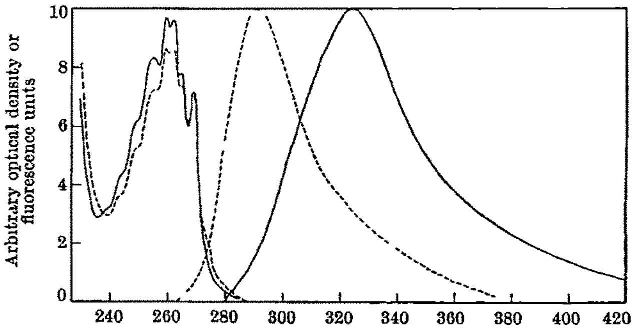

Fig. 1. Absorption and fluorescence spectra of 'atactic' and isotactic polystyrene in 1,2-dichloroethane. Solid line, isotactic; broken line, 'atactic'

The red band is developed in dilute solutions of isotactic polystyrene to about the same degree as in very concentrated solutions of benzene and it may be concluded that interaction between phenyl rings on the polymer is very efficient. It is reasonable to suppose that each phenyl ring can form an excimer with either of its nearest neighbours. If so, congruent plane-to-plane contacts between phenyl rings are not necessary for excimer formation, since such contacts are not possible in isotactic polystyrene.

In Table 1 the absolute quantum yields, Q_f, the wave-lengths of maximum fluorescence emission, λ_{max}, and the band-width of emission at half height are given for toluene, 'atactic' polystyrene and several copolymers of styrene and methyl methacrylate. The most notable features of these fluorescence characteristics are the increase in Q_f with decreasing proportion of styrene, the increase in λ_{max} with increasing styrene content and the increase in peak broadening with increasing styrene content. The behaviour of λ_{max} and of the peak width appears to be due to excimer formation here, too, since just these changes are observed in toluene solutions of increasing toluene concentration. However, the shift in λ_{max} is somewhat larger than might be expected on this basis and may be due in part to the increasing local polarizability as the styrene content is increased.

Since the 324 mμ band was never detectable in these solutions, the number of pairs of phenyl rings able, by reasons of geometry or time, to form excimers must be much smaller than in the case of isotactic polystyrene. It is not possible to decide, on the basis of our data, whether the small amount of excimer formation which does occur in these polymers is between adjacent phenyl rings on the polymer or between phenyl rings brought close together by close approach of two sections of polymer distant from each other along the polymer chain. Both situations would seem to be possible, but the high efficiency of excimer formation in isotactic polystyrene suggests that most excimers in the 'atactic' polystyrene and the copolymer are formed between adjacent phenyl rings. If so, quantitative measurement of the parameters of the emission spectra will provide an index of the number of 'isotactic pairs' of such groups. Of more utility, perhaps, would be the measurement of the 324 mμ band as a simple criterion to distinguish between isotactic and 'atactic' polystyrene.

The absolute values of Q_f, all but toluene, as given in Table 1, would appear to be reliable within ±10 per cent. The Q_f values for toluene solutions were erratic and will bear further study. Care was taken to deoxygenate solutions by repeated freezing. The decrease of Q_f in oxygen-free solutions can be seen to parallel the increase in λ_{max} and thus presumably the increase in excimer formation in the excited state. This suggests that the intrinsic lifetime of the excimer is significantly shorter than that of an isolated excited phenyl side-chain. However, Ivanova, Mokeeva and Sveshnikov[6] have reported

Table 1 FLUORESCENCE QUANTUM YIELDS (Q_f) OF POLYSTYRENE, STYRENE METHYL METHACRYLATE COPOLYMERS AND TOLUENE (0 002–0 015 PER CENT IN 1,2-DICHLOROETHANE, 20 0 ± 0 1°)

		λ_{max} mμ	Half-height width (mμ)	Q_f N_2	Q_f Air	Q_f O_2
Polystyrene, 'atactic'		292	30	4	3 5	3
Polystyrene, isotactic		324	47	1 5		0 7
Styrene methyl methacrylate copolymers						
Styrene methyl methacrylate feed ratio	Mol fraction of styrene in copolymer					
75 25	69	288	28	5	4	3
50 50	53	286	26	6	5 4	3
25 75	33	286	26	10	6	3 4
10 90	16	285	25	12	8 5	5 5
Toluene		281	26	8 ± 2		3

for benzene and toluene a longer lifetime and a larger fluorescence yield when excimer formation is increased by lowering the temperature In contrast to our results, benzene solutions have larger Q_f and presumably a longer fluorescence lifetime, the higher the benzene concentration It may be that the decrease in Q_f for the polymer is a consequence of the close association of phenyl rings, which might make resonance-transfer mechanisms possible In such a situation the migration of the quantum along the random chain of phenyl rings could lead to enhanced inter-system crossing to a non-radiative state, or, if traces of quenching agents still remained in these experiments, the efficiency of these substances could be enhanced by the rapid migration of quanta to a phenyl group already in contact with a quenching molecule[7]

It will be noted in Table 1 that the efficiency of oxygen as a quencher decreases as styrene content increases Hence, there is no net enhancement of fluorescence quenching as conditions are made more favourable to resonance energy transfer There is furthermore no evidence from absorption or emission spectra to indicate the presence of situations favourable to long-range migration of electronic quanta For example, there is no hypochromism in the spectra of 'atactic' or isotactic polystyrene From investigations of deoxyribonucleic acids[8,9] and helical polypeptides[10–14], hypochromism might be expected if the conditions for the occurrence of exciton behaviour were present in the polystyrenes We note also that Ivanova *et al* [6] have found self-quenching even in pure toluene or benzene to be very inefficient

Yet our experiments in systems with and without oxygen show that the Q_f of both excited monomeric side-chains and excimer formed from such side-chains decreases rapidly with increasing content of styrene Since these reductions in Q_f presumably imply a reduction in fluorescence lifetime and cannot be attributed to enhanced efficiency of quenching by external quenching agents, it seems necessary to suppose that linkage of the phenyl groups to the polymer introduces a new internal quenching mechanism or that the natural lifetimes of the excited states of monomeric and dimeric side-chains are reduced by their linkage to the polymer The reduction in lifetime is proportional to the styrene content of the polymers Hence, isolated phenyl groups are not strongly influenced, although some change in these units may occur

The predominant effect, however, appears to depend on the density of styrene units in the polymer The orientation of these units governs the amount of excimer formation but has only a secondary effect on the lifetime, since Table 1 shows that the fluorescence yield of isotactic polystyrene is reduced by only a factor of two when compared with Q_f of 'atactic' polystyrene If quenching by terminal groups is unimportant, as seems probable at this time, it might be supposed that there is an electronic coupling between the phenyl side-chains mediated by the polymer backbone which enhances quenching of the first excited singlet state of the phenyl groups However, we cannot suppose that the natural lifetime changes, since changes in Q_f would require a five-fold decrease in lifetime passing from toluene to atactic' polystyrene This change would require a very large decrease in area of the absorption spectrum, which is not observed Hence, it is necessary to postulate a quenching process in the polymer not involving in a significant way the energy or crossing probabilities for low-lying excited states, but rather a physical quenching dependent on the density of phenyl groups Apparently, the quenching process involves an approach of phenyl rings in such a way that excimer formation does not occur, or, if it does occur, has little bearing on the quenching reaction Such a process appears to be consistent with our several observations and particularly with the observation that the integrated area of the absorption band does not undergo any significant change with increasing styrene content The behaviour is inconsistent with the poor efficiency of self-quenching in liquid benzene or toluene and thus appears to be peculiar to the polymers

A preliminary investigation of the fluorescence of polymer films has also been carried out and provides information complementary to that supplied by solution spectra Films (about 2 cm² in area) were prepared by evaporating a 1,2-dichloroethane solution of the polymer on a 'Spectrosil' plate, which was then placed diagonally in the cell compartment in such a way that the fluorescence was analysed from a direction opposite to that of the reflected light Films of the copolymers containing the lowest proportions of styrene exhibited fluorescence spectra very similar to those of the solutions However, the fluorescence of the homopolymer film was very different from that of the solution, λ_{max} being shifted to much longer wavelength The thinner the film, the more striking was the effect Thus, a film prepared from a solution containing 0 25 mg of 'atactic' polymer per ml exhibited a λ_{max} at about 320 mμ, whereas a film prepared by evaporation of a solution containing 2 0 mg/ml had a λ_{max} at about 310 mμ The fluorescence spectra are evidently highly sensitive to differences of chain orientation occurring during evaporation

We are continuing work with styrene polymers and other polymers containing aromatic fluorophors We have also examined the fluorescence of a number of small molecule model compounds having aromatic rings strained and oriented in various ways Investigations of styrene polymers may provide useful information about the behaviour of excited states of benzene and related compounds Perhaps the most interesting result of this work is the difference in fluorescence properties of concentrated solutions of benzene and the polymers of high styrene content

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CHARACTERISTIC BEHAVIOUR OF SOME CARBOXYLIC EXCHANGERS IN RELATION TO THE ABSORPTION AND DESORPTION OF POLYELECTROLYTES

By Dr C S KNIGHT, V C. WEAVER and B N BROOK

Whatman Research Laboratories, W and R Balston, Ltd , Springfield Mill, Maidstone, Kent

ALTHOUGH the usefulness of ion-exchange celluloses for the isolation, purification and separation of large ions is now established, the selection of a suitable derivative for a particular purpose is still based on a relatively small amount of fundamental information It is known that desirable properties are imparted to ion-exchange celluloses for use in the foregoing context by the physical and chemical characteristics of the cellulosic structure Thus, cellulose exhibits a very large surface area which is not dependent on macro-particle size In addition, the hydrophilic character of the cellulose molecule has the effect of enhancing accessibility of the ion-exchange sites in aqueous media Rapid rates of absorption and desorption, the latter under relatively mild conditions, are features of chromatography of large ions on selected ion-exchange cellulose derivatives

This article is an attempt to correlate the absorption and desorption characteristics of some new cellulose derivatives with the chemical and physical characteristics of the products It has been found convenient to compare the performance of cellulose derivatives with that of corresponding derivatives of 'Sephadex' (cross-linked dextran) and synthetic resin

Crystalline insulin was selected for use in this investigation because of its availability in pure form and its well-characterized properties

A standard solution was prepared by dissolving 1 0008 g of dry crystalline insulin in a mixture of 9 2 ml of 0 1 N hydrochloric acid and 100 ml of distilled water After solution the total volume was made up to 250 ml

Table 1 lists the derivatives used in this investigation together with their properties .

Table 1 CHARACTERISTICS OF SOME CARBOXYLIC EXCHANGERS

Exchanger	Ion-exchange capacity m equiv /dry g	Water regain	Physical form	Ionic form
'*CMC* (*a*)'	0 25	1 50 H 1 60 Na	Powder	Sodium
'*CMC* (*b*)'	1 0	1 78 H 2 57 Na	Powder	Sodium
'*CMC* (*c*)'	4 5	1 71 H 3 35 Na	Powder	Sodium
'*CM* Seph *C* 25'	4 4	4 85 Na	Powder	Sodium
'*CM* Seph *C* 50'	4 4	6 04 H 19 85 Na	Powder	Sodium
'Zeo Karb 226'	10	0 8–1 0 H	Beads	Hydrogen

The exchangers were cycled through their sodium and hydrogen forms on a Buchner funnel using 0 5 N sodium hydroxide and 0 5 N hydrochloric acid and retained in their hydrogen form Excess acid was removed by washing with de-ionized water until the filtrate was of neutral *p*H In the case of the cellulose and 'Sephadex' exchangers the samples were shaken with de-ionized water for a further 24 h, filtered, and finally washed to neutral *p*H All the exchangers were retained for use in a swollen state

A calculated excess of standard insulin solution, the same for each sample, was rapidly added to a sample of exchanger (about 0 5 g) with continuous stirring Filtered samples (1 ml) of the ambient solution were taken at timed intervals Each sample was diluted with 5 ml de-ionized water and its ultra-violet absorption at 276 5 $m\mu$ measured

The exchanger, saturated with insulin, was filtered off and washed with de-ionized water until free of excess insulin A calculated excess of 0 01 N hydrochloric acid was added rapidly to the exchanger with continuous stirring Filtered (1 ml) samples of the ambient solution were taken at timed intervals, diluted with de-ionized water (5 ml) and their ultra-violet absorption measured at 276 5 $m\mu$

Any insulin still remaining bound to the exchanger was removed by washing with 0 1 N sodium hydroxide Excess alkali was washed from the exchanger which was then reconverted to the hydrogen form, washed, dried and weighed

A summary of the results obtained on the carboxylic exchangers is recorded in Table 2 The significance of the measurements recorded in this table is as follows

The fraction of the theoretical capacity used up by the absorbed insulin is dependent on the relative affinity of the exchanger for, and the accessibility of the exchanger to, the insulin ion whereas the fraction of the absorbed insulin which is afterwards released is dependent only on the relative affinity of the exchanger for the insulin

The time taken to complete 50 per cent of the absorption provides a convenient means of comparing the relative speeds of the absorption process on the different exchangers and provides an indication of the part played by the accessibility factor in determining the fraction of the theoretical capacity used up during absorption

Table 2 ABSORPTION OF INSULIN ON AND DESORPTION OF INSULIN FROM CARBOXYLIC EXCHANGERS

Derivative	Nominal capacity m equiv / dry g	Water regain	Insulin Absorption				Insulin Desorption			
			Insulin absorbed m equiv / dry g	Fraction of theoretical capacity used (%)	Time taken to complete 50% reaction (min)	Order of reaction	Insulin released m equiv / dry g	Fraction of absorbed insulin released (%)	Time taken to complete 50% reaction (min)	Order of reaction
'*CMC* (*a*)'	0 25	1 50 H 1 60 Na	0 072	29	<1	2	0 072	100	<1	2
'*CMC* (*b*)'	1 0	1 78 H 2 57 Na	0 308	31	2 5	2	0 205	67	1 5	2
'*CMC* (*c*)'	4 5	1 71 H 3 35 Na	0 521	12	30	2 5	0 213	41	16	2
'*CM* Seph *C* 25'	4 4	4 85 Na	0 563	13	42	6→3 5 →2	0 170	30	20	4→2 5 →2 1
'*CM* Seph *C* 50'	4 7	6 04 H 19 85 Na	0 680	15	20	5 5→4 →2 5	0 367	54	28	7→3 →2 5
'Zeo Karb 226'	10	0 8– 1 0 H	0 040	0 4	<1	—	0 008	20	2	2

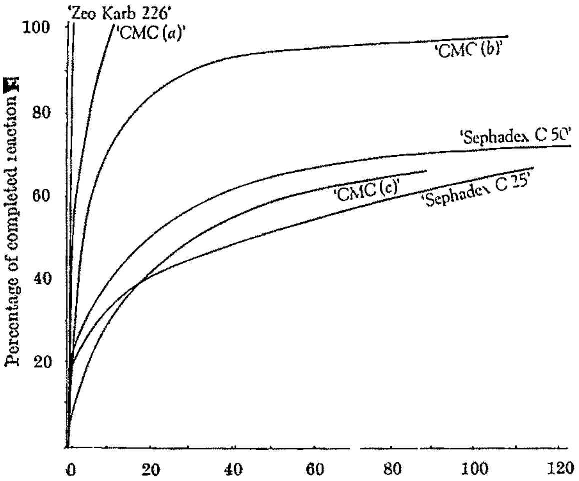

Fig 1 Absorption of insulin on carboxylic exchangers

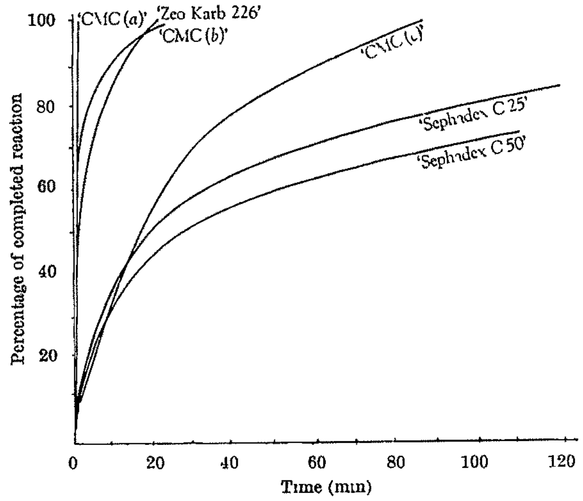

Fig 2 Desorption of insulin from carboxylic exchangers

The water regain value, together with the relationship between the rates of absorption and desorption, indicates the resistance of the system to changes in volume with changing ionic form and hence to the dimensional stability of the exchanger

The order of the reaction provides an indication of the manner in which the ion-exchange sites are disposed in the exchanger, an order of 2 being consistent with reaction at readily available surfaces

The relative rates of absorption and desorption of insulin are further compared in Figs 1 and 2

Cellulose, in its natural state, possesses a type of molecular structure which is able to confer unique characteristics on later derived ion-exchange materials In contrast to various other types of synthetic ion exchangers which offer a more or less uniform network of molecular pores, it is possible to prepare ion-exchange celluloses which are characterized by aggregates of polysaccharide chains in various states of order and disorder, within and between which are located pores of widely differing size The largest pores are readily accessible even to polyelectrolytes At the same time the regions of higher order provide a supporting matrix which effectively controls expansion and contraction of the pore structure, enabling high accessibility to be combined with high dimensional stability

The results recorded in Table 2 reflect the fundamental structural differences between synthetic resin, cross-linked dextran and cellulose ion-exchange derivatives

It is significant that the experiments were carried out using pre-cycled, undried exchangers Although the hydroxyl character of cellulose and dextran matrices is an asset in the application of these materials to large ion investigations this same character necessitates special precautions in the use of the exchangers For example, intermolecular hydrogen bonding induced on drying the exchangers tends to create inaccessible regions in the structure It is because of this that it is necessary to pre-cycle the exchangers prior to use and to retain them in a swollen state

The highly cross-linked, high capacity 'Zeo Karb 226' with its backbone of hydrophobic polymer chains is virtually inaccessible to the insulin ion Exchange is confined to the near surface of the resin particles Thus, absorption of insulin, accounting for only 0 4 per cent of the nominal capacity of the exchanger, is completed in less than a minute Subsequent desorption of even this small amount of insulin at pH 2 is relatively slow and incomplete, however, due presumably to the de-swelling of the surface structure on conversion to the hydrogen form Low accessibility, low dimensional stability and resultant irreversible absorption combine to characterize the application of a synthetic resin of this type to problems involving insulin or larger ions

In the 'Sephadex' ion exchangers the hydrophobic backbone of the resin has been replaced by a hydrophilic structure The effect of this hydrophilic backbone on the swelling of the exchanger in aqueous media increases the accessibility of the structure to large ions Thus, in spite of lower nominal capacities the 'Sephadex' exchangers absorb larger quantities of insulin than the 'Zeo Karb 226' (Table 2). Penetration of the insulin into the internal structure of the exchanger is reflected in the slow rate of absorption The relatively low dimensional stability of the 'Sephadex' exchangers, however, results in an initial rapid absorption being followed by a relatively slow uptake of insulin as the exchanger de-swells Desorption of insulin from the 'Sephadex' exchangers follows a pattern similar to the absorption 'Sephadex *C* 50' is more highly swollen than 'Sephadex *C* 25' and is also less stable dimensionally The effect of swelling changes on absorption and desorption of insulin is particularly marked with this exchanger The rate of desorption of insulin from Sephadex *C*.50' is slower than that of the initial absorption process for this reason

In dextran-derived exchangers, therefore, the occurrence of a hydrophilic backbone structure increases the accessibility of the exchanger to insulin relative to that of a synthetic resin exchanger The retention of the more or less uniform molecular porous network of a gel structure, however manifests itself in relatively large dimensional changes during absorption and desorption of large ions This effect is particularly pronounced in the higher swelling variants and leads to relatively slow kinetics, particularly during desorption A fraction of the insulin thus has the appearance of being preferentially held on the exchanger

Viewed in the foregoing context, cellulose exchangers differ in their performance in being able to combine, by virtue of their unique molecular structure, high accessibility with high dimensional stability The extent to which these characteristics are shown is dependent on the ion-exchange capacity of the exchanger as is indicated in Table 2 The percentage of insulin desorbed at pH 2 from the '*CMC*' variants shows that selectivity for insulin increases with increasing ion-exchange capacity The higher selectivity of '*CMC* (*c*)', however, is achieved at the expense of accessibility and kinetics Table 2 illustrates the similarity between '*CMC* (*c*)' and the 'Sephadex' exchangers in relation to selectivity for, and rates of absorption and desorption of, insulin A significant difference in performance is shown in Figs 1 and 2, however The higher dimensional stability of the cellulose exchanger gives rise to more regular rates of absorption and desorption of insulin. It is consequently easier to remove the more deeply penetrated insulin from cellulose compared with cross-linked dextran exchangers even when all other features such as total absorption, capacity and selectivity are the same.

Significant departure from synthetic resin and cross-linked dextran exchanger type behaviour, as recorded in Table 2 and Figs. 1 and 2, is shown by '*CMC.(a)*' and '*CMC.(b)*'. Because of their relatively low capacity the selectivity of these exchangers for insulin, particularly that of '*CMC.(a)*', is lower than that of the previously discussed exchangers. The influence of this lower selectivity on total absorption of insulin is compensated for by the higher accessibility and faster kinetics on the lower capacity '*CMC*' variants. The performance of '*CMC.(a)*' and '*CMC.(b)*' reflects those characteristics which are peculiar to cellulose-based exchangers and which could not be reproduced on the other particular exchangers investigated. Rapid absorption and desorption. relatively high accessibility and the lack of any tendency for irreversible absorption even when maximum loadings are used are significant features arising from the results recorded on these two exchangers.

To sum up, therefore, the comparative results obtained in this investigation have indicated that although a hydrophilic matrix is a highly desirable feature of an exchanger used in large ion studies, for more versatile and efficient use, under both batchwise and chromatographic conditions, an exchanger must also possess specialized structural features at a molecular level. A combination of these features has been found to exist in selected cellulose exchangers.

We thank the Directors of W. and R. Balston, Ltd., for permission to publish this work.

LAVA TUNNELS AND SOME POSSIBLE GAS EFFECTS IN BUFUMBIRA

By E. J. WAYLAND, C.B.E.

MR. L. A. J. WILLIAMS'S account of "Lava Tunnels in Suswa Mountain, Kenya"[1] recalls records of similar phenomena in Bufumbira, the Uganda embayment of the remarkable Birunga volcanic field (Virunga of the Belgians) lying to the north of Lake Kivu in the Western rift valley of East Africa.

The following hitherto unpublished facts are embodied in the first of two field journals covering my preliminary reconnaissance of the southern and western portions of the Uganda Protectorate, undertaken more than forty years ago.

Under the sub-head "4.4.19, Exploration of Kegezi Camp" it is recorded that a line drawn from the right front corner of the camp compound towards Bihungi and on to the highest peak of Chansi crater (Chansi and Mount Muhavara appear to be one and the same) passes through a tall, conspicuous and solitary tree by the side of which is the mouth of a large lava cave, or tunnel, running below the flat surface of the ground. I went along this tunnel for about eighty yards, at which point it becomes very narrow, low, damp and infested with bats. Its direction parallels the strike of the high sedimentary country to the east of it (S. 15° E.). In numerous places lava tunnels have responded to structural control (lines of strike and jointing) imposed by pre-lava rocks well below them. A case in point is provided by the Kegezi tunnel, as for convenience I call the one considered here.

In vertical section the tunnel, where I traversed it, is nearly semi-circular with plenty of headroom. There are about 6 ft. of soft deposit on its floor (black at bottom and top with a white zone in the middle). A grindstone, presumably of no great age, was unearthed near the top and a few microlithic artefacts were near the bottom. Among the latter was a beautifully made 'lunate', almost certainly belonging to the Wilton culture.

It seemed obvious that the tunnel was in a measure consequent on differential chilling of flowing lava. Temperature control at the ground beneath the molten rock and at the interface between the latter and the covering air could provide the required primary conditions at opposing surfaces and, given a slope sufficient to prevent stagnation, provide a temporary flowage of lava between solidified sheets of itself. But this would not make a tunnel, for the molten lava would in its turn solidify sourcewards. Thus would the stony sheets thicken and coalesce; and it is not difficult to visualize conditions in which the flow of sub-surface lava would cause sheet-collapse of a solidified and jointed zone above it. Such results are seen.

Clearly, a factor conducive to tunnel production in these circumstances would be a channelled ground surface disposed to increase the speed of lava flow to a maximum controlled by viscosity and temperature along its course, and indeed beyond its beginning to a starting point of lava movement until the source-supply was abolished by solidification or, more likely, by cessation, temporary or otherwise; but the channelled molten rock would continue, for a while, its onward journey, only to be frozen in its turn; a long gap, a tunnel, in fact, would thus appear between the proximal and distal portions of the once recalcitrant sub-surface lava stream.

Apart from penecontemporaneous additions such as lava stalactites of which very few were seen in the Kegezi tunnel, its walls and roof are smooth.

Old tunnel entrances are in most cases concealed by vegetation, and because they are unassociated with tip heaps they are not easily found. The Duke Adolphus Ferdinand of Mecklenburg, who, with his exploring party, entered Birunga (Virunga) in 1907, states that:

> "Spacious caverns formed under the crust of the lava streams have remained to the present day. We searched around for them, and crept into some of them so as to inspect the interiors. The entrances mostly lie concealed under shrubbery, and it occasionally takes a long time to find an opening."[2].

One is moved to ask, "Have any of these tunnels been put to sustained use?". At least one in Bufumbira has functioned as a vault. It was seen to house an astonishing number of human bones, some of which are deeply patinated and possibly of great antiquity. It is conceivable that they may have been collected as bones, from elsewhere.

I was shown this communal mausoleum, for the place has imposing features, under oath of secrecy; nor was I permitted to remove any bones—an unfortunate restriction, for it became my practice to send all crania and pieces of human skull that I chanced to come across or to discover to Sir Arthur Keith.

I have visited the Bufumbira country and the Belgian Congo (Birunga) beyond it several times, but nineteen years were to elapse after the first occasion before I was privileged to behold lava tunnels in action.

Mount Nyamlagira (summit 10,026 ft. above M.S.L. at Mombasa and rising 5,000 ft. above its base) in Birunga had awakened again, as it does from time to time, to belch forth lava and all the products of seeming ill temper to which volcanoes are prone. J. Verhoogen, the volcanologist, was flown out from Belgium to study the phenomena[3], and I, with similar intent, hurried westwards.

Here I must restrict my remarks to lava tunnels and to some ancillary matters. To recall all the experiences and observations that emerged from my stay on the Mountain would be to fill a book; they begat, however, at a later date much detailed field work, mapping and profound petrological enquiries, all of which matters are set forth in two official volumes and several scientific papers[4].

There is a tendency for the tops of lava tunnels to drop in for a few yards from place to place, and in some cases for extended stretches to do so, thus producing lava rifts which have sometimes been wrongly attributed to tectonic activity. Commonly, however, in Birunga, bridge-like roof-remains provided chances to stand over the thalweg of a lava stream, thus to peer down and watch the incandescent turmoil until driven away by unbearable heat. Through one such opening on Nyamlagira the racing magma passed over a point where in other circumstances a small waterfall might have danced onwards, so to say, to the tune of its own music; but now molten lava poured over the site in ominous treacly silence. A rock tossed into the glowing stream occasioned no splash. It was rapidly absorbed and ceased to exist.

There are numerous large caves of one sort or another in Uganda. Some of them have been used for refuge during tribal raids or wars; others, or the same caves at different times, for impounding cattle, but I was unable to discover that they had been utilized for either of these purposes in Bufumbira. In Birunga, on the other hand, to continue the Duke of Mecklenburg's pronouncement:

"These caves served the Wanjaruanda not seldom as secret lurking places Even as late as 1906 Captain von Grawert had arrows showered upon him from the mouth of one of these caves whilst engaged on a punitive expedition."

Some of the tunnels, I was told, were shunned by everyone, but I did not learn why this was so. Perhaps it was for the same reason as certain of the Elgon caves were, and likely enough still are, avoided because of fear of revengeful wraiths or because they have been cursed by evil beings of malicious intent. In either case the result is said to be the same—a distressing illness ending, more often than not, in death.

The supernatural or occult can be discredited. Not so the sickness, it seems, for it is claimed to follow inhalation of certain airborne fungal spores. A similar explanation has on occasion been applied to ancient Egyptian tombs of sinister reputation.

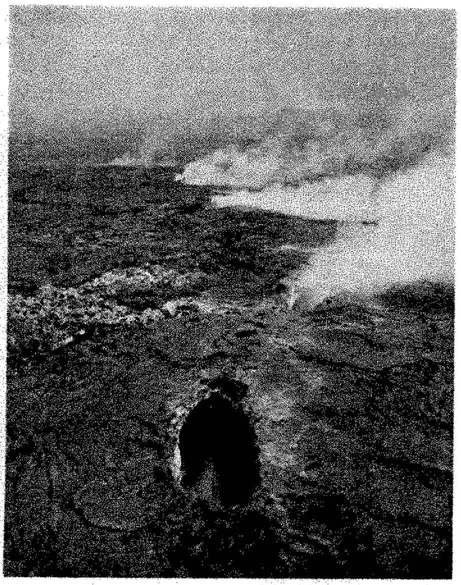

Fig. 1. A lava tunnel opened in four places by falls of roof. While three of the openings emit vapour, the nearest shows a breach to an older tunnel below it. The vapour-giving lava stream appears to have entered the lower tunnel at a spot between the third and the nearest roof collapse

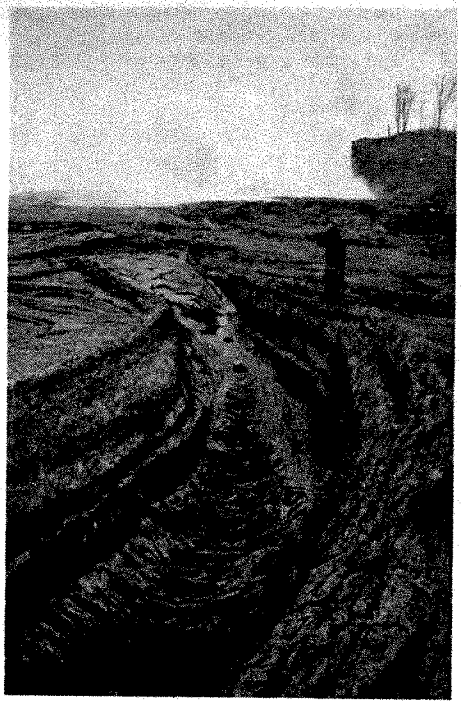

Fig. 2. A lava stream in an open channel produced by lengthy collapse of a lava tunnel roof

Williams writes: "Although the complete pattern of the Suswa tunnels is as yet unknown, it seems remarkable at first sight that they should have developed immediately above each other in successive lava flows. Such an arrangement is readily explained, however, because the dividing lava was thin, allowing the mobile central portion of the overlying flow to drain away through collapsed areas of its floor. Lava flow in open channels, if it ever occurred, probably played only a minor part in the development of these features, and formation of the earliest tunnels probably depended on unusual circumstances, such as extrusions of the lava over patches of hot ground which helped to keep portions of the flow mobile".

These may be unusual circumstances; certainly the claimed effect would scarcely have been anticipated. The patches of hot ground are unlikely to have been lineal unless they were themselves already channelled. Lava flows in open channels, by the way, are by no means unknown in the Bufumbira–Birunga field (Fig. 2).

Lava tunnels in other widely flung volcanic terrains are well known: some are almost famous. Williams goes on to say that such openings "have not been reported from surrounding areas in the rift valley where many of the volcanic rocks are phonolites and soda trachytes" (like those of Mount Suswa ?).

As already mentioned, the fluidity of a molten material depends in large measure on its viscosity: the higher the viscosity the lower the degree of fluidity of a lava, and the flatter the cone built of it. Nyamlagira, it may be mentioned, is a flat cone.

In general the viscosity of a lava is a function of the percentage of its silica. Phonolite and trachyte are silica-rich (about 60 per cent according to the type) and would not be expected to display a propensity to tunnelling, except where the molten rock was so canalized that gravity hastened its progress while the upper and lower portions froze solid, after the manner already explained.

Lavas would seem to have a tendency to shrink on cooling. Hence the 'moat' to be seen on a number of volcanoes, Suswa included, surrounding more or less completely the consolidated lava columns which otherwise fill their craters.

Nyamlagira provides an instructive example, and vividly I recall the occasion when a small hut-like construction which had been erected over a narrow part of the moat was suddenly and unexpectedly shot into the air, together with its occupant, by reason of a singular and powerful belch up the moat of hot gas and 'steam'. This unique missile landed, fortunately, clear of the gaping chasm, into a cushion of fine black volcanic dust. No bones were broken.

Owing to the comparative freedom of movement occasioned by the presence of the surrounding moat, a solidified central lava column, compelled by gas or ascending magma, or by both, may rise high above the volcanic mass—like the spine of Mount Pelée.

But against thermal shrinkage in a cooling lava (setting aside for convenience paramount glass production, obsidian, for example) one must place expansion resulting from crystallization of most of its constituents as they congregate to form mineral species. These opposing states (the introverted strain of shrinkage, or I.S., and the extroverted strain of crystallization, or E.S., one might call them) will balance at one stage, or at a few stages only of the cooling process.

If the top and bottom of a lava flow have solidified sufficiently to permit of tunnel-formation, surface effects may occur vertically above a stretch wherein the tunnel-lava has been halted and has filled all available space in its vicinity. Should crystallization begin concurrently with cooling (it will scarcely start impressively before it) no consequent surface phenomena will result, provided that the cooling and the crystallization proceed *pari passu*, and that the introvert and extrovert stresses cancel out (I.S. = E.S.). When E.S. is effectively greater that I.S. it will be reinforced from below in consequence of thrust occasioned by the unyielding bottom and a lineal swelling may appear at ground surface and split longitudinally. When E.S. is effectively less than I.S. a channel or a shallow lineal collapse may occur revealing no tunnel beneath it.

The trace of a lava tunnel, or tube, is likely to have been the thalweg of a relatively flat valley-form hollow. Of no great size but very much wider than the molten stream that later came to fashion a tunnel, the hollow-way or valelet may or may not have positioned a predisposing water channel in pre-lava days. In either event, the result would have been very much the same. Had the lava filled much or all of the hollow-way, melt-chilling would have been vertically convergent and progressive, and a similar convergence would have operated laterally in accordance with lava thickness; so, too, with crystallization. Over the tunnel site E.S. would additionally operate slowly downwards, but it would not deform the roof-shape of the developing tunnel. None the less, a shallow linear sag might well appear at surface later to initiate another tunnel if and when a further copious lava spread occurred. Thus may one tunnel be superimposed, wholly or in part, above another.

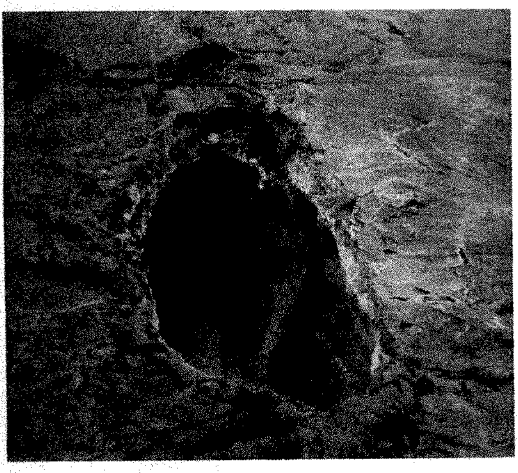

Fig. 3. Close view of nearest collapse depicted in Fig. 1

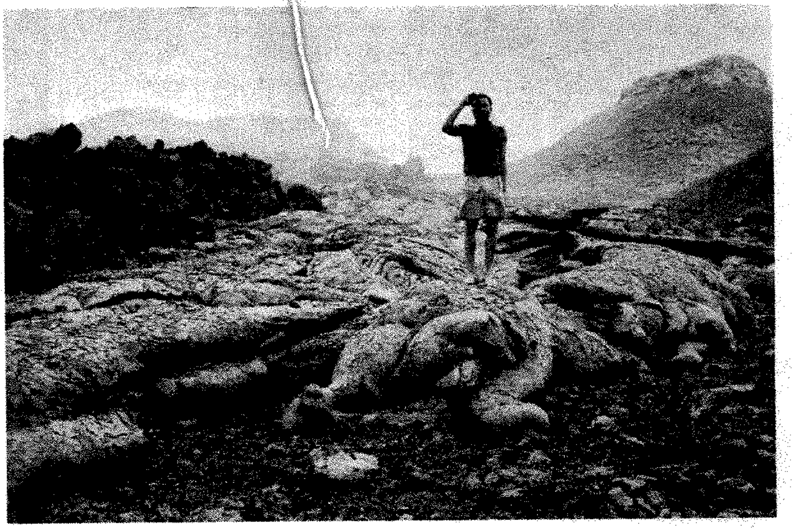

Fig. 4. Lava 'slugs'

(All photographs—unofficial—by the late A. D. Combe, of the Geological Survey of Uganda)

Williams writes of minor explosions caused by the combustion of gases generated from inundated vegetation. Nothing appears to be known of this phenomenon in the Bufumbira–Birunga field, but in this category perhaps may be placed some obscene-looking bubbloid excrescences, suggestive of connubial slugs of enormous proportions, occurring on Nyamlagira and some associated volcanoes. When first I clambered on to one of the 'slugs' I crashed through the top of it, but not to descend, as momentarily I feared I should, into an inferno below, but to solid ground at about 4 ft. 6 in. (Fig. 4).

There is much evidence of true volcanic explosion and gas activity in Bufumbira–Birunga. The following is extracted from my notes of April 3, 1919.

"Near Chanse crater there are many hummocks of lava without craters which require explanation. They suggest bubbles, but are up to 20 ft. in height; each has a base diameter of about three times the vertical measurement." Much bigger examples were later discovered, some breached to the base by emergent lava.

It was later discovered that these mamilliform excressions are not formed from lava but made up of ash and scoriæ of extremely frothy and pumiceous lava. Although not titanic bubbles, they represent a phase of an explosive episode. Thus they differ widely from the mamelons of Reunion Island and some of the puys of the Auvergne, to quote two well-known examples of lava plugs.

There is evidence in plenty that explosive volcanic action was widely developed in the Bufumbira–Birunga field. Indeed, it appears to have been the initial stage of the volcanicity.

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[1] *Nature*, **199**, 348 (1963).

[2] Adolphus, Duke of Mecklenburg, *In the Heart of Africa*, 119 (London, 1910).

[3] Verhoogen, J., "Les eruptions 1938–1940 du Volcan Nyamuragira", *Mem. Inst. des Parcs Nationaux du Congo belge*, **1** (1948).

[4] (*a*) Simmons, W. C., *Geol. Mag.*, No. 797, 491 (1930). (*b*) Combe, A. D., and Simmons, W. C., "The Volcanic Area of Bufumbira, South-west Uganda", *Mem.* 3, Part 1, *Geol. Surv. Uganda* (Entebbe, 1933). (*c*) Holmes, A., *Min. Mag.*, No. 155, 408 (1936). (*d*) Holmes, A., and Harwood, H. F., "The Volcanic Area of Bufumbira", *Mem.* 3, Part 2, *Geol. Surv. Uganda* (Entebbe, 1937). (*e*) Brammal, A., *Nature*, **143**, 649 (1939).

MULTIPLE SOLUTIONS OF X-RAY DIFFRACTION DATA

By Dr. R. K. BULLOUGH

Manchester College of Science and Technology, Manchester, 1

It has been known for many years[1-3] that two quite different configurations of atoms can show the same X-ray scattering pattern. Equivalently the Patterson interatomic vector diagram can have two equally good alternative solutions. Such solutions are homometric. Weighted periodic or non-periodic homometric pairs of point sets (that is, sets with Dirac δ-functions of positive integral weight z_i at points $\mathbf{x}_i$) exist in any number m of dimensions[3,4]: it is known[3] that if two sets each of total weight Z (assumed integral) constitute a homometric pair in q dimensions they may be used to derive non-trivial homometric pairs of weight Z in $m > q$ dimensions and it has been shown[4] that they may also be used to generate homometric multiplets with 2^s members (that is, of order 2^s) and weight Z^s in $m \geqslant qs$ dimensions. The investigation of one-dimensional homometric multiplets therefore has an important bearing on multiple solutions of X-ray data in 3 dimensions.

In one dimension it is now known[5] that homometric r-tuplets exist for sets of $n + r$ points of equal weight for arbitrary $n, r \geqslant 2$. It follows: (1) that there are sets which constitute homometric multiplets of order $Z-2$ for every $Z \geqslant 4$; (2) that there are, therefore, multiplets of arbitrarily large order, that is, it is possible to construct one dimensional vector sets with as many equally good solutions as one pleases. From multiplets of order r and weight Z in one dimension can be constructed multiplets of order r^m and weight Z^m in m dimensions. It is evident here that although the maximum order of a multiplet is without limit the large order is achieved only at the expense of increasing the total weight Z—and this proves to be true whether this is done by increasing the total number of points N, or their individual weights z_i, or both.

It has been proved by me that for a given total integral weight Z the maximum order R of any multiplet of point sets is bounded above. In so far as the restriction to ('continuous') δ-function point sets and vector sets restricts the available solutions of the latter to ones approximately conforming to the known physical characteristics of a system of discrete atoms of positive density the existence of the number R demonstrates the existence under these physical constraints of a solution (albeit possibly still multiple) of the 'phase problem'. In contrast it has been proved that, if solutions are restricted only by a condition of continuity, R does not exist and the maximum multiplicity is not bounded: a further condition that solutions be everywhere positive is not of itself then sufficient to imply the existence of R. Since R exists for δ-function point sets and, as noted here, is $\geqslant Z-2$, R is an increasing function of Z for these sets. Clearly it is important for the assessment of the validity of crystal structures determined by X-ray diffraction to know how fast R increases with Z: it is also important to know how R is reduced by the additional constraints offered by symmetry, or by other physico-chemical information, or, for example, by the direct determination of a few phases.

The chance of any structure occurring in Nature being one of several homometric alternatives is probably very small; but errors in observed intensities and from their finite number could mean that for large enough Z the observed Patterson diagram differs from a vector diagram with multiple solutions by less than the uncertainty in the observed diagram. We shall need to know more about the dependence on the number Z of each of the errors in Patterson peak positions, of R, and of the number $Q(r, Z)$ of vector sets with precisely r solutions, to form a valid conclusion; but the fact that $R \geqslant Z-2$ suggests how dependent on other physico-chemical information may be the structures of large molecules elucidated by X-rays. In particular it seems at least possible that, because any observed vector diagram probably 'almost always' has a unique solution, that solution would always be obtained by any routine method of solution at the expense of a possibly very different solution equally acceptable when the error in the observed intensities is included.

The practical implications of the dependence of R on Z when the only constraints are those offered by δ-function point sets must be drawn cautiously. Nevertheless it seems of sufficient interest to report that, for one-dimensional sets of Z points of equal weight, R is almost certainly not asymptotically equal to $Z-2$ as it was earlier thought to be[5]. Indeed, the brief remark in the earlier paper[5] must be withdrawn because it is in fact easy to construct multiplets of equal points for which r increases not as Z but essentially as Z^2. It can be proved that $R = 1$ for $Z < 4$; $R(4) = 2$ and $R(5) = 3$; but $R(6) \geqslant 5$ as the example (2) below shows and its precise value is not known.

One of the only two families of pairs which can be constructed for $Z = 4$ is the periodic pair with co-ordinates[5]:

$$S_1 = (0, 1/13, 3/13, 9/13);\ S_2 = (0, 1/13, 4/13, 6/13) \quad (1)$$

with a unit cell of unit length and each point chosen for convenience with unit weight. This pair has a remarkable vector diagram consisting of 12 points of unit weight at 1/13, 2/13, . . . , 12/13, and with a point of weight 4 at the origin. For $Z = 6$ the following analogous pentuplet:

$$\begin{aligned} S_1 &= (0, 1/31, 3/31, 8/31, 12/31, 18/31)\\ S_2 &= (0, 1/31, 4/31, 6/31, 13/31, 21/31)\\ S_3 &= (0, 1/31, 8/31, 11/31, 13/31, 17/31)\\ S_4 &= (0, 1/31, 4/31, 10/31, 12/31, 17/31)\\ S_5 &= (0, 1/31, 3/31, 10/31, 14/31, 26/31) \end{aligned} \quad (2)$$

exists; the vector diagram has a zero vector of weight 6 and vectors of unit weight at 1/31, 2/31, . . . 30/31. The order r of this type of multiplet seems to increase about as $Z^2/10$. I have calculated them for Z = 3, 4, 5, 6, 8, 9, 10, 12, 14, 17 and find corresponding multiplet orders r = 1, 2, 1, 5, 6, 4, 6, 18, 20, 6; $r = 0$ for $Z = 7$, that is, there is no set at all of this type. It is not clear from this that r is even an increasing function of Z: however, each r satisfies the remarkable formula:

$$r = \varphi(M)/6k \quad (3)$$

where $M = Z^2 - Z + 1$, $Z - 1 = p^k$ with k an integer and p always prime, and $\varphi(M)$ is Euler's number theoretic function—the number of numbers prime to and less than M. It is easily proved that $6k$ is always a factor of $\varphi(M)$ and I have also proved (1) that $r \leqslant \varphi(M)/6k$; (2) that there are certainly $\varphi(M)/6k$ 'generators' of (probably distinct) sets with vector diagram ($1/M$, $2/M$, . . . , $(M-1)/M$) and a zero vector with weight Z (the 'generators' are distinct cubics irreducible over a Galois field of characteristic p: the problem is to show which distinct cubics generate distinct homometric sets); (3) that the 'average order' of r increases with Z: all the evidence suggests the truth of (3). The 'average order' of $\varphi(n)$ is $6n/\pi^2$, so that the normal order of r is (probably) at least $\sim Z^2/\pi^2 k$ and could be slightly higher if the numbers M contain a high density of primes for which $\varphi(M) = Z^2 - Z$ Examples are $Z = 18$, $M = 307$ prime and r (probably) 51, or $Z = 42$, $M = 1723$ prime and r (probably) 287, or $Z = 60$, $M = 3541$ prime and r (probably) 590

These sets are somewhat divorced from reality because they show a dearth of short bonds (it would be interesting to know the reduction in R if point sets are required to satisfy additional conditions on the relative distribution of vector magnitudes), but this apart they seem important to me because. (1) they show distributions of vectors spread uniformly over the unit cell and except for the dearth of short bonds and especially when convoluted[6] with a (Gaussian or other) peak of finite width approximate to the featureless vector diagrams of many organic molecules, (2) any particular homometric multiplet of weight Z and order r is a degenerate example of a large class of multiplets of weight ZZ_0 ($> Z$) and order r (ref 6), (3) they seem capable of giving a clearer idea of 'what sort' of multiplicity r of solution we can expect for large values of Z We may add that these particular sets have considerable number theoretic interest, they all constitute examples of 'finite projective geometries' and as such were first examined by Singer[7] to whose key theorem my method of calculation of the multiplets for $Z = 8$, , 17 is essentially due Singer himself also calculated some of these sets and in effect conjectured the remarkable formula (3)

Examination of some of these sets suggests that each can exist when and only when all its points have equal weight In this they show some indication of achieving an optimum arrangement with maximum multiplicity so that (3) may indeed be the precise value of R when $Z = p^k + 1$ On the other hand, if the points can take on unequal weights the possibility for multiplicity with a fixed number N of points is enormously increased it has already been reported[5] that homometric pairs of sets of the same or different unequal weights can then exist, but we must add here that multiplets of only three points exist and with order as large as one likes Again the order can be increased only at the expense of increasing Z, the total (positive) integral weight of the 3 points This example indeed is only of theoretical interest since typical data are $r = 2$, $Z = 168$, $r = 3$, $Z = 1157$, $r = 4$, $Z = 8088$, and $Z\ (r = r_0 + 1) \approx 7 \times Z\,(r = r_0)$ but from it can be constructed almost homometric multiplets with integral weights of practical significance Moreover with larger numbers of points we might expect similar behaviour for a smaller average weight of each point

If rational non-integral individual weights are permitted multiplets of arbitrary order and three points can be constructed for any Z one cares to choose As the order increases for any fixed Z, solutions remain discrete but become progressively 'closer' From these can be constructed, of course, homometric multiplets of three Gaussian (say) functions of arbitrary order and with different total non-integral density in each comparable 'atom' if the atoms are not restricted to pre-assigned or similar shapes possibilities of multiplicity again increase This again forcibly illustrates the fundamental importance of approximately positive integral (or at least positive and finitely discrete) atomic numbers and essentially spacially discrete atoms of similar or *a priori* known shapes to the existence of a solution of the phase problem It is easily proved that all continuous periodic sets can be deformed continuously to continuous sets by infinitesimal deformations which leave their convolution stationary, and even by finite deformations other than simple finite translations which leave their convolution invariant it follows that R does not exist in this case In contrast it has been proved that it is never possible to deform a non-negative point set of integrally weighted δ-functions continuously by other than translation and leave its vector diagram unchanged Indeed. in this case a majority (in some sense) of sets of data have a unique solution (with the necessary corollary that a unique phase determining method then exists) Nevertheless even in this very restricted but practically important case there remain multiple solutions of arbitrary order some of which are reported here, and if the restriction to atoms of the same shape is removed so that they cannot be represented by δ-functions the theoretical possibility of the existence of multiplets of an at least enumerably infinite order has still to be excluded This might put some limitation on the validity of the determination of peak shapes and thermal motion by X-rays It would be interesting to know what minimal sets (if any) of conditions implicit in the physical situation are sufficient for the proof of the existence of an always unique or at most boundedly multiple solution of the X-ray 'phase problem'

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MAXWELL–MICHELL THEORY OF MINIMUM WEIGHT OF STRUCTURES

By PROF T M. CHARLTON

Department of Civil Engineering, The Queen's University of Belfast

IN 1869 [1] Maxwell stated the theorem "In any system of points in equilibrium in a plane under the action of repulsions and attractions, the sum of the products of each attraction, multiplied by the distance of the points between which it acts, is equal to the sum of the products of the repulsions multiplied each by the distance of the points between which it acts" He applied this theorem to plane pin-jointed frameworks as follows ' In a plane frame, loaded with weights in any manner, and supported by vertical thrusts, each weight must be regarded as attracted towards a horizontal base line, and each support of the frame as repelled from that line Hence the following rule

"Multiply each load by the height of the point at which it acts, and each tension by the length of the piece on which it acts, and add all these products together

"Then multiply the vertical pressure on the supports of the frame each by the height at which it acts, and each pressure by the length of the piece on which it acts, and add the products together This sum will be equal to the former sum If the thrusts which support the frame are not vertical, the horizontal components must be treated as tensions or pressures borne by the foundations of the structure, or by the Earth itself

"The importance of this theorem to the engineer arises from the circumstance that the strength of a piece is in general proportional to its section, so that if the strength of each piece is proportional to the stress which it has to bear, its weight will be proportional to the product of the stress multiplied by the length of the piece Hence these sums of products give an estimate of the total quantity of material which must be used in sustaining tension and pressure respectively "

Maxwell proved the theorem applied to frames by an argument using the concept of virtual work

In 1904[2] Michell deduced from Maxwell's theorem that "A frame therefore attains the limit of economy of material possible in any frame structure under the same applied forces, if the space occupied by it can be subjected to an appropriate small deformation, such that the strains in all the bars of the frame are increased by equal fractions of their lengths, not less than the fractional change of length of any element of the space"

In the following paragraphs Michell's theory is explained in modified form by means of the principle of virtual work, which is first explained in detail

If any system of forces in equilibrium is imagined to be subjected to any system of displacements which is compatible with the geometry of the force system, the net work done during the imaginary process is zero This is the principle of virtual work and it depends on the law of conservation of energy Application of the principle to frameworks is, perhaps, most easily appreciated by reference to pin-jointed frameworks Each joint of a loaded framework is in equilibrium under the influence of forces exerted by bars and external forces (loads or reactions), so that for an arbitrary imaginary or virtual displacement of a joint which is compatible with the geometry of the loaded framework

$$\Sigma f\Delta_B + \Sigma F\Delta_L = 0 \qquad (1)$$

where f is the force exerted by any bar connected to the joint, F is an external force acting on the joint, Δ_B and Δ_L are the components of the virtual displacement of the joint in the line of a bar and external force, respectively, and the summations embrace every bar and external force at the joint It should be noted that the products $f\Delta_B$ and $F\Delta_L$ are positive when the sense of the force and displacement component is the same Also the limit on the nature of virtual displacements is set by the condition of their interrelation on the basis of the geometry of the loaded framework, that is, they must not be such that gross distortion of the loaded framework is implied

The virtual work equations of all the joints of a framework may be added to give the virtual work equation of the whole, as follows

$$-\Sigma fel + \Sigma Fed = 0 \qquad (2)$$

in which the summations include all bars and all external forces, respectively Now the virtual work of the bars is expressed in terms of their forces and virtual changes in length, *el*, where *e* is a small fraction and the minus sign in equation (2) is to denote work done on a bar when it is subjected to a virtual change in length of the same sign as the force which it exerts, for example, virtual extension of a bar in tension The virtual displacements of the external forces F (loads and reactions), *ed*, are in the form of a fraction *e* times a length *d* which specifies the point of application of the respective force

Consideration of equation (2) indicates that if the system of virtual displacements of a framework is such that the loaded framework is imagined to shrink uniformly so that the same fraction *e* is applicable throughout, then

$$\Sigma fl = \Sigma Fd \qquad (3)$$

This is in accordance with Maxwell's concept for the purpose of proving his theorem applied to frameworks, as set out in the first paragraph

It is clear that for a definite system of loads and reactions the points of application of which are defined, the quantity on the right-hand side of equation (3) is constant, say, C An infinite variety of structures can, however, be used to transmit the loads and the fact that Σfl is the same for all of them is the salient feature of the analysis in relation to the least-weight aspect

Michell writes equation (3) here as follows

$$\Sigma f_p l_p - \Sigma f_q l_q = C \; (= \Sigma fl) \qquad (4)$$

where now f_p is the numerical value of the tension in any tie bar of length l_p and f_q is the numerical value of the thrust in any strut of length l_q He then goes on to say that if P is the greatest allowable tensile stress and Q is the greatest allowable compressive stress, the least volume of material in any frame (among those suitable for withstanding the specified system of loads and reactions) consistent with security is

$$V = \Sigma l_p \frac{f_p}{P} + \Sigma l_q \frac{f_q}{Q} \qquad (5)$$

The condition for V to be a minimum is the same as that for the expression

$$2PQV - (P - Q)C \qquad (6)$$

to be a minimum because V is the only variable Substitution from equations (4) and (5) in (6) shows, however, that V is a minimum when

$$(P + Q)\{\Sigma f_p l_p + \Sigma f_q l_q\} \qquad (7)$$

is least That is, V is least when the arithmetical sum of the products of lengths of bars and their forces is least This means that of all the frames which could be constructed in the space bounded by the points of application of the loads and the reactions, that which has the least volume of material has the smallest arithmetical sum of bar force and length products (This condition follows directly from equation (5) if $P = Q$)

Michell proceeds to derive the condition for $\Sigma f_p l_p + \Sigma f_q l_q$ to be least His method is rather tortuous and abstract and it is replaced here by what is believed to be a more practical, if less general, approach

Confining attention to the problem of the framework with the least volume of material to support a specified system of loads acting at specified points, with the aid of a specified system of reactions, the 'space' available is defined by the points of application of the loads and reactions Now the virtual work of the loads and reactions due to specified virtual displacements of their points of application causing virtual displacements throughout the structural space such that no bar suffers a change of strain numerically greater than ε is a definite quantity W The virtual work of the bars of any framework in the space for which the virtual displacements are compatible with those specified for the loads and reactions must then be equal to W, in spite of the fact that the number of bars and geometry of each possible frame are different Thus

$$W = \Sigma efl \qquad (8)$$

(where the summation is, of course, carried out algebraically taking account of the signs of *e* and *f* and includes all bars) for each of all possible frames within the limits of the space

Having regard to equation (8), it appears that the frameworks for which each of the terms *efl* ($e \leqslant \varepsilon$) has the same sign, that is, ties subjected to virtual extensions, struts to virtual contractions, make up the range of frameworks with the smaller values of $\Sigma f_p l_p + \Sigma f_q l_q = \Sigma |f| l$ If, in addition, all the virtual strains of the bars can be increases of the same amount ε, it follows that $\Sigma f_p l_p + \Sigma f_q l_q = W/\varepsilon$ is the smallest possible value This means that a framework attains the limit of economy of material possible in any framework subjected to the same system of applied forces with specified points of application, if the co-ordinates of its joints with reference to any specified origin can be imagined subjected to small changes such that the strains of all the bars receive equal virtual increases (It is emphasized that strain is defined as a fraction an increase of strain implies additional extension or contraction depending on whether the bar functions as a tie or a strut) Although the analysis is presented with reference to plane pin-jointed frameworks the implications appear to be quite general

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LETTERS TO THE EDITOR

PHYSICS

Kallman's Bipolar Negative Resistor

Kallman has described a bipolar negative resistance element using a few grains[1,2] or thin layers[2] of semiconductor material Later[2] he describes a technique using "plain iron wire" The purpose of this communication is to emphasize the elegant simplicity of the latter method, it is found possible to demonstrate many of his results in less than 1 h, using only readily available materials

About 60 cm of 20 s w g soft iron wire was cleaned with metal polish, and heated in air by a current transformer to just below red heat (judged in semi-darkness) After 25 min heating, the wire was removed, cut in half and connected to a 'Tektronix' type 575 transistor-curve tracer The wires were arranged to cross and touch lightly

After suitable adjustment of contact position and pressure, a curve (Fig 1) was obtained A rounded turnover at +4 8 V and 0 3 m amp is indicated followed by a negative resistance region up to 0 7 m amp A slope resistance of about −1 0 kΩ is obtained Further adjustment produced Fig 2, where the turnover is at about +5 5 V and 0 2 m amp, followed by a negative resistance region up to 0 7 m amp A slope resistance of about −1 7 kΩ is indicated, together with a burst of oscillation presumably due to energy storage circuits within the curve tracer being maintained in self-oscillation at the most active part of the negative resistance characteristic Both curves were taken with a 20 kΩ limiting resistance, the scales being 0 5 V/div horizontally and 0 2 m amp/div vertically The sweep voltage was a full-wave rectified 50 c/s sinewave

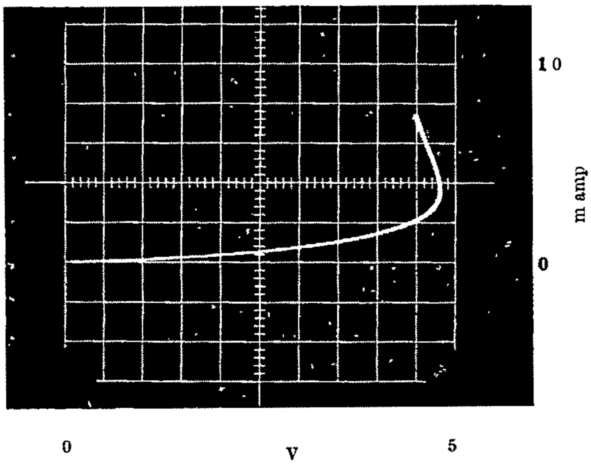

Fig 1

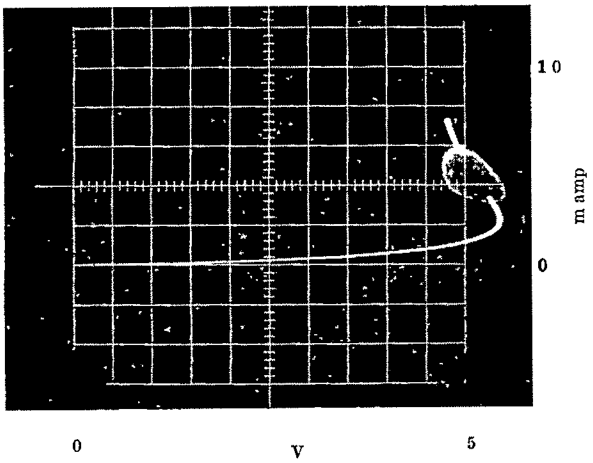

Fig 2

It was observed that a negative slope region could sometimes be formed from an all-positive-slope characteristic by suitable adjustment of the sweep drive, and that the negative slope could usually be destroyed by an increase of drive Kallman implies[2] that his characteristics were displayed with 1 2-kc/s sinewaves often gated to 60/sec 15 per cent duty factor so as to minimize heating

With the simple arrangement described here the characteristics shown were not as easily or stably produced or reproduced as suggested by Kallman, but the use of thinner wire (Kallman used 0·010 in diam) and a simple jig for adjusting the contact point and pressure should give a great improvement

H B S Brabham

The General Electric Co , Ltd ,
Electronics Research Laboratories,
Hirst Research Centre,
Wembley, Middlesex

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Ultrasonic Excitation of Nuclear Magnetic Resonance of the Copper-63 and Copper-65 Nuclei in a Single Crystal of Copper

This communication reports the observation of the ultrasonic excitation of nuclear magnetic resonance in a metal single crystal Using the direct method of Bolef and Menes[1] it has been found possible to detect the additional attenuation of an ultrasonic wave due to the nuclear spin-level transitions for both copper-65 and copper-63 nuclei in a copper single crystal

The specimen was in the form of a half centimetre copper cube of purity 99 999 per cent, the cubic faces being aligned along the [100], [010] and [001] directions Because of its high electromechanical efficiency and good acoustic impedance match to copper, a barium-titanate ceramic (80 per cent $BaTiO_3$, 12 per cent $PbTiO_3$, and 8 per cent $CaTiO_3$) (refs 2 and 3) transducer was used This was in the form of a 0 47-cm disk, plated on one flat surface The fundamental thickness resonance frequency of the transducer was 5 0 Mc/s and it was possible to detect mechanical resonances of the specimen over a bandwidth of 4 Mc/s (ref 3)

The specimen was coupled into a Pound Knight Watkins marginal oscillator[4] using a double tuned transformer similar to that utilized by Bolef and Menes[1] The magnetic field was provided by a Newport Instruments electromagnet which was swept by applying a sweep voltage to the control circuits

The magnetic field was modulated at 250 c/s and synchronous detection was used

The specimen was driven at a mechanical resonance frequency (ν) of 5 223 Mc/s and the magnetic field was swept through the field value where the Larmor precession frequency of the nucleus concerned was approximately $\frac{1}{2}\nu$, that is, the ultrasonic excitation of transitions with magnetic quantum number changes of $\Delta m = \pm 2$ were observed The magnetic field was measured using a proton resonance magnetometer[5]

The line shape of the ultrasonically induced resonance broadens and the ratio of signal strength to ultrasonic power decreases with increasing transducer voltage, due to saturation of the nuclear spin-levels The unsaturated line width of the resonance is difficult to detect with the present experimental equipment but is approximately 25 gauss for the copper-63 resonance and 50 gauss for the copper-65 resonance Both lines are shifted to higher field values from that corresponding to the ultrasonic frequency equal to twice the Larmor frequency, by 85 gauss and 170 gauss respectively The relative sensitivities of the two lines can be estimated both from the strength of the absorption signal and the signal necessary to saturate the resonances The ratio in both cases is approximately 2 3 1, in agreement with the isotopic abundance ratio

Quantitative experiments are to be carried out with an improved experimental system, and the theoretical possibility that the line width observed may be due to exchange interaction between the nuclei via the conduction electrons[6,7] is being investigated

D J **Barnes**

Physics Department,
Imperial College of
Science and Technology,
London, S W 7

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Dislocation Loops in Irradiated Iron

It has been suggested many times that the hardening of iron and steel on irradiation may result from the interaction of glide dislocations with dispersed clusters of point defects Two observations of such clusters using the technique of transmission electron microscopy have been reported in the literature[1,2] The work reported in this communication was undertaken to determine the crystallography of dislocation loops observed in pure iron

Thin foils of iron (approx 1000 Å thick) were irradiated with protons and Fe^+ ions of energy 150 KeV at temperatures in the range 20°–600° C Dislocation loops produced by this treatment had diameters ranging from 50 Å at low temperatures to 1500 Å at the higher temperatures The projected shape of the loops was elliptical in the proton irradiated foils and lozenge shaped in the foils irradiated with Fe^+ ions

A full crystallographic analysis has so far only been made for loops produced by irradiating with Fe^+ ions to a total dose of $\sim 1\ 7 \times 10^{16}$ ions/cm^2 at a rate of $\sim 4 \times 10^{14}$ ions/cm^{2}sec The specimen temperature was 500°–600° C

The crystallographic directions of the sides of the loop were determined by measuring the projected directions of the loop sides in a plane perpendicular to the electron beam On tilting the specimen through a large angle and repeating this procedure, the true directions of the sides were found Determining the directions of a pair of non-parallel sides gave the plane of the loop unambiguously All the observed loops were shown to be square, lying on $\{100\}$ planes with sides in the $<100>$ directions Sometimes loops were observed edge on and in these cases it has been possible to confirm that their poles lay very close to $<100>$

The Burgers vectors of the loops were determined uniquely by the standard diffraction contrast technique as pure $<100>$ edge dislocations[3] In the absence of any diffraction contrast within the loops which would indicate the presence of a stacking fault, it is likely that the Burgers vector is $a <100>$

The sense of the Burgers vector defines whether the dislocation loop has been formed by the condensation of vacancies or of interstitial atoms Methods[4,5] of determining the sense of b from diffraction contrast observations confirm that the loops result from the condensation of interstitial clusters

Estimates of the energies of various modes of condensation of point defects in the body-centred-cubic lattice indicate that the observed mode has low energy Other low-energy modes have $b = \frac{1}{2}a <111>$ since this is the slip vector, such loops may be sucked out of the foil by the image forces and hence would not be observed

B C **Masters**

Central Electricity Generating Board,
Berkeley Nuclear Laboratories,
Berkeley,
Gloucestershire

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METALLURGY

X-ray Asterism and Work-hardening in Crystals

This communication reports some observations pertaining to an experiment on the plastic behaviour of age-hardenable alloy single crystals The experiment formed a part of a senior year laboratory course and was intended to illustrate the strengthening effects due to well-known[1] precipitates in aluminium base copper alloys and also the fact that predictable lattice rotations are obtained for a given amount of tensile deformation in cases where only one slip system prevails[2] To this end it was planned that a number of crystals would be grown, their orientations determined, various heat treatments performed and elongations predetermined so as to give limited amounts of crystal rotation on the $<110>\{111\}$-system which could be checked by a second orientation determination after tensile deformation

It was found possible to grow single crystals of commercial 'Alcoa 2011' alloy by the strain anneal technique in spite of the high impurity concentration The nominal composition of this alloy is copper 5 5 per cent, lead 0 5 per cent, bismuth 0 5 per cent, silicon 0 4 per cent, iron 0 7 per cent, and zinc 0 3 per cent with the balance aluminium Half-inch diameter rod was rolled to a reduction of 57 per cent Tensile specimens were then machined, given a 5-min anneal at 550° C, pre-strained 2 per cent in tension and lowered into a 550° C salt bath at a rate of 0 5 in /h This produced single crystals throughout gauge-lengths several inches long Six crystals were given heat treatments which produce well-known[1] conditions in pure binary aluminium base copper alloys These were (*a*) Guinier–Preston zones of the first kind (*GP*I) The first coherent metastable decomposition product of the supersaturated solid solution—quench plus age 24 h at 130° C, (*b*) Guinier–Preston zones of the second kind (*GP*II)—quench plus age 24 h at 190° C, (*c*) θ′ (first over-aged, partially incoherent metastable precipitate)—quench plus age 24 h at 240° C, (*d*) θ, the equilibrium precipitate—quench plus age 24 h at 460° C, (*e*) θ, as produced by slow furnace cool from 550° C, (*f*) supersaturated solid solution—quench from 550° C Treatments *e* and *f* presumably yield θ and the supersaturated solid solution respectively Each crystal was then deformed in tension at 300° K in an 'Instron' testing machine at a rate of about 10^{-3} sec^{-1}

Pure binary aluminium–copper crystals, either as quenched or containing *GP*I or *GP*II zones, show crystal rotations characteristic of slip on only one system of the

<110>{111} type[2,3] In crystals containing θ′ or θ rotation is not detectable because sharp spots on X-ray patterns soon become smeared into arcs of Debye rings as plastic strain increases, indicating bending of lattice planes and probably simultaneous slip on many systems[2,3] In the present work, over-aged crystals (treatments *c*, *d* and *e*) behaved as expected in that they showed no lattice rotation, but unexpectedly crystals given zone hardening or quenching treatments (*a*, *b* and *f*) also behaved as just described

In all conditions the mode of fracture resembled that observed in binary aluminium–copper crystals in a zone-hardened condition[2,3], for example, extensive shear in a narrow band of {111} planes It is also of interest to compare values of critical resolved shear stress (CRSS) and rate of work hardening between 2011 alloy and binary aluminium–copper These comparisons follow

CRSS (kg/mm^2)

Treatment	*a* 24 h 130° C	*b* 24 h 190° C	*c* 24 h 240° C	*d* 24 h 460° C	*e* Furnace Cool	*f* Quench
2011 alloy	9 3	11 7	3 4	3 8	3 4	8 2
Al-3 85% Cu[2]	7 0	8 5	4 5	1 5	—	5 0[4]

Initial rate of work hardening $d\sigma/d\epsilon$ in kg/mm^2 from nominal stress versus percentage of elongation curves (σ versus ϵ)

Treatment	*a*	*b*	*c*	*d*	*e*	*f*
2011 alloy	0 94	0 40	2 34	2 80	1 87	0 35
Al-Cu avg [5]	------58---		----------360---------			

In summary it appears that for equivalent heat treatments 2011 alloy crystals are slightly stronger, work harden at about one-tenth the rate of zone hardened binary aluminium–copper crystals and one-hundredth the rate of precipitate hardened binary aluminium–copper crystals Neither the 'zones' nor the precipitates formed in 2011 have been studied in detail, however, it is possible that they produce higher coherency strains than their counterparts in binary aluminium–copper alloys, thus imparting higher strength The lower work-hardening rates are not understood, but it is possible that dislocations still simply shear through the 'zones', and concurrently produce new distortion of the slip plane as new interface is exposed This distortion could be responsible for the loss of the Laue spots and would also help explain why fractures resembled those of zone-hardened binary aluminium–copper crystals

The low rate of work hardening (1/100 of that of over-aged binary aluminium–copper crystals) and the observed mode of fracture for conditions *c*, *d* and *e* suggest that some precipitate shearing may also occur in the over-aged commercial material with dislocations more restricted to remain in one slip system than in the binary alloys Again, the loss of Laue spots could be due to the creation of increased distortions in the slip planes as further shearing of precipitates occurs rather than to slip on many systems

J G Byrne

Department of Metallurgy
Stevens Institute of Technology,
Hoboken, New Jersey

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CHEMISTRY

Surface Conduction in Volatile Organic Crystals

The relatively good reproducibility found for the dark conductivity of crystals of π-electron molecules[1] does not apply to the smaller aromatic molecules ($\leqslant C_{14}$)

Thus for anthracene, $\Delta\varepsilon$ values lie between 1 1 and 2 7 eV, where $K = K_0 \exp(-\Delta\varepsilon/2kT)$ The highest value would be that expected on theoretical grounds (cf ref 1, Fig 4) Pulse photo-conduction in anthracene, known to be a bulk property, leads to a mobility value μ of 1 cm^2/V sec[2], hence assuming band theory, where $K_0 \sim 4\mu$ ohm^{-1} cm^{-1}, a $\Delta\varepsilon$ of 2 7 eV would give a conductivity $K = 10^{-16\ 4}$ $ohm^{-1}cm^{-1}$, at 400° K Since the observed dark conductivities, largely for polycrystalline specimens, are around 10^{-12} $ohm^{-1}cm^{-1}$, at 400° K, there is clearly a big discrepancy

We have accordingly measured the electrical conductivity of a number of small aromatic molecules, as plates about 50 $mm^2 \times 1$ mm thick cleaved from crystals grown by the Bridgeman technique from zone-refined material The measurements were carried out under nitrogen or argon in a silica and glass cell designed to reduce so far as possible the rate of sublimation of the crystal A field strength of 1,000 V cm^{-1} was used with electrodes of silver paste, mercury, pressed aluminium and platinum and all gave similar results Provision was made to attach a guard ring in such a way as to avoid appreciable distortion of the field between the electrodes Resistance values of 10^{16} ohms were measurable in favourable cases

It has been found that without a guard ring a single crystal showed very similar behaviour to a disk formed by compressing the crystalline powder The conductivity values were found to depend, to a certain extent, on the form and thermal history of the specimen, but within the limits of the rather poor reproducibility the results for anthracene and naphthalene agreed with the earlier values However, when a guard ring was attached to the single crystal, the values fell below the limit of measurement, corresponding to an upper value for the specific conductivity for the temperature concerned, as below

Anthracene	K	10^{-15} ohm^{-1} cm^{-1}	at 180° C
Naphthalene	K	10^{-15} ohm^{-1} cm^{-1}	at 70° C
m-Dinitrobenzene	K	10^{-15} ohm^{-1} cm^{-1}	at 70° C
Benzoic acid	K	10^{-15} ohm^{-1} cm^{-1}	at 120° C

Electrode contacts were checked since a measurable conductivity existed between electrode and guard ring in all cases On the other hand, polycrystalline disks and single crystals with visible cracks showed no reduction in conductivity on attaching the guard ring

It therefore appears that the conductivity earlier found for these crystals was a surface conductivity Because of their volatility at the temperatures of measurement the crystal surfaces will hold a certain coverage of adsorbed molecules of the substances concerned in a two-dimensional mobile layer If we suppose these surface molecules become ionized and form the charge carriers we may expect, because of the high mobility and entropy of the charge carriers, a very high value of K_0 This could explain the measurable conductivities K observed in spite of the high $\Delta\varepsilon$ values observed (4 eV for some benzene derivatives[1])

Such a mechanism for surface conduction would predict a possible parallelism between $\Delta\varepsilon$ and molecular sublimation energy which is in fact observed This mechanism clearly becomes impossible for large involatile molecules such as phthalocyanine, where there is now good evidence for bulk currents[3] Also it cannot hold for pure polymeric systems such as proteins, where the μ values (about 10^4 cm^2/V sec) although large are still within the possibilities predicted by band theory and its modifications[1] Where very high K_0's arise in polymeric systems we may suspect a surface transport of carriers by plasticizer or impurity molecules

D D Eley
A S Fawcett
M R Willis

Department of Chemistry,
University of Nottingham

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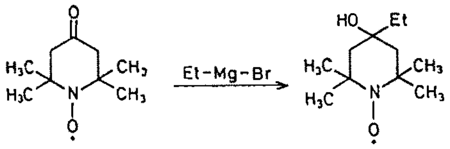
A New Organic Radical Reaction involving No Free Valence

THE discovery of a fundamentally new type of organic radical reactions involving no unpaired electron has been already reported[1–3] The work recorded here was concerned with a reaction between a free radical, triacetoneaminoxyl and the Grignard reagent The reaction proceeding with conservation of free valence yielded a new organic free radical with a hydroxyl group



After distillation in vacuum, the crude product appeared as a bright red oily substance readily soluble in water and in organic solvents Analysis by means of paper chromatography has shown that the oil was a mixture of two radicals 2,2,6,6-tetramethyl-4-ethyl-4-hydroxypiperidinoxyl and triacetoneaminoxyl (Fig 1)

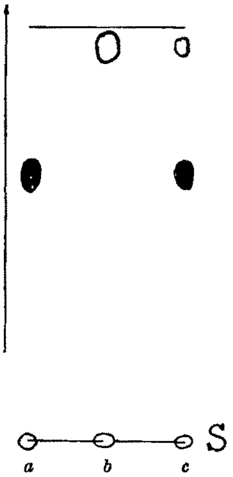
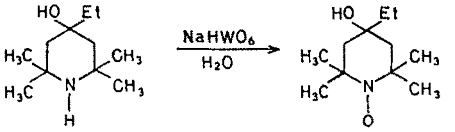


Fig 1 Paper chromatography (methanol acetone = 1 1) (a) 2,2,6,6-tetramethyl-4-ethyl-4-hydroxypiperidinoxyl, (b) triacetoneaminoxyl, (c) paramagnetic red oil

An individual paramagnetic compound with a melting point of 67°–69° was obtained on chromatographic purification of the crude product on aluminium oxide (found 0 480 per cent H (active), molecular weight 199 4, spin/mole $5\ 0 \times 10^{23}$, calculated 0 503 per cent H (active), molecular weight 200 3, spin/mole $6\ 0 \times 10^{23}$)

The new free radical represented an orange-yellow crystalline compound, soluble in water and in organic solvents, suffering no change for a year under the usual conditions

The ultra-violet (λ_{max} 227 and 235 mμ) and infra-red absorption spectra (Fig 2) were found to be identical to those of 2,2,6,6-tetramethyl-4-ethyl-4-hydroxypiperidinoxyl obtained by direct catalytic oxidation of 2,2,6,6-tetramethyl-4-ethyl-4-hydroxypiperidine by the scheme



The electron spin resonance spectrum of the crystalline radical represented a singlet type curve When the sample was solved in benzene, it turned into a triplet similar to the initial ketone radical spectrum (Fig 3)

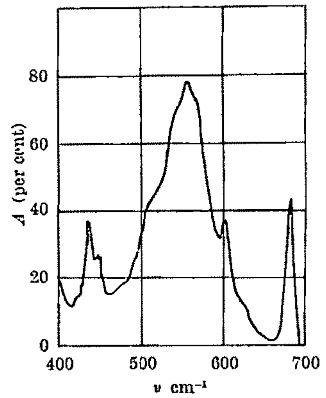


Fig 2 Infra-red spectrum of a solid free radical

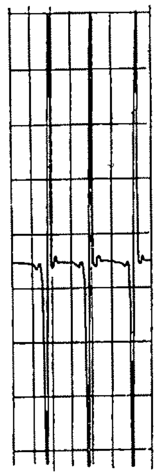


Fig 3 Hyperfine structure of a free radical electron spin resonance spectrum

The low-intensity doublets in the hyperfine structure of the spectrum (splitting of 5 4 gauss) seem to be due to an admixture of a natural carbon-13 isotope (spin 1/2)

M B NEIMAN
E G ROZANTZEV
YU G MAMEDOVA

Institute of Chemical Physics,
Academy of Sciences,
Moscow

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A Direct Hydrocarbon/Air Fuel Cell

A CONSIDERABLE amount of effort is being expended at present to develop fuel cells capable of burning hydrocarbons directly and efficiently to carbon dioxide and water Most of the work so far has been confined to temperatures below 100° C using sulphuric acid as the electrolyte and platinum metals as the catalytic surface Under these conditions it has been possible to demonstrate current densities of up to 10 or 20 m amp/cm² at very low cell voltages, leaving in considerable doubt the feasibility of making efficient working devices

We have carried out investigations using phosphoric acid of various concentrations as the electrolyte up to temperatures of 220° C, also using platinum for the electrode catalytic surface Fig 1 shows the polarization obtainable on simple platinum black electrodes using propane as the fuel Polarization is shown versus the reversible hydrogen electrode in the same electrolyte It can be seen from these results that current densities

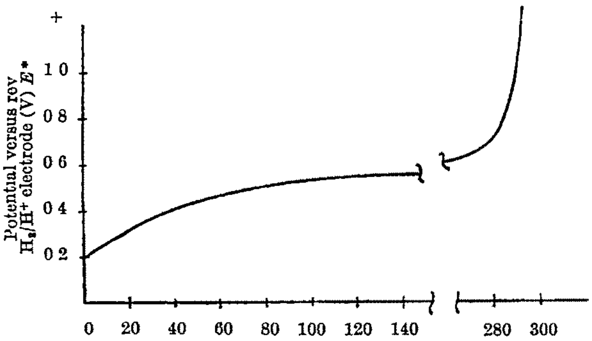

Fig 1 Polarization curve for propane/platinum black electrode 85 per cent phosphoric acid, 160° C

of the order 100–200 m amp/cm^2 are attainable under these conditions and at over-all cell voltages corresponding to thermal efficiencies between 20 and 35 per cent It has been observed with propane and higher hydrocarbons that the major product of oxidation is indeed carbon dioxide, but that methane also can be formed The formation of methane is favoured under open circuit conditions, which leads us to suspect that the reaction is purely a heterogeneous catalytic process involving cracking of the hydrocarbon to form "surface carbide complexes" and methane It is not likely that the formation of methane is an electrochemical process since at potentials of 200–300 mV positive to E^* (reversible hydrogen potential) the formation of methane would not be thermodynamically favoured

It would seem that it is a 'simple' cracking process which would require a finite value for the surface coverage with respect to hydrogen, that is, θ_H The rate of formation of methane will be a function of θ_H and it is well known that at potentials in the region of 200–300 mV E^* θ_H on platinum is very small

We feel that these results conclusively demonstrate the feasibility of a direct hydrocarbon/air fuel cell A considerable amount of work must now be directed towards demonstrating that such cells can be made to function in a practical fashion and can be built and operated economically

H G Oswin
A J Hartner
F Malaspina

Leesona Moos Laboratories,
Division of Leesona Corp ,
Great Neck, New York

Photolysis of Sulphate Ion

The sulphate radical ion has been postulated as an intermediate in thermal reactions[1] and in the photolysis of peroxydisulphate ion in aqueous solution[2] Recently, it has been suggested as a possible intermediate in the flash photolysis of aqueous ferrous sulphate solutions[3]

We have determined the absorption spectrum of aqueous sulphate ion in neutral solution at 25° C using a Hilger Uvispek with nitrogen flushing as previously described[4] The spectrum corresponds to previously reported data[5,6] The value of the molar absorption coefficient of sulphate ion at 1849 Å was found to be 202 l mole^{-1} cm^{-1}

In view of the fact that many photolyses are carried out in systems containing sulphate ion, it was decided to investigate the possibility of direct photolysis of the ion by 1849 Å radiation

A de-aerated aqueous solution consisting of 1 0 × 10^{-1} M sodium sulphate and 1 0 × 10^{-1} M methanol was irradiated using a low-pressure mercury vapour discharge lamp, the apparatus and technique being similar to that described previously[7] The fractions of 1849 Å radiation absorbed by the components of this solution were $SO_4^{=}$ 0 905, CH_3OH 0 028 and H_2O 0 067 Conditions of complete absorption were realized in the photolysis, the results of which showed a yield of hydrogen of 1·62 × 10^{-5} mole l^{-1} min^{-1} The quantum yields for water and methanol[7] photolysis are 0 6 and 1 0 respectively at the wave-length used so that hydrogen yields arising by direct photolysis of these components can be calculated for the foregoing experiment They are 10 3 × 10^{-7} mole l^{-1} min^{-1} from water and 7·2 × 10^{-7} mole l^{-1} min^{-1} from methanol Thus 1 45 × 10^{-5} mole l^{-1} min^{-1} of hydrogen must arise from direct absorption by sulphate ion This result would indicate a value of 0 63 for the quantum yield for the direct photolysis of sulphate ion in the presence of 1 0 × 10^{-1} M methanol

We have observed that the quantum yield for sulphate photolysis varies with the methanol concentration This is due to several factors, one of which is a change in the absorption characteristics of the solutions as the methanol concentration increases Further work is being carried out together with a detailed investigation of the effect of methanol addition on the spectrum of sulphate ion

Although the intensity of absorption by sulphate ion is low compared with intensities associated with many electron transfer spectra it is possible that the initial absorption process may be represented by

$$SO_4^{=}\,H_2O \rightarrow (SO_4^{-}H_2O^{-})^{*} \rightarrow SO_4^{-} + H + OH^{-} \qquad (1)$$

followed by

$$SO_4^{-} + H_2O \rightarrow HSO_4^{-} + OH \qquad (2)$$

the net effect being the indirect photodissociation of water

Free radicals can be detected by including organic compounds in the system which act as scavengers by providing extractable hydrogen atoms The scavenger used in this work was methanol In the presence of methanol reaction (2) may be replaced by the energetically more favourable process

$$SO_4^{-} + CH_3OH \rightarrow CH_2OH + HSO_4^{-}$$

CH_2OH radicals can also arise by

$$H + CH_3OH \rightarrow CH_2OH + H_2$$
$$OH + CH_3OH \rightarrow CH_2OH + H_2O$$

subsequent reactions of the radical being

$$2\,CH_2OH \rightarrow (CH_2OH)_2$$
$$CH_2OH + OH \rightarrow H_2O + CH_2O$$
$$\cdot CH_2OH + SO_4^{-} \rightarrow HSO_4^{-} + CH_2O$$
$$CH_2OH + H \rightarrow H_2 + CH_2O$$

so that the expected products would be gaseous hydrogen, and in solution formaldehyde and ethylene glycol, the product balance being

$$\varphi_{H_2} = \varphi_{CH_2O} + \varphi_{(CH_2OH_2)_2}$$

Investigation of the solution products does reveal a balance between the gas yield and the yields of formaldehyde and ethylene glycol as suggested Formaldehyde was found to be 15 per cent of the gas yield and ethylene glycol 85 per cent, which are identical to those found in the water–methanol system[7] This suggests that the photolysis of sulphate ion may give radicals identical to those present in the direct photolysis of water

One of us (M F F) thanks the Hertfordshire County Council for the award of a research assistantship

Jack Barrett
M F Fox
A L Mansell

Department of Science,
Hatfield College of Technology,
Hatfield, Herts

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Donnan Equilibria in Polyelectrolyte Solutions

MEMBRANE equilibria, involving a macromolecule, impermeable through the membrane, and a permeable salt, were first investigated by Donnan[1]. He found that at equilibrium the salt was unevenly distributed between the two sides of the membrane, and he advanced a theory to explain the effect. Since then this effect has been intensively examined, first especially with proteins, later even with linear polyelectrolytes.

In recent years numerous theoretical[2–6] papers on the subject have appeared whereas much less attention has been given to the experimental side of the problem, and accurate data are, in general, lacking. In the work recorded here precision measurements on a linear polyelectrolyte and a non-electrolyte polymer are reported. It is shown that the classical Donnan theory alone does not explain the behaviour of linear polyelectrolytes and that in addition an 'excluded volume' effect is present. In this it is assumed that the permeable salt is partly excluded from the interior of the polyelectrolyte molecule. The excluded volume effect has also been considered by Strauss and Ander[7] in an investigation of polyphosphate solutions.

The polymers used in the present experiments were poly-methacrylic acid (PMA) and poly-oxy-ethylene (POE $(CH_2CH_2O)_n$). The permeable electrolytes were sodium hydroxide and sodium hydroxide in the presence of sodium chloride. The dialysis cell was essentially an enlarged block-type osmometer cell, made of polyethylene and with a membrane of 'Cellophane'. The equilibration of the solutions was carried out at 25° C for 24 h. This was well in excess of the actual time necessary for the equilibrium to be established.

The concentration of sodium hydroxide was determined by titration with hydrochloric acid. In solutions containing PMA a sharp equivalence point was found near *p*H 10, where the excess sodium hydroxide was neutralized, but the PMA was still in the form of its sodium salt. This point could be accurately determined with thymolphthalein as indicator. In the actual experiments 25 ml. of the equilibrated solutions were transferred with a pipette to glass stoppered flasks and weighed. Most of the alkali in the solutions was then neutralized by adding to each flask 25 ml. of a standard hydrochloric acid solution with a precision pipette. The final neutralization of the alkali was carried out by titrating with a 0.05 M hydrochloric acid solution using a microburette and thymolphthalein as indicator. By this method the concentrations of sodium hydroxide in the two solutions could be determined with a relative accuracy of about 0.01 per cent. The concentrations were expressed in weight units with the following designations

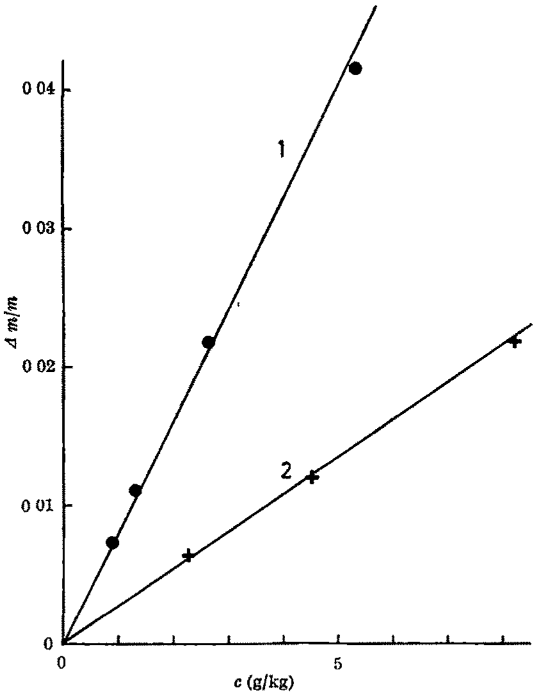

Fig. 1. Measurements at constant sodium hydroxide concentration. Curve 1 for PMA with m = 0.25 M/kg, curve 2 for POE with m = 0.5 M/kg

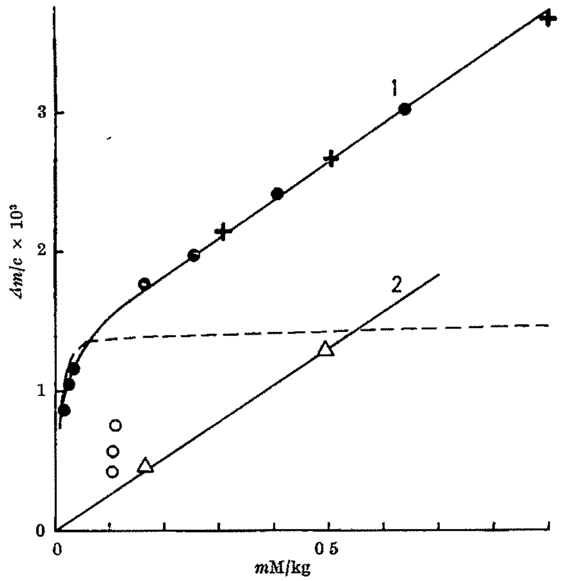

Fig. 2. Measurements at constant polymer concentration. Curve 1 for PMA with c = 5.2 g/kg, curve 2 for POE with c = 4.5 g/kg. Open circles represent Δm for sodium hydroxide in PMA solutions in the presence of sodium chloride, from top to bottom m_{NaCl} = 0.20, 0.40 and 0.80 M/kg. The crosses on curve 1 represent the corresponding Δm values referring to the total permeable electrolyte concentration. The dotted curve represents equation (1) with c = 5 g/kg and with ε = 0.25

permeable electrolyte	m moles per kg of solution
polymer	c g per kg of solution

The results are shown in Figs. 1 and 2. They are expressed in terms of $\Delta m = m - m_1$, where m_1 and m are the m-values for the solution containing the polymer and for the solvent respectively. Fig. 1 shows some results from measurements at constant sodium hydroxide concentration and varying polymer concentrations. We see that with both polymers Δm is very nearly proportional to the polymer concentration. In Fig. 2 the polymer concentration is kept constant and the concentration of the permeable electrolyte varied. With PMA some measurements were carried out with sodium hydroxide in the presence of varying amounts of sodium chloride. We see that Δm for sodium hydroxide is depressed by the presence of sodium chloride. However, if we assume that chloride is displaced to the same extent as sodium hydroxide, and we calculate Δm for the total amount of permeable electrolyte, we find that the points fall on the curve for pure sodium hydroxide. This indicates that both electrolytes behave similarly in this connexion.

The classical Donnan theory gives for Δm the following formula

$$\Delta m = \frac{\dfrac{c\varepsilon}{M}}{1 + \sqrt{1 + \dfrac{c\varepsilon}{Mm_1}}} \qquad (1)$$

where ε is the degree of dissociation of the polyelectrolyte and M its equivalent weight. In Fig. 2, together with the experimental results, a curve representing equation (1) for PMA with c = 5 g/kg and with ε = 0.25 is shown. We see that equation (1) cannot be made to fit the experimental results.

For the excluded volume effect we have in the first approximation

$$\Delta m = \alpha m c \qquad (2)$$

where α determines the excluded volume (in the present units it equals the mass of solution in kg/g polymer, from which the permeable electrolyte is excluded)

Comparing equation (2) with the experimental results, we see that with POE the agreement is excellent From both diagrams we obtain $\alpha = 0\ 0027$ kg/g As the polymer mass itself is included in α this means that for each gram of the polymer there are 1 7 g of (solvating) water from which sodium hydroxide is excluded With PMA the situation is more complicated A comparison of the experimental results in Fig 2 with the predictions of the Donnan theory and the excluded volume effect reveals that the experimental curve may be obtained by a superposition of these two effects It is interesting to note that the excluded volume effect for PMA happens to be of the same magnitude as for POE

I may conclude by stating that the experiments recorded here indicate that in linear polyelectrolyte solutions two effects are present, namely, the classical Donnan effect and the excluded volume effect The latter occurs even with non-electrolyte polymers These matters are treated further in a paper to be published in *Acta Chemica Scandinavica*

H Vink

Institute of Physical Chemistry,
University of Uppsala

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Partial Differentiation, with Special Reference to Thermodynamics

From time immemorial savants and scholars have taken liberties with well-known words and phrases and by 'new definitions' have imparted highly specialized meanings to familiar expressions While in some cases such actions can be justified on the grounds of greater clarity of meaning and greater precision of definition, as a general practice the imparting of specialized meanings to well-known words and phrases tends to obscure any language, even the language of mathematics, particularly when the specialized meanings involve largely meaningless 'new definitions'

In a recent communication, Elliott[1] has suggested a new definition for the partial derivatives occurring in thermodynamics The approach is based on the form

$$dz = a dx_1 + b dx_2 + c dx_3 \qquad (1)$$

and it is stated that a, b, and c in the limit of small displacement dx_1, dx_2, dx_3 are to be identified with the new partial derivatives and indicated as $\frac{\partial z}{\partial x_1}$, etc

No restrictions are imposed on the manner in which the limit is to be taken and, in our opinion, the failure to do this renders the definition meaningless

As an illustration of our criticism let us introduce the symbols Z_1, Z_2, Z_3 to represent the 'old' derivatives of z, that is, Z_1 is the rate of change of z with respect to x_1 keeping x_2 and x_3 constant Then to a first order of small quantities

$$dz = Z_1 dx_1 + Z_2 dx_2 + Z_3 dx_3 \qquad (2)$$

Evidently by keeping x_2 and x_3 constant we can deduce from (1) and (2) that

$$a = \frac{\partial z}{\partial x_1} = Z_1$$

On the other hand, for $dx_1 = dx_2$ and x_3 kept constant

$$\frac{\partial z}{\partial x_1} = Z_1 + Z_2$$

and so on The symbol $\frac{\partial z}{\partial x_1}$ can have an infinity of values depending on how we approach the limit

Dr Elliott implies that the freedom from restrictions in taking the limit is one of the merits of his definition We believe it can only lead to confusion in a field where precise definitions are essential

G C Benson
E A Flood

Division of Pure Chemistry,
National Research Council of Canada,
Ottawa

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The preceding communication shows that if the partial differential indicated by $\partial z/\partial x_1$ is defined as the limiting value of the rate of change of z with respect to x_1, and if we include changes due to the variation of x_2 (which is dependent on x_1) then the result obtained depends on the relation between x_1 and x_2 and in particular if $dx_1 = dx_2$ the expression is

$$\partial z/\partial x_1 = Z_1 + Z_2$$

This 'definition' is therefore quite useless, and on this point there is no dispute

On the other hand, if we define the partial differential in the way proposed in my previous communication we always obtain the result $\partial z/\partial x_1 = a$ and no other answer is possible The symbol $\partial z/\partial x_1$ is to be regarded simply as a rather clumsy but time-honoured method of indicating the coefficient a or rather its limiting value when the neglect of differentials of higher order than the first in forming equation (1) above is strictly justified The new definition is strictly independent of any relations between the variables

G A Elliott

Department of Chemistry,
University of Western Australia,
Perth

BIOCHEMISTRY

Enzymatic Evidence for the Configuration of *d*-Isocitric Acid

The configuration of the α-carbon atom of the *d*-isocitric acid of the Krebs tricarboxylic acid cycle was designated L_S on the basis of an application of the Levene displacement rule[1], but subsequent chemical[2-4] and X-ray[5] studies indicated the D_S configuration Other investigations have shown that the α- and β-asymmetric centres of *d*-isocitric acid possess opposite configurations[6,7]. Although *d*-isocitric acid may be distinguished by the action of isocitric dehydrogenase from the three isomeric isocitric acids, none of the isomers of α-aminotricarballylic acid, the α-amino analogue of isocitric acid, has thus far been found to be attacked by enzymes Greenstein and Winitz[1], who prepared both DL-α-aminotricarballylic and DL-allo-α-aminotricarballylic acids, were unable to find an enzyme capable of resolving these and therefore resorted to the use of brucine They thus obtained the corresponding four optical isomers, which they converted to the respective isocitric acids by treatment with HNO_2

Application of the Clough–Lutz–Jirgensons rule permitted assignment of configuration to the D- and L-isomers of *allo*-α-aminotricarballylic acid, but assignment of the configurations of the isomers of α-aminotricarballylic acid was not possible by this procedure. Application of the Levene displacement rule[8] to the derived isocitric acid isomers indicated the L_S configuration for *d*-isocitric acid and therefore also for *l*-α-aminotricarballylic acid, the amino-acid isomer from which it was derived.

The enzyme recently obtained in this laboratory[9] from mammalian kidney that catalyses the optically specific cyclization of D-glutamic acid to D-pyrrolidone carboxylic acid also catalyses the cyclization of the D-isomers of α-methylglutamic acid, γ-methylglutamic acid, and one-half of several racemic glutamic acid derivatives including α-aminotricarballylic and *allo*-α-aminotricarballylic acids, which may be considered as glutamic acids possessing β-carboxyl groups. DL-*Allo*-α-aminotricarballylic acid on treatment with HNO_2 was previously shown to give isocitric acid that was unreactive with isocitric dehydrogenase[10].

DL-α-aminotricarballylic acid was prepared by acid hydrolysis of the corresponding DL-pyrrolidone-α,β-dicarboxylic acid (kindly provided by Dr. Milton Winitz). Incubation of DL-α-aminotricarballylic acid with the mouse kidney enzyme[9] led to disappearance of approximately 50 per cent of the ninhydrin-reactive material; on addition of more enzyme no further amino-acid disappeared. The reaction mixture was deproteinized and placed on a column of 'Dowex 50' in the H^+ form. The water effluent containing the enzymatically formed D-pyrrolidone-α,β-dicarboxylic acid was evaporated to dryness and hydrolysed with 2 N hydrochloric acid for 2 h at 100°. The L-α-aminotricarballylic acid was eluted from the column with 2 N ammonium. The D- and L-α-aminotricarballylic acids thus obtained were converted to the corresponding isocitric acid isomers by reaction with HNO_2. Assay with the isocitric dehydrogenase–NADP system indicated that the isocitric acid obtained from the amino-acid isomer that had cyclized was enzymatically active and accounted for 80 per cent of the *d*-isocitrate obtained from a control sample of DL-amino-acid, which was not treated with the cyclizing enzyme. That the amount of *d*-isocitrate recovered was less than theoretical may be ascribed to incomplete conversion of amino-acid to hydroxy acid under the conditions adopted, and to losses during elution and other manipulations. The isocitrate obtained from the α-aminotricarballylic acid isomer that was eluted from the column with ammonium contained only 7 per cent as much *d*-isocitrate as found in the isocitrate preparation obtained from the cyclized product; this finding is consistent with the observation that cyclization reaches equilibrium at about 95 per cent of completion[9]. These enzymatic studies are therefore in agreement with the chemical and X-ray findings and provide additional evidence that the isocitric acid of the Krebs cycle has the D_S-configuration and may, in accordance with the nomenclature proposed by Vickery[11], be designated *threo*-D_S-isocitric acid.

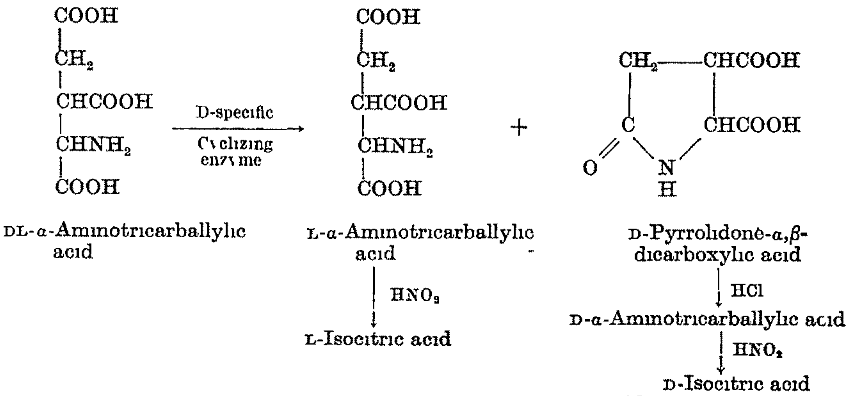
COOH
CH₂
CHCOOH
CHNH₂
COOH
D-specific
Cyclizing
enzyme
COOH
CH₂
CHCOOH
CHNH₂
COOH
+
CH₂—CHCOOH
C
CHCOOH
O
N
H
DL-α-Aminotricarballylic acid
L-α-Aminotricarballylic acid
HNO₃
L-Isocitric acid
D-Pyrrolidone-α,β-dicarboxylic acid
HCl
D-α-Aminotricarballylic acid
HNO₂
D-Isocitric acid
[threo-Ds-Isocitric acid]

Fig. 1

The work was supported by the U.S. National Institutes of Health and the National Science Foundation.

ALTON MEISTER
MARYELLEN STRASSBURGER

Department of Biochemistry,
Tufts University
School of Medicine,
Boston, Mass.

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Distribution of Carbon-14 in Polysaccharide after Photosynthesis in Carbon Dioxide labelled with Carbon-14 by *Anacystis nidulans*

THE reductive pentose phosphate cycle[1] has been proposed as the means of formation of hexose during photosynthesis. The enzyme fructose-1,6-diphosphate aldolase is an integral part of the cycle. It was therefore unexpected that Richter[2] could not detect aldolase in extracts of the photoautotrophic blue-green alga, *Anacystis nidulans*. This observation was confirmed and extended by Fewson, Al-Hafidh and Gibbs[3] to include other members of the Cyanophyceae. Recently, Kandler[4] reported that the distribution of carbon-14 in the products formed during photosynthesis in $^{14}CO_2$ by *A. nidulans* was similar to those obtained with *Chlorella*, an alga which contains aldolase. It therefore became of interest to examine the distribution pattern of carbon-14 in the polysaccharide formed during photosynthesis in $^{14}CO_2$ by the two types of algae.

A. nidulans and *C. pyrenoidosa* were grown as described in a previous publication[3]. After collection, the cells were suspended at a concentration of 2 g of wet weight per 100 ml of 0.025 M potassium phosphate, *p*H 7.0. Twenty-five ml of cell suspension were placed in a glass 'lollipop', which was illuminated from each side by two incandescent spotlights each of which gave 4,000 ft.-candles at the surface of the 'lollipop'. After 10 min of illumination with air being continuously bubbled through the suspension, $NaH^{14}CO_3$ (348 μc./m.mole) was introduced. When the required time-period had elapsed (Table 1), the algal suspension was dropped into 250 ml of boiling 90 per cent (v/v) ethanol. The suspension was centrifuged and the solid material was extracted with absolute ethanol, 20 per cent (v/v) ethanol, and finally with water. The residue remaining was heated with 15 ml of 1 N hydrochloric acid for 1.5 h in a 100° water bath. The neutralized solution was deionized by passing it through 'Dowex 50 *W* (H^+)' and then through 'Amberlite *CG*-4*B*(OH^-)'. The effluent was evaporated under vacuum at 30° and then streaked on Whatman No. 3 MM paper along with unlabelled glucose and galactose. The chromatogram was developed with a mixture of *n*-butanol, 95 per cent ethanol and

Table 1 DISTRIBUTION OF CARBON-14 IN POLYSACCHARIDE GLUCOSE FORMED DURING PHOTOSYNTHESIS USING *A nidulans* AND *C pyrenoidosa*

Time (sec)	Relative specific activity of the carbons of glucose on the basis of C-4 = 100					
	C-1	C-2	C-3	C-4	C-5	C-6
			Chlorella pyrenoidosa			
5	5 0	5 4	73	100 (1 0)	0 8	0 8
10	5 2	5 4	80	100 (7 5)	1 5	2 4
20	14	12	86	100 (21)	8 5	9 6
30	20	17	86	100 (19)	14	18
			Anacystis nidulans			
5	5 6	1 6	55	100 (0 66)	0 9	0 9
10	9 3	5 5	68	100 (6 1)	0 8	2 1
20	18	8 0	77	100 (11)	3 9	9 0
30	26	21	73	100 (27)	11	19

Value in parenthesis is the specific activity of C-4 expressed in mμc /mg carbon

water (52 32 16) Only one band of radioactivity was found This band corresponded to glucose and was identified by cochromatography with authentic sugar and by its colour reaction with aniline hydrogen phthalate Each glucose sample together with 600 μmoles of carrier glucose was degraded by the *Leuconostoc mesenteroides* method[5] Table 1 shows the results The asymmetric pattern of distribution of carbon-14 in the glucose of the polysaccharide isolated from both algae is almost identical at any given time

These results and those of Kandler[4] suggest the following (1) aldolase is present in *Anacystis* and *Chlorella* and the reductive pentose phosphate cycle functions in both organisms, but the enzyme is not detected in extracts of *Anacystis* for various reasons[3], (2) aldolase is not involved with the conversion of carbon dioxide to carbohydrate during photosynthesis, (3) different mechanisms of photosynthetic assimilation of carbon dioxide operate in the two algae but both mechanisms give rise to the same distribution of carbon-14 pattern

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PAUL KINDEL*
MARTIN GIBBS

Department of Biochemistry,
Cornell University,
Ithaca, New York

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* Predoctoral Fellow of the U S Public Health Service Present address Max-Planck-Institut fur Zellchemie, Karlstrasse 23, Munich, Germany

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Natural Occurring Variations in Rat Liver DNA Content

THE content of deoxyribonucleic acids (DNA) in non-dividing somatic cells is believed to be constant[1,2] For this reason the nucleic acid content is a reference standard in several biochemical investigations On the other hand, there are few data which lead to the conclusion that DNA content is not very constant[3] and one has to assume that DNA occurs also in a metabolic form[4]

In an investigation of the influence of sex and season on the diurnal rhythm of rat liver content, nucleic acids were biochemically and histologically determined This investigation was carried out with untreated animals of an inbred Wistar strain of both sexes kept under constant conditions (temperature, humidity, free access to food and water) The determination of nucleic acids after fractionated extraction and ultra-violet spectrography and the Dische test has shown that the DNA-content varies very little during day-time in the spring The content is about 18 γ/100 mg liver wet weight In the night there is a strong increase of the DNA with a maximum of 73 γ/100 mg There are no differences between sexes by the time the peak is reached Assays carried out in winter (January 1963) show quite different patterns of amount of DNA when estimated every 2 h for 24 h Here I found two marked peaks (12 h and 18 h) during the day, while at night the content is low and without very strong variations Sexual differences are marked in winter by a time difference of 2 h reaching the maximum peaks The proportions of the values are the same when DNA-content is calculated for the dry weight of total protein content

This leads to the conclusion that DNA changes not only in a diurnal rhythm pattern, but also that this pattern is influenced by the sex and season

GABRIELLE HORVÀTH

Laboratorium voor Cyto-Histologie,
Universiteit Nijmegen, Holland

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Disassociation of Heart Cells by Collagenase

THE disassociation of cells from various parent tissues by digestion with trypsin has been often applied by workers on tissue cultures[1-3] There are, however, several defects in the trypsinization method when the goal is use of the cells for physiological investigations We have examined various possible methods for avoiding these difficulties and have found that the substitution of collagenase for trypsin satisfied most of our requirements

The criteria for a suitable disassociating enzyme were (*a*) cell damage must be minimized, (*b*) cell yield should be high; (*c*) the mucus-like strands which appear during trypsinization should be absent, (*d*) the ionic environment during disassociation should be normal It was observed that, using trypsin, the proportion of injured or dead cells as indicated by the nigrosin uptake method[4] was fairly high, particularly when old embryonic hearts (11–16 days) were used It was also noted that quite large amounts of non-cellular material and ropes of mucus-like substance were always present following tryptic digestion Furthermore, many workers[2] when using trypsin have found it desirable to reduce the concentrations of divalent cations in the digestion medium In the work recorded here it was felt that the physiologically important factors to be studied (ion transport, transmembrane potentials, etc) might be seriously, possibly irreversibly, damaged by exposure to such an abnormal environment

The mucoid material in trypsinized suspensions suggested that collagen was present, accordingly, collagenase

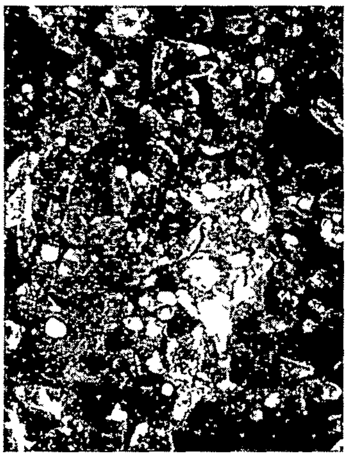

Fig 1 Collagenase 24 h culture (× *c* 245)

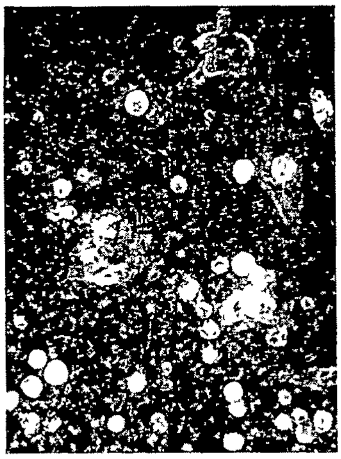

Fig 2 Trypsin 24-h culture (× *c* 245) Note large amount of non-cellular material, still rounded cells (many characteristically flattened cells were present elsewhere in culture, see Fig 4)

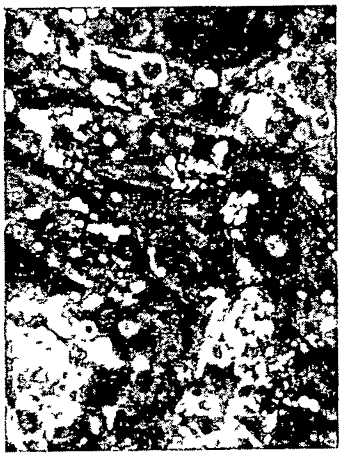

Fig 3 Collagenase 24-h culture (× *c* 700)

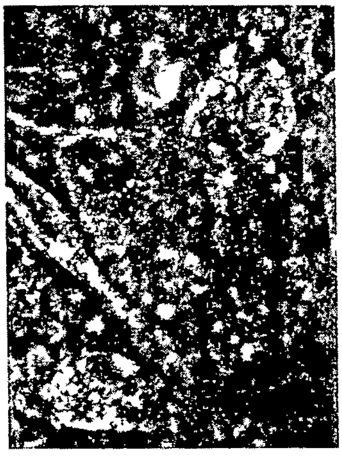

Fig 4 Trypsin 24-h culture (× *c* 700) Note that collagenase and trypsin yield essentially the same cytological characters but that the cell density is somewhat greater per field than with trypsin and there is less debris present

(partially purified collagenase, Worthington Biochemical Corp , Freehold, New Jersey) was tested alone and in mixtures containing trypsin or elastase or both Collagenase alone was as effective as trypsin, elastase was a very poor disassociating agent

The results of collagenase disassociation were (1) No mucoid strands were present (2) As measured with an

Table 1 COMPARISON OF CELL SIZE DISTRIBUTIONS

Trypsin distribution curve		Collagenase distribution curve	
Size plateau (μ^3)	Cell No /0 5 ml	Size plateau (μ^3)	Cell No /0 5 ml
15 3–16 9	990	12 25–13 8	2,121
	981		2,045
18 4–19 9	840	16 9–18 4	1,376
	839		1,372
24 5–26	525	24 5–26	755
	527		769
30 6–35 2	641	29 1–35 2	1,553
	688		1,595

For each digest, eleven 6 5–7-day-old embryos were dissected and the scissors-minced hearts were incubated in the enzyme solutions Trypsin was the '1 250' commercial (Microbiological Associates) preparation in normal saline Trypsin digestion period, 45 min Collagenase, 5 mg/ml in Gey's solution, digestion period, 60 min In each case 0 1 ml calf serum was added per ml enzyme solution and the hearts were added to 2 0 ml of the mixture Size distribution and cell counts were made on freshly prepared suspensions from the digests after washing and re-suspending in avian Ringer to give a 1 500 dilution Aperture, 70μ, instrument, Coulter counter model *B*

electronic counting and sizing apparatus (Coulter counter) (Table 1), the subjective appearance of fewer broken cells and more complete disassociation with collagenase were confirmed (3) Excellent cell separation and viability, as demonstrated by culture of the disassociated cells (Figs 1 and 3), were obtained using collagenase in a normal saline containing full complements of calcium and magnesium Using trypsin in calcium, magnesium-free media, suspensions were decidedly inferior (Figs 2 and 4) (4) A fairly high proportion of pulsating cells was present shortly after disassociation when collagenase was used Trypsinization yielded virtually no pulsating cells (5) The initial appearance of the cells in the two cases was quite different With collagenase, about 30 per cent of the cells retained an elongated, flattened shape, while tryptic digests always contained many more rounded cells (6) The speed of initial attachment to the glass of culture dishes and subsequent flattening and stretching were much greater when collagenase was used

In general, it appears that, at least for cells from muscular elements, collagenase may be considered superior to trypsin as a disassociating agent

The completeness of disassociation with collagenase suggested that some form of collagen was a major component of the inter-cellular binding material

D J CAVANAUGH*
W O BERNDT
T E SMITH

U S Army Chemical Research and Development
Laboratories,
Edgewood Arsenal, Maryland

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* Present address Medical Bacteriology Division, Fort Detrick, Frederick, Maryland

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A Simple Technique for the Isolation of $\beta_1C(\beta_1 A)$ Globulin

SERUM submitted to immuno-electrophoresis shows 4 distinct lines in the β_1-region The best known of these precipitation lines is $\beta_1 S$, that corresponding to siderophilin , this line has been identified by use of labelled iron $\beta_1 B$ is the line corresponding to a protein, with combining power for cytochrome and similar compounds , it was first isolated by Biserte, *et al* [1] Different names have been proposed for it, such as chromophilin, hæmopexin and β-haptoglobin The two remaining lines $\beta_1 A$ and $\beta_1 C$ show a somewhat complex behaviour Indeed, fresh serum will give the line corresponding to $\beta_1 C$ and aged serum the $\beta_1 A$ line However, a transient aspect is very common, showing a double or even triple curved line[2]

Muller-Eberhard *et al* have isolated both $\beta_1 C$ and $\beta_1 A$-globulin[3] and they have shown that the latter protein is a derivative formed from $\beta_1 C$-globulin by an ageing process This protein is the slowest of the 4

β_1-globulins The sedimentation rate is 9 5 S whereas that of β_1A, the most rapid globulin of this group, is only 7 S Muller-Eberhard, *et al*, also supplied evidence that β_1C-globulin is related to the complement system and more especially with $C'3$ (ref 4) β_1A shows no activity The technique used by Muller-Eberhard *et al* for the isolation of both these proteins is rather complex as is another procedure proposed recently by Schultze *et al*[5] The examination of the mechanism of the complement system would be greatly facilitated if workers in this field could easily dispose of the isolated components

The technique developed in our laboratory is simple and gives β_1C-globulin in good yield and purity without special equipment, not more than 24 h, including time for dialysis, being necessary to obtain this result

Plasma (6 per cent protein, pH 7 5–8) is adsorbed by bentonite (2–2 5 per cent) during 15 min at room temperature Bentonite adsorbs mainly fibrinogen, lipoprotein and certain clotting components[6,7]

This mixture is centrifuged, the precipitate discarded, and the pH of the supernatant immediately adjusted to pH 6 3 An aqueous solution of rivanol (0 5 per cent) is now added (50 ml for 100 ml of plasma) The mixture is stirred for 30 min, then left an additional hour at room temperature

The mixture is then centrifuged (30 min) The precipitate is quickly washed with ice-water or 0 05 per cent rivanol solution and centrifuged again It is then extracted by 1/5 of the initial plasma volume of 3 per cent sodium chloride The crude saline extract is again adsorbed by bentonite (1 5–2 per cent) during 15 min (pH should be maintained at 8, bentonite adsorbs anions and the pH usually rises to 9 or more)

The supernatant obtained after centrifugation is dialysed against acetate buffer (0 01 M) at pH 5 3 at 4° The precipitate is centrifuged, washed with buffer and dissolved in saline at neutral pH The solution contains mainly β_1C-globulin, which may be accompanied by some lipids and lipoprotein Acidification at pH 6 3, adsorption on silica or treatment by dextran-sulphate (or similar products) may be used for the elimination of these lipidic contaminants This purification step might be unnecessary and the redissolved euglobulin precipitate can be used directly for certain purposes

ACD plasma as well as serum can be used as a starting material From fresh and frozen plasma we almost always obtained β_1C-globulin This protein migrates as a β_1-globulin on paper and agar, its position on starch gel, as revealed by a microtechnique[8], is approximately in the middle between α_2-macroglobulin and siderophilin When β_1C is left in the ice-box for some time, it will slowly undergo transformation into β_1A-globulin Two lines will be seen after starch gel electrophoresis and the preparation submitted to immunoelectrophoresis will reveal the characteristic double or triple curved lines (Fig 2) After several weeks β_1C has completely disappeared, leaving β_1A and some break-down products, as revealed by electrophoresis on starch gel and immunoelectrophoresis The isolated β_1C–β_1A-globulin is also revealed by antiserum obtained from horse 1299 (Institut Pasteur) (We thank Dr J M Fine for this analysis) Hence, this antiserum contains only one single antibody against β_1-globulins, namely, anti-β_1A, this result gives a further proof for the identity of our protein with β_1C- β_1A-globulin

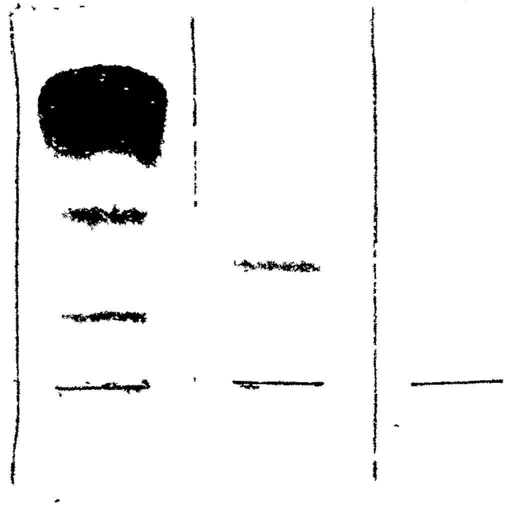

Fig 1 Starch-gel electrophoresis (microtechnique) (*a*) Normal serum (*b*) β_1C freshly prepared, (*c*) same preparation after storage at + 4° undergoing transformation to β_1A globulin

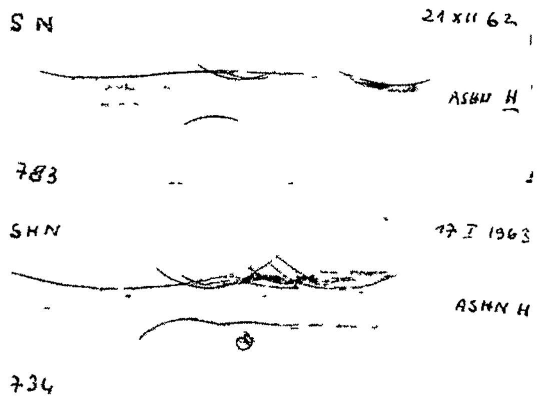

Fig 2 Immunoelectrophoresis of β_1C–β_1A-globulin Above, β_1C-globulin (0 8 per cent) freshly isolated, below, same preparation after storage at + 4° during 4 weeks showing partial transformation to β_1A

M STEINBUCH
M QUENTIN
L PEJAUDIER
Travail du Centre National de Transfusion Sanguine,
6 Alexandre-Cabanel, Paris

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Sodium – Potassium-activated Adenosine Triphosphatase in Schizophrenic Erythrocytes

SINCE Skou's[1,2] description of a sodium–potassium-activated adenosine triphosphatase in crab nerve, many reports have indicated that this enzyme has properties virtually identical to those exhibited by the Na^+ extrusion mechanism in many tissues[3–8] The intracellular concentrations of Na^+ and K^+ ions are of importance in regulating cell membrane excitability in nerve, muscle[9], secretory cells, etc It is also known[10] that the amount of this enzyme in sheep erythrocyte membranes correlates with the intracellular concentration ratio of K^+/Na^+, which is in turn regulated by a gene[11]

The ætiology of schizophrenia remains unknown Kallmann's[12] statistics as well as Slater's[13,14] suggest that the disease is predominantly a manifestation of an autosomal recessive gene Because of this strong genetic character of schizophrenia, it would seem likely that some

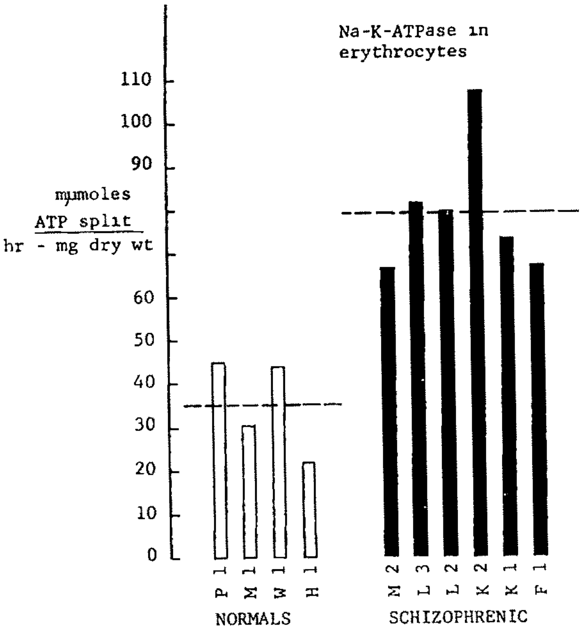

Fig 1

protein abnormality might be found On the assumption that schizophrenia may be a 'disease of cell excitability' we decided to investigate a membranal enzyme intimately associated with cell excitability, namely, sodium–potassium–ATPase

Blood (20 ml) was drawn from 4 normal volunteers and 6 sub-acute paranoid schizophrenic patients The erythrocyte membranes (ghosts) were prepared according to the method of Bonting and Caravaggio[6] with minor modifications The *tris*–EDTA washes were alternated with 0 3 per cent sodium chloride instead of with 0 986 g/l sodium chloride At the end of the procedure the ghosts were again washed twice in *tris*–EDTA For sodium–potassium–ATPase assay 0 1-ml aliquots of the final resuspended sediment were added to 0 1 ml of medium *A* and medium *D* as outlined by Bonting *et al* [15] After correction for the blanks the difference in activity between media *A* and *D* corresponded to sodium–potassium–ATPase Triplicate enzyme determinations were within 3 per cent Duplicate dry weights of the ghost suspensions were within 5 per cent The results are shown in Fig 1

The mean sodium–potassium–ATPase for the 4 normals is 35·2 ± 11 6 (standard deviation) mμmoles ATP split/h/mg dry weight The value for the patients is 79 1 ± 14 On Student's *t* test these mean values are significantly different with *P* less than 0 001 Subtracting the sodium–potassium–ATPase from the total ATPase gives the 'residual ATPase' this is 180 ± 10 for the normals and 246 ± 90 for the patients The difference between these two means is not significant ($P = 0\ 2$) Only 2 patients had received any tranquillizer in the week preceding *M*2 and *K*1 had received 200 mg hora somni and 150 mg 'Thorazine' daily, respectively The patient with the highest value of sodium–potassium–ATPase, *K*2, had been off all medication for 4 weeks

The normal sodium–potassium–ATPase of 35 units agrees fairly well with that of Post *et al* [3], who found an average of 20 units using 4 ghost preparations It is known that enzyme-levels vary with the age of the erythrocyte, young red cell fractions, containing 12 times the number of reticulocytes than old cell fractions, have about 30 per cent greater enzyme activity[16] These schizophrenic patients, however, have approximately normal reticulocyte counts It will be necessary to examine many more bloods of patients not on tranquillizers and it might be preferable to examine the enzyme level in cerebro-cortical biopsy or autopsy material

We are indebted to Dr Herman C Denber, director of research at Manhattan State Hospital, New York, for his co-operation

PHILIP M SEEMAN
The Rockefeller Institute, New York, 21

EILEEN O'BRIEN
New Dorp School, Staten Island, New York

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Compounds interfering in the Diphenylamine Test for Deoxyribonucleic Acid

THE use of DNA (deoxyribonucleic acid) as a reference standard in biochemical investigations[1], which is now well established, rests on the observed constancy of DNA content in each somatic cell in a species With certain exceptions, the number of cells in a sample may be related to the amount of DNA[2]

One procedure for determination of DNA, namely, the use of the diphenylamine reagent[3], has been reviewed[4], and the variables examined Considerable interference has been noted in some cases[5], but in general the reaction provides a reliable method of assay

Briefly, the method involves extracting the tissue with hot trichloroacetic acid, and then boiling the extract with the diphenylamine reagent[3] Within 5 min there is rapid development of a purple-blue coloration, which increases in intensity somewhat on longer heating The solution shows a characteristic absorption band in the ultra-violet region at 5800–6000 Å

When interfering substances are present, as shown by an alteration in the colour observed, the utility of the reaction may be preserved by suitable modifications[4] If the correct colour is produced it is generally assumed that no interference is occurring However, we have observed, on occasion, a normal colour with material, which on closer examination gave no maximum in the region expected Instead a peak occurred at 5000–5500 Å, often accompanied by a second peak at 6500–6800 Å, sometimes having a shoulder at 5700–6000 Å Reference to the literature[6] showed that the cause of this type of interference was suggested to be mucopolysaccharides

With pure DNA it is usually considered that boiling for 15 min is required for full colour development[7] although 10 min was suggested to be sufficient by other authors[8] When small amounts of interfering material are present considerable inaccuracies appear with 10–15 min heating and to obtain reliable estimates it is best, I find, to restrict heating to 5 min, since the colour from the interfering material develops more slowly than the colour from the DNA To obtain reliable figures for DNA when large amounts of interfering substances are present I find it most useful to plot absorbance against time of heating, in the region 5800–6000 Å

Since the pure DNA/diphenylamine-reagent colour shows only weak absorption in the region of 5400 Å and of 6600 Å, a similar plot at these wave-lengths gives a good indication of the rate of development of interfering colour Plots in these two regions usually give smooth curves

In the 5800–6000 Å region, however, such plots produce curves with a sharp break at about 5 min Back-extrapolation of these curves from 30 min through 10 min to zero time gives a good measure of DNA concentrations, since there is little increase in intensity due to DNA after this time

Fig 1 shows the variation in the absorption spectrum with time for a typical test Fig 2 shows the plot of absorbance against time at different wave-lengths to illustrate the foregoing points

Alternative methods of assay[9] should be used as a further check, if possible, although considerable divergences of view exist as to the interpretation of some of these methods[10]

To gain further evidence concerning the nature of the materials interfering in the Dische test, a number of substances were subjected to the test Table 1 lists those compounds which gave similar colours under the test conditions, together with the absorption peaks associated with the colour

Although in certain cases a careful comparison of the colours produced, when a compound and DNA were separately tested, distinguished the compound from DNA, if tested in admixture the resultant colour was visually indistinguishable from the colour produced by DNA alone

In each case, however, the rate of development of interfering colour was slower than the initial increase in intensity at 5900 Å The addition of known amounts of DNA to test solutions of these compounds showed that reasonable accuracy was obtained by the extrapolation suggested, and that the measurement of intensity after 5 min heating, although less accurate, gave moderately satisfactory results under controlled conditions

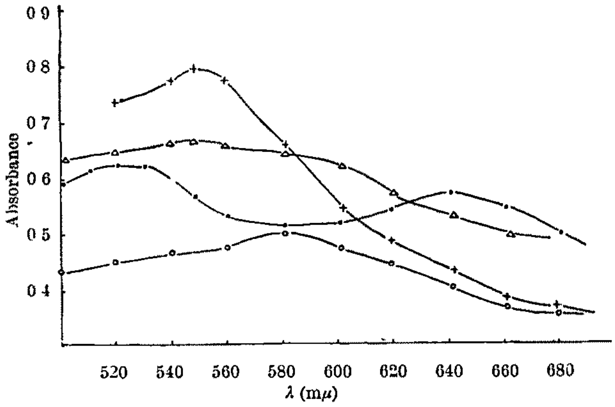

Fig 1 Spectra obtained from interfering substances compared with those from lactating mammary gland +, 5-Methyl furan heated 30 min, ●, fructose heated 30 min, O, mammary gland, heated 6 min (*B*), Δ, mammary gland, heated 30 min (*C*)

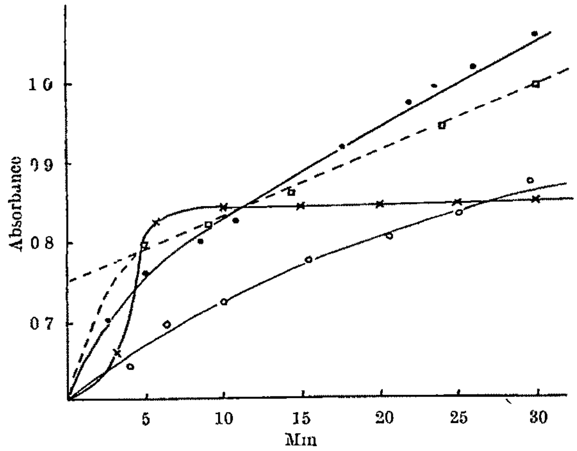

Fig 2 Change of spectra with time of heating □, Mammary gland at 590 mμ, O, mammary gland at 660 mμ, ●, mammary gland at 540 mμ, ×, DNA at 590 mμ

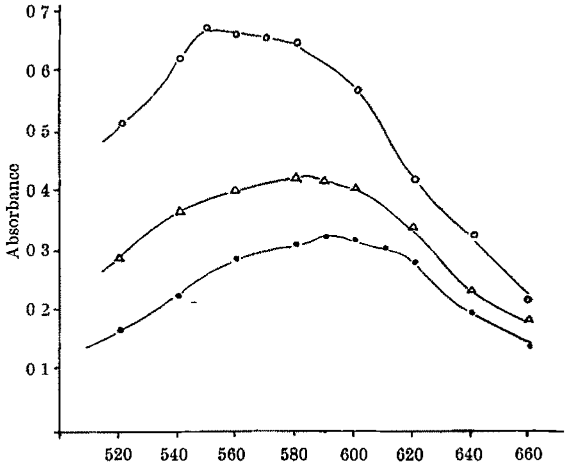

Fig 3 Absorption spectra from DNA and from DNA – 5-methyl furan mixture Compare Fig 1, *B* and *C* O, Mixture heated 30 min, Δ, mixture heated 10 min, ●, DNA alone

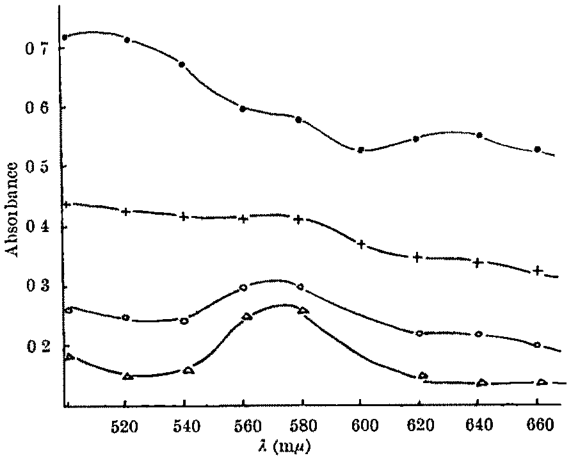

Fig 4 Spectra from DNA – 5-hydroxymethyl furfuraldehyde mixture (aldehyde obtained from sucrose treated with trichloroacetic acid) ●, Mixture heated 30 min, +, mixture heated 10 min, O, mixture heated 5 min, Δ, DNA alone Compare Fig 1, *B* and *C*

Some of the compounds listed (Nos 1–5, 16, Table 1) are known to occur often in tissues[11], while others (Nos. 6–15, Table 1) are either uncommon or have not been noted to occur

However, since treatment with hot trichloroacetic acid is a preliminary to the test, it should be noted that hot acids convert the methylpentoses, rhamnose and fucose[12], to 5-methylfurfuraldehyde (compound 12, Table 1) and convert ketohexoses[13] and sucrose[13] to 5-hydroxymethyl furfuraldehyde (compound 7, Table 1)

Table 1

	Compound	Absorption maxima (Å)
1	Fructose	5200 and 6300
2	Sorbose	
3	Fructose 1-phosphate	
4	Fructose 1,6-diphosphate	
5	Sucrose	
6	Levulinaldehyde	5550
7	5-Hydroxymethyl furfuraldehyde	5200 and 6300
8	2-Deoxy-glucose	5100
9	2-Deoxy-galactose	
10	Glucal	
11	Galactal	
12	5-Methyl furfuraldehyde	5500
13	2-Methyl furan	5850
14	5-Methyl furfuryl alcohol	
15	4,5-Dihydroxy valeraldehyde	5550
16	Sialic acids (various samples)	Deep violet-blue colour, maximum not determined

In certain plant tissues, where interference has occurred, high concentrations of methyl-pentoses are probably responsible in some cases, and high concentrations of sucrose and/or fructose in some other cases In animal tissues interference of this type has so far been encountered in normal serum, in lactating mammary gland, and in certain disease conditions, for example, some types of rheumatoid arthritis, among other cases

I suggest that the nonulosaminic (sialic) acids are a common cause of interference Not only are these acids often associated with methyl-pentoses (6-deoxy-hexoses) (for example, fucose), which may give an interfering colour by conversion to methyl-furfuraldehyde, but in addition they themselves give a blue-violet coloration when treated with the Dische reagent In the past few years these acids have been found to occur in a large number of tissues (Table 2) including those found to give trouble in this test

Table 2 OCCURRENCE OF NONULOSAMINIC (SIALIC) ACIDS, AND THE CARBOHYDRATES FOUND IN ASSOCIATION (REF 18)

Origin	Associated sugars	Ref
Mucin of bovine submaxillary gland	*N*-Acetyl-glucosamine, glucose, glucosamine, fucose, mannose	14
Brain (especially gangliosides), serum*, cerebrospinal fluid, erythrocytes, saliva, vitreous humour, urine, bronchial and epithelial tissue, liver*, mammary gland (lactating)*	Mannose, fucose, galactose, galactosamine, glucosamine	15
Milk (human)*, colostrum (bovine)*	Lactose, galactose, glucose, fucose, *N*-acetyl-galactosamine	17
E coli		16

Abnormal Dische tests so far found with the tissues marked with an asterisk (cf ref 19)

The main purpose of this communication is to point out the need for careful checking of the absorption spectrum when using the test, particularly on materials not previously characterized, or where alternative assays are not carried out A subsidiary purpose is to suggest that where interference of this type is encountered, this may be partially diagnostic of unusual concentrations in the tissue of some of the substances listed, and it may be useful in some cases to carry out further tests for these compounds

I thank Prof Wesley Cocker and Dr R F Phillips for their advice

J B LEE*

University Chemical Laboratories
Trinity College, Dublin

* Present address College of Technology, Loughborough

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Variants of α_1-Acid Glycoprotein

IN a recent publication[1], α_1-acid glycoprotein, a human plasma glycoprotein, was shown to be polymorphic At pH 2 9, that is, near its isoelectric point, seven zones were observed if the mentioned protein had been isolated from pooled normal blood Moreover, it could be demonstrated that, following enzymatic removal of essentially all sialic acid, the modified pooled α_1-acid glycoprotein resolved at pH 4 8 into two major and a minor faster moving component[2]

In the present investigation experiments were carried out to study further the latter observation α_1-Acid glycoprotein was prepared from serum of normal adults and analysed by starch-gel electrophoresis at pH 4 8 Three types of patterns were observed (Fig 1) Type 1 distinguished itself in that the slower moving main band exhibited the maximum colour intensity, whereas Type 2 showed the maximum colour at the faster moving main band Type 3 revealed the mentioned two bands to be almost equally coloured To evaluate the three types of patterns with regard to the homozygous and heterozygous properties, further analyses are needed

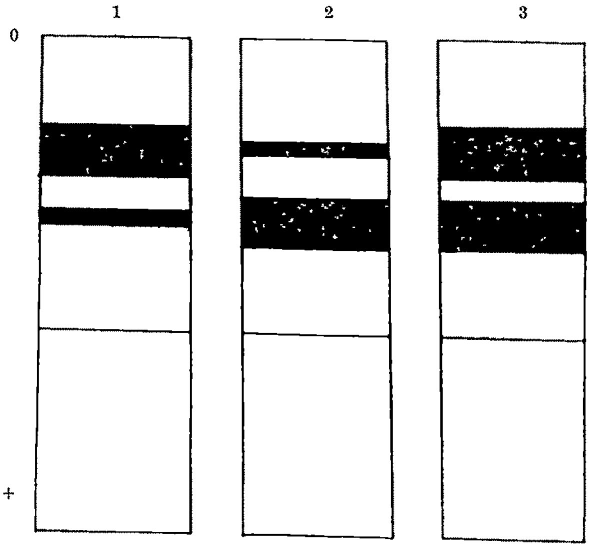

Fig 1 Starch-gel electrophoretic patterns of type 1 (1), type 2 (2) and type 3 (3) of α_1-acid glycoprotein modified with neuraminidase The electrophoresis was carried out at pH 4 8 in $\Gamma/2$ 0 02 acetate buffer The protein was applied at the positions indicated by ○ and migrated towards the anode (+)

An additional series of experiments was performed to establish the relationship between the patterns obtained at pH 2 9 using native α_1-acid glycoprotein and those observed at pH 4 8 utilizing native α_1-acid glycoprotein from which sialic acid had been removed enzymatically α_1-Acid glycoprotein preparations from apparently healthy adults were analysed by starch-gel electrophoresis at the mentioned two pH values As judged from the relatively small number of experiments it appeared that Types 1, 2 and 3 were largely associated with pH 2 9 patterns showing 7, 6 and 7 bands, respectively

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K TOKITA
K SCHMID

Department of Biochemistry,
Boston University School of Medicine
Boston University Medical Center,
Boston 18, Mass

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Sorption of Copper by Wood Constituents

Belford, Myers and Preston[1] have directed attention to the fact that cellulose and wood absorb copper ions and that they appear to be regularly spaced on a grid pattern not corresponding to the unit cell of cellulose. Bayley and Rose[2] consider that α-cellulose has only a small cation binding power and that the complexes formed in wood consist of metal cations bound on the non-cellulosic constituents. Belford *et al.*[3] observed that in wood impregnated with preservatives containing copper-salts the middle lamella in particular stained very deeply with dithio-oxamide. In the work now reported the copper sorption of isolated wood constituents has been measured and the copper has been located in sections of treated wood by staining methods and electron microscopy.

'Milled wood lignin' prepared by the method of Björkman[4], wood α-cellulose (40 mesh) and purified cotton cellulose (40 mesh) have been examined for their copper binding capacity by the following method. A specimen of each material, 0·1–0·5 g, was shaken for 1 h in 5 ml. of 0·3 per cent copper acetate solution at about 20° C. The mixture was then filtered, the specimen washed with distilled water and the filtrate discarded. It was then washed three times with 10 per cent acetic acid and the combined acetic acid filtrates evaporated to dryness on a steam bath. The organic matter present was destroyed by heating on a hotplate with sulphuric–nitric acid mixture and the copper determined by the spectrophotometric rubeanic acid (dithio-oxamide) method of Center and MacIntosh[5]. The results are given in Table 1. A similar test on the residual lignin after the acetic acid washing showed that it retained no significant amount of copper.

These copper sorptions of cellulose are much lower than those recorded by Belford, Myers and Preston[1] and consistent with those observed by Michie[6], allowance being made for the different conditions by his equation (1). However, the absorption of copper by the milled wood lignin was, as shown in Table 1, much higher than for cellulose.

Table 1. Copper Sorption of Wood, Cellulose and Lignins (Grams of Copper per 100 g)

Eucalyptus regnans wood	0·033
Wood α-cellulose	0·040
Cotton—purified	0·038
E. regnans milled wood lignin	0·361
E. goniocalyx milled wood lignin	0·325
E. regnans methanol lignin	0·154
P. radiata compression wood milled wood lignin	0·258

It has been shown by me[7] that these milled wood lignins retained some uronic acid and that their infra-red spectra showed a band at 5·8μ attributable to carboxyl groups. Milled wood lignin from compression wood was exceptional in that this band was absent. No such band was observed in the spectrum of methanol lignin[8]. However, it may be seen from Table 1 that a significant sorption of copper by both these lignins occurred.

Examination of the infra-red spectra of copper-treated milled wood lignins showed only a small reduction in intensity of the band at 5·8μ. This indicates that, as would be predicted by Mitchie's equation for the conditions of our experiment, only a fraction of the carboxyl groups reacted with copper. Furthermore, there is extensive sorption of copper on lignin by some other mechanism. This suggests that groups other than carboxyl groups are also involved, but appears to be in accord with the view of Bayley and Rose that most of the copper sorption is on the non-cellulose constituents of the wood.

General confirmation of this conclusion has been obtained from the optical and electron microscopic examination of sections treated with a solution of copper sulphate. Fig. 1*a* shows a transverse section of *Pinus radiata* impregnated with copper sulphate and stained with dithio-oxamide. Fig. 1*b* shows the same section photographed between crossed nicols. It can be seen that copper is observed in or external to the layer S_1 of the secondary wall. An electron micrograph of a similar section of copper impregnated wood is shown in Fig. 2, the dark area indicating the presence of copper. It is apparent from the greater resolution in this picture that most of the copper was taken up by the primary wall *P* and intercellular layer. From ultra-violet absorption measurements, Lange[9] concluded that more than 70 per cent of the cell wall lignin is located in this region, so that these results are consistent with the suggestion that most of the copper taken up by the cell wall is attached to the lignin.

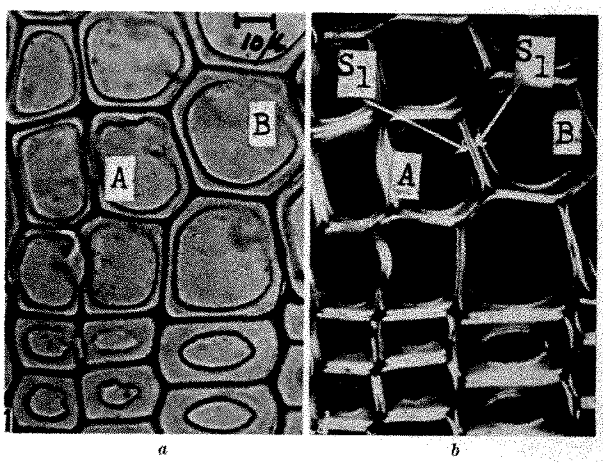

Fig. 1. Transverse section of *Pinus radiata* copper treated. (*a*) Stained with dithio-oxamide; (*b*) same section between crossed nicols

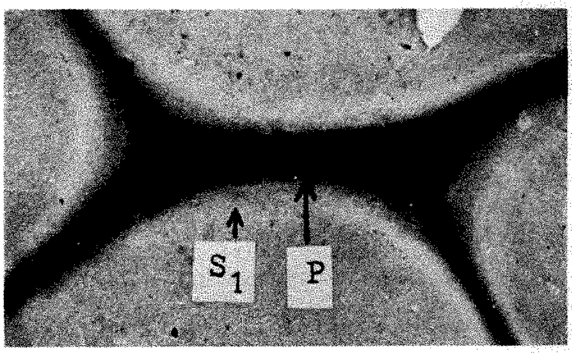

Fig. 2. Electron micrograph showing intercellular layer in copper-treated *Pinus radiata*

I thank Dr. A. B. Wardrop for discussion of the results of microscopic examination of the treated sections and M. Menshun for assistance.

David E. Bland

Commonwealth Scientific and Industrial
Research Organization,
Division of Forest Products,
South Melbourne.

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PHYSIOLOGY

Perinatal Changes in the Fluid and Electrolyte Content of Rat Liver

One of the difficulties of providing a satisfactory description of the fluid and electrolyte content of a tissue is the choice of a reference framework to which these contents can be related[1,2]. It is usually sufficient to relate the composition to the weight of the dry solids of the tissue, but in cases where some component of the dry solids may be subject to physiological variation, misleading conclusions may result from a comparison of data expressed in such terms. A striking example of variation in the composition of the dry solids is seen in the developing rat liver. Thus, recalculation of data reviewed by Shelley[3] shows that in this tissue the glycogen content increases from nil to more than 40 g per 100 g of the total dry solids during the last four days of gestation, and then falls to 5 g per 100 g within the first day after birth.

Table 1. Composition of Developing Rat Liver. Values given represent Mean ± Standard Error of Mean (Number of Observations)

Age (days)		Protein g/kg FFDW*	Water (kg/kg)		Potassium (m.moles/kg)		Sodium (m.moles/kg)	
			FFDW	Protein	FFDW	Protein	FFDW	Protein
Fœtal:								
17–18		653±39 (4)	4·43±0·29 (9)	7·14±0·68 (4)	702±45 (9)	1,185±108 (4)	282±25 (9)	—
19–20		590±22 (5)	4·24±0·06 (11)	7·13±0·16 (5)	596±16 (11)	959±39 (5)	233±12 (11)	422±5 (5)
21		473±19 (5)	3·51±0·08 (8)	7·55±0·27 (5)	440±12 (8)	951±15 (5)	193±19 (8)	437±47 (5)
22		384±9 (10)	3·14±0·05 (15)	8·38±0·11 (9)	370±11 (15)	975±41 (9)	170±10 (15)	421±38 (9)
Post-natal:								
0·5–1·0	Fed	663±10 (6)	3·89±0·06 (12)	6·01±0·09 (6)	514±17 (12)	849±34 (6)	245±14 (12)	408±32 (6)
	Unfed	491±22 (3)	3·46±0·04 (3)	7·06±0·33 (3)	440±21 (3)	904±87 (3)	190±16 (3)	391±45 (3)
4–5		618±13 (5)	3·16±0·07 (8)	5·08±0·15 (5)	448±17 (8)	744±20 (5)	191±24 (8)	339±34 (5)
Adult		594±37 (5)	2·39±0·04 (13)	4·02±0·14 (5)	325±13 (13)	604±13 (5)	140±26 (13)	246±50 (5)

* FFDW, fat-free dry weight.

We have previously shown that there is a rapid increase in liver water and potassium contents relative to the fat-free dry solids during the first day after the birth of the rat[2]. Fenn[4] and Edlund *et al.*[5] have shown that quite large variations in the glycogen content of the liver of fed and fasted adult rats do not affect the liver water and potassium contents per unit dry solids, because deposition of glycogen in the cells is accompanied by an influx of water and potassium and vice versa. This observation would suggest that the change relative to the dry weight in the water and potassium content of the rat liver immediately after birth may not be entirely due to the varying contribution of glycogen to the total tissue solids. Nevertheless, the temporal association between the fall in the liver glycogen and the increase in the liver water and potassium per unit fat-free solids was sufficiently striking to warrant further investigation. We now report the protein, water, potassium and sodium contents of liver tissue taken from rats of differing perinatal ages.

The rats used were of local albino stock. The methods for the preparation of the tissue and for analysis of water, electrolytes and protein have been described previously[1,2,6,7]. The results, presented in Table 1, show that total protein forms a markedly decreasing fraction of the fat-free dry solids of rat liver during the late fœtal life. By the age of 12–24 h post-natal, however, the liver protein has increased to account once again for more than half the fat-free solids. These changes in protein content occur simultaneously with the marked perinatal changes in liver glycogen content[3]. However, 12–24 h old rats which were prevented from suckling had a lower liver protein content than did suckled animals of this age-group (Table 1), despite the fact that the unfed animals have a lower glycogen content[8]. The changes in protein content per unit fat-free solids that occur during the perinatal period are thus not solely a consequence of the changing contribution of glycogen to the total fat-free solids of the tissue.

Expressed in terms of the fat-free solids, both the water and the electrolyte content of the liver decrease during late fœtal life. In confirmation of our earlier findings[2], the water and potassium contents per unit fat-free solids were found to increase during the first day after birth and then to decline during later growth. On the other hand, when expressed in terms of the protein content there emerges a somewhat different picture of the changes in liver composition during the perinatal period. Thus, just before birth, the water content per unit protein increases; immediately after birth there is a large decrease in the water content, followed by a slower decrease which continues until adulthood. Both the sodium and potassium contents remain relatively constant during the last 3–4 days of fœtal life. During the first day after birth the potassium content falls significantly, but the sodium content only shows a significant fall later, that is, between the ages of 1 and 4 days. The changes in water and electrolyte composition during the 12–24 h immediately following birth are partly, but not entirely, accountable in terms of intake of food during this period. New-born rats which were not suckled during this time are found to have a higher water content per unit protein than fed animals (Table 1); the sodium and potassium contents of the fed and unfed rats are, however, not significantly different.

Table 2. Tissue Water Compartments of Developing Rat Liver

Age (days)		n	α_{Na}	(kg/kg protein) Extracellular water	Intracellular water
Fœtal:					
19–20		5	0·40±0·01	2·87±0·04	4·26±0·17
21		5	0·39±0·05	2·89±0·38	4·66±0·48
22		9	0·33±0·03	2·81±0·28	5·58±0·29
Post-natal:					
0·5–1·0	Fed	6	0·46±0·03	2·77±0·22	3·27±0·20
	Unfed	3	0·37±0·03	2·67±0·31	4·40±0·12
4–5		5	0·46±0·05	2·30±0·23	2·77±0·27
Adult		4	0·47±0·11	1·88±0·36	2·11±0·50

α_{Na} is calculated as (Na concentration in liver water)/(Na concentration in extracellular water) and is taken as a measure of the fraction of the whole water content of liver which is extracellular. The water contents of the extracellular and intracellular compartments can be calculated from α_{Na} and from the total water content of liver tissue (W)kg/kg protein from the relations:

Extracellular water $= \alpha_{Na}\ W$ kg/kg protein.

Intracellular water $= (1 - \alpha_{Na}\ W$ kg/kg protein).

In Table 2 are shown estimates of the quantities of liver 'extracellular' and 'intracellular' water per unit protein calculated on the two assumptions that all the liver sodium is extracellular and that the concentration of sodium in the blood plasma remains constant during growth[9] at a value of approximately 161 m.moles/kg water[10]. It can be seen that the volume of 'extracellular' water per unit protein remains constant during the last 3–4 days of fœtal life and the first day after birth; during this period the changes in total water content are largely due to changes in the quantity of 'intracellular' water per unit protein. During later growth the changes in the liver water content are due to decreases in the amounts of both 'extracellular' and 'intracellular' water per unit protein.

Our results indicate that very different pictures of the changes in liver water and electrolyte contents during growth are given by the use of different reference materials. It is clear that, for comparative purposes, an adequate description of the fluid and electrolyte composition of tissue requires a knowledge of the content of organic material as well as that of the water and minerals.

One of us (G. D. V. v. R.) was a NATO Research Fellow and thanks Prof. E. C. Slater for his hospitality.

D. S. Parsons

Department of Biochemistry,
University of Oxford.

G. D. V. van Rossum*

Laboratorium voor Fysiologische Chemie,
Universiteit, Amsterdam,
The Netherlands.

* Present address: Johnson Research Foundation, University of Pennsylvania, Philadelphia 4.

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Strontium – Calcium Discrimination by the Human Placenta

The ability of the placenta to discriminate between strontium and calcium has been demonstrated by Comar *et al.* in double tracer experiments using rats and pigs[1]. They found that the strontium/calcium ratio of the fœtus was considerably less than that of the mother. In the case of rats, the strontium/calcium ratio of the fœtus was 0·55, and 0·65 times that of the mother, while for rabbits the fœtus had a strontium/calcium ratio 0·49 times that of the mother.

An estimate of placental discrimination in human beings has been made by Bryant and Loutit by comparing the stable strontium/calcium ratio of bones obtained from adults with that found in the bones of still-births and new-born infants[2]. The authors assume that there is no discrimination between blood and bone and that, therefore, the placental discrimination is measured by the ratio of strontium/calcium in the infant bone to the strontium/calcium ratio in adult bone. In the subjects studied by them this ratio was 200/320 or 0·62.

In this laboratory we have attempted to measure placental discrimination in human beings somewhat more directly, by determining the concentration of stable strontium in serum from blood taken from the fœtal and maternal sides of the placenta at birth. If the calcium concentration of the blood on either side of the placenta is assumed to be the same, then the ratio of the stable strontium concentration in the infant's serum to that of the mother is a measure of placental discrimination.

Approximately 10–20 c.c. of whole blood were taken from the maternal and fœtal side of the placenta immediately after birth and placed in sterile test-tubes. The blood samples were reamed and centrifuged and the serum removed. The serum samples were then sent to a commercial laboratory for stable strontium determination by neutron activation analysis.

The method used was to lyophilize known masses of blood serum, weigh the dried samples and enclose them in small aluminium-foil capsules. These samples, along with duplicates prepared from a standard strontium sample, were then sealed in an aluminium irradiation capsule and irradiated in a neutron flux of $\sim 1 \times 10^{13}$ n cm^{-2} sec^{-1} for about one week. After irradiation, the samples and standards were removed from the aluminium foils, dissolved in 16 N nitric acid in the presence of strontium carrier, and duplicate aliquots of the solution were taken for analysis. The strontium fraction was purified radiochemically by repetitive precipitation of $Sr(NO_3)_2$ with fuming nitric acid, followed by scavenging precipitations of $Fe(OH_3)$ and $BaCrO_4$. The strontium was recovered as $SrCO_3$ and weighed to determine the chemical yield. The strontium-85 (64-day half-life) content of each sample was then measured by γ-spectrometry.

The stable strontium content of each blood serum sample was determined from the respective strontium-85 counting rates and chemical yields of the samples and standards. The accuracy of individual analyses was estimated to be ± 10 per cent.

Table 1. STABLE STRONTIUM IN MATERNAL AND NEW-BORN BLOOD SERA AND THEIR RATIO

Case	(μg Strontium/ml.) Maternal (M)	New-born (N)	Ratio (N/M)
1	0·134	0·064	0·48
2	0·066	0·057	0·86
3	0·037	0·045	1·22
4	0·078	0·032	0·41
5	0·060	0·053	0·88
6	0·040	0·042	1·05
7	0·148	0·069	0·47
8	0·080	0·134	1·68
9	0·080	0·084	1·05
10	0·148	0·145	0·98
11	0·066	0·077	1·17
12	0·151	0·040	0·26
13	0·055*	0·139*	2·53*
14	0·246*	0·080*	0·33*
15	0·060	0·043	0·72
16	0·167	0·069	0·41
17	0·146	0·103	0·71
Average (17 cases)	0 0948	0 075	0·89
S.D.	±0·045	±0·036	±0·57
Average (15 cases)	0·097	0·070	0·823
S.D.	±0·045	±0·034	±0·38

* Excluded in second average.

Results of the analyses of 17 pairs of samples are presented in Table 1. The average concentration of stable strontium in the mothers' sera was 0·10 μg/ml. with a standard deviation of 0·06 μg/ml. The average concentration of stable strontium in the new-born infants' sera was 0·08 μg/ml. with a standard deviation of 0·04 μg/ml. The ratios of the concentration of stable strontium in the serum of the new-born to that of the mother ranged from 0·26 to 2·53. The average and standard deviation of these ratios was 0·89 ± 0·57.

Because of the wide range of values obtained, the results were analysed statistically to see if any of the observations could be rejected on the ground that they were from a population with a different mean. Using a test devised by Dixon[3], the concentration of strontium in the mother of Case 14, and the new-born/maternal ratio of Case 13 were considered to be atypical of their respective populations and were consequently rejected. Average values for the new population of 15 cases were: mothers' sera 0·10 ± 0·05 μg/ml., new-borns' sera 0·07 ± 0·03 μg/ml., and new-born/mother sera of 0·82 ± 0·38.

If the assumption that the concentration of calcium in the new-born infant's blood is the same as that of the mother's blood, then the observed statistically significant difference in the strontium concentration of blood taken from the two sides of the placenta is an indication of strontium–calcium discrimination by the placenta. The magnitude of this discrimination is given by the ratio of the concentration of strontium in the new-born's serum to that in the mother's serum. This ratio was 0·82 ± 0·38, a result which is in general agreement with the findings of Bryant and Loutit that placental discrimination in human beings is less than that observed in rats and rabbits, but that it does occur.

If, on the other hand, the concentrations of calcium in the mother's and infant's blood are assumed to be different, and if further the placental discrimination is 0·62 as found by Bryant and Loutit, then the serum calcium of the mother can be expected to be 0·62/0·82 or 76 per cent that of the new-born. This increased concentration of calcium in the infant's serum as compared with that of the mother is consistent with Comar's findings in animal experiments[4], but requires verification in examination of human beings.

I thank Dr. Arthur Weinberg for obtaining the blood samples for this work.

JOSEPH RIVERA

U.S. Atomic Energy Commission,
Health and Safety Laboratory,
New York, New York.

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Influence on Capillary Permeability of Lymph discharged from a Scald

ATTENTION has recently been directed to the protein permeability factor while investigating the pathogenesis of disturbance of the capillary permeability[1–4].

Investigations were carried out to determine the properties of lymph discharged from a scald. The lymph was collected from the popliteal nodes of rabbits (2·0–3·0 kg) and proteins were separated by paper electrophoresis (veronal buffer, μ0·023, *p*H 8·6). The influence of lymph on capillary permeability was investigated by introducing the lymph (0·1 ml.) intracutaneously into a rabbit previously injected intravenously with *T*–1824 or trypan blue (10 mg/kg). The scald was caused by dipping the hind-leg into water of $80 \pm 2°$ C for 1 min, and $50 \pm 0·5°$ C for 5 min.

In the lymph collected from 15 rabbits, 30 min and 24 h after the burn at 80° C (Table 1) protein concentration increased, the *A*/*G* coefficient decreased and β-globulin concentration increased ($P < 0·001$). In lymph obtained 24 h and, in several cases, 30 min after the burn, an additional globulin fraction was registered, located between β- and γ-globulin (β_2-globulin).

Results from scalds made at a lower temperature (50° C) with 10 rabbits were somewhat different. The protein concentration in the lymph increased ($P < 0·001$), but the *A*/*G* coefficient did not decrease; β-globulin concentration did not increase, nor was any additional β-globulin fraction discovered. At 50° C the actual swelling appeared after 2·0–2·5 h and continued increasing during 24 h. In contrast to the experiments at 80° C no destructive changes were noted in scalded paws.

The lymph obtained 24 h after scalding at 80° C in all cases caused acute disturbances in the capillary permeability. The influence of the lymph obtained 30 min after the scald was weaker. Lymph collected 24 h after scalding at 50° C did not provoke permeability derangement, while that obtained 30 min after scalding was active only in 7 out of 15 experiments.

Antihistamines ('Dimedrol', 'Alphadril') practically eliminated the influence of the capillary permeability of lymph collected 30 min after scalding at 50° and 80° C, and there was a slight weakening of the influence of the lymph obtained 24 h after scalding at 80° C. This might be explained by the participation of histamine in the first phases of the inflammation and the subsequent inclusion of active globulins[5,6].

Table 1. ELECTROPHORETIC ANALYSIS OF LYMPH

	Albumin (%)	Globulins (%)					*A*/*G* coeff.	Protein (g/%)
		α_1	α_2	β_1	β_2	γ		
Before burn	50·2	6·9	12·9	14·0	—	16·0	1·03	3·2
30 min	45·7	7·0	11·1	11·5	7·7	17·0	0·87	3·4
P	< 0·001	< 0·5	< 0·1			< 0·5	< 0·001	< 0·01
24 h	43·2	7·3	11·2	10·0	10·7	17·6	0·78	3·8
P	< 0·001	< 0·5	< 0·5			< 0·5	< 0·001	< 0·001

The appearance of unusual β-globulins in the rabbit's lymph during scalding coincides with previous investigations made in our laboratory[7]. It seems that during inflammation not only are the existing globulin permeability factors activated but also unusual globulins are formed[8].

In spite of intensive swelling at the site of the scald at 50° C, the lymph after 24 h did not cause capillary permeability disturbances and did not contain histamine and globulins with unusual electrophoretic mobility. It is possible that under the influence of moderate temperatures the mediators causing capillary permeability derangement in rabbits, just as in rats, are other factors affecting permeability[9,10].

I. A. OYVIN
S. M. SHEGEL
E. G. YAGODKINA

Department of Pathological Physiology,
Kuban Medical Institute,
Krasnodar, U.S.S.R.

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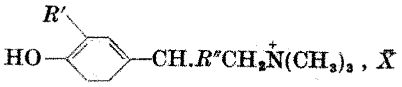
PHARMACOLOGY

Quaternized Sympathomimetic Amines

IN their paper on sympathomimetic amines, Barger and Dale[1] described the action of hordenine (the *N* : *N*-dimethyl derivative of tyramine), and showed that the quaternary derivative, hordenine methiodide (1; $R' = R'' = H$), had a nicotine-like action.



(1)

($\overset{-}{X}$ = chloride, bromide or iodide)

They also found that the trimethylammonium derivative (1; $R' = OH$, $R'' = H$) of dopamine had a stronger nicotine-like action, about as strong as nicotine itself.

These compounds, together with the trimethylammonium derivative (1; $R' = R'' = OH$) of noradrenaline, have recently been made again by our colleague, Dr. A. M. Creighton, and have been compared with nicotine on the blood pressure of a spinal cat, and on other preparations. The full results will be described in detail elsewhere. Calculations were made in terms of active base. The general statement can be made that: (1) hordenine methiodide was found to be approximately equal to nicotine in pressor action; (2) the quaternary derivative of dopamine was about four times as active as nicotine; (3) the corresponding derivative of noradrenaline possessed only one-tenth of the action of nicotine. Thus the nicotine-like action was greatly reduced when $R'' = OH$.

These results led us to compare the pressor effects of ethyltrimethylammonium bromide with that of choline chloride. Both compounds have a pressor action in the spinal cat which is abolished by hexamethonium. Ethyltrimethylammonium was found to be about 40 times more active than choline expressed in terms of active base. Thus in this pair of compounds, also, the presence of an

–OH group attached to the β-carbon atom greatly reduced the nicotine-like action.

J. H. Burn
M. F. Cuthbert
R. Wien

Research Laboratories,
May and Baker, Ltd.,
Dagenham, Essex.

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Skin Reaction to Intradermal Injection of Rare Earths

The availability of highly purified rare earths, elements 21 and 58–71, has stimulated interest in their chemical and physical properties[1]. Haley *et al.*[2–6] have examined their biological effects and reported that although they can produce non-specific liver damage they exert their greatest toxicity on the ocular and dermal tissues. However, even this damage can be repaired with time and results in no permanent injury. Recently, it was observed that the intradermal injection in guinea pigs of 0·05 ml. aqueous solutions of 1×10^4–1×10^6 concentrations of praseodymium, neodymium, europium, dysprosium, holmium, erbium or lutetium chlorides resulted in the formation of 2-mm diameter nodules containing crystalline deposits (Fig. 1). Observation for periods of 45 days showed no decrease in nodule size or any resorption of the crystalline deposits.

Differential staining of histological sections with Dominici, Prussian blue and von Kossa stains indicated the presence of histiocytes and foreign body giant cells surrounding the crystals with fibroblasts and granulation tissue extending into the area. There was an indication of calcium but no iron in the crystals. Considering the total amount of rare earth injected, 0·05–5 μg, further analysis was not possible to establish the exact composition of the crystals. Furthermore, analytic procedures for the rare earths are non-specific and the presence of other ions would interfere to the extent of negating a more complete analysis. Of prime importance is the tissue reaction to such low levels of element and its implication to industrial prevention of injury during chemical processing. A re-evaluation of the other members of the series is being undertaken to determine whether or not they also can induce such changes in tissue at the same concentrations.

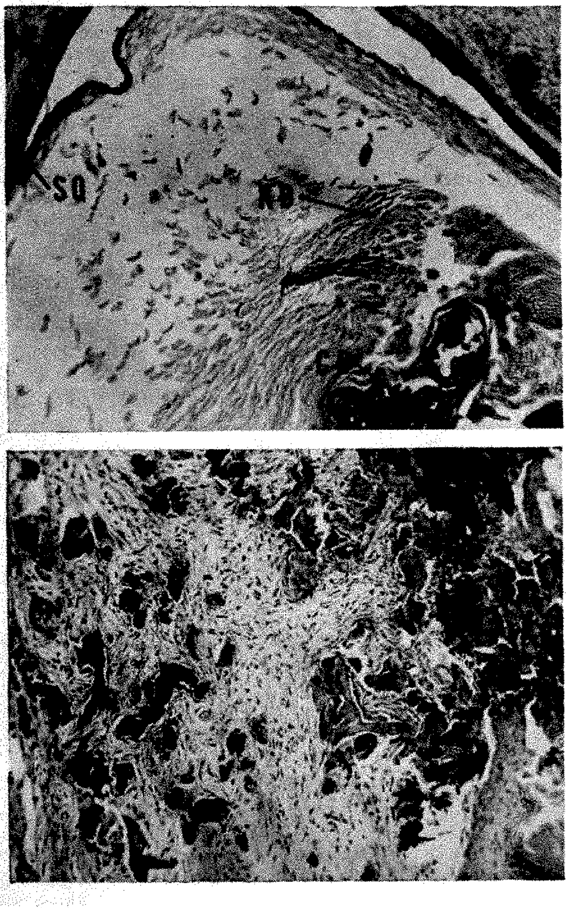

Fig. 1. Dermal reaction to injection of 0·05 ml. of 10^6 lutetium chloride in guinea pigs. Upper photomicrograph illustrates presence of keratotic debris (*KD*), stratified squamous epithelium (*SQ*) and crystalline deposits (*CD*). Lower photomicrograph illustrates foreign body giant cells in lower right-hand corner of upper photomicrograph

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Thomas J. Haley
Harvey C. Upham

Laboratory of Nuclear Medicine
and Radiation Biology,
Department of Biophysics and
Nuclear Medicine,
University of California at
Los Angeles.

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Some Biological Properties of 'Ibufenac', a New Anti-rheumatic Drug

For some years we have been investigating the biological properties of substituted phenylalkanoic and phenoxyalkanoic acids and related compounds. We wish to describe briefly the properties of one of these, 'Ibufenac', which Chalmers and Duthie have recently shown to be effective in the treatment of rheumatoid arthritis[1].

'Ibufenac' (British Pharmacopœia Commission approved name) is 4-isobutylphenylacetic acid, a colourless crystalline solid, melting-point 85°–87° C. It is relatively insoluble in water, but readily soluble in most organic solvents.

This compound has been tested by the oral route in a number of different anti-inflammatory and analgesic tests, and the results obtained in some of these are reported here. The anti-inflammatory activity of 'Ibufenac', as assessed by an ultra-violet erythema test in guinea pigs[2], is 2–4 times that of aspirin. Its analgesic activity, measured by a modification of the method described by Randall and Selitto[3], is also 2–4 times that of aspirin. The antipyretic activity is approximately 4 times that of aspirin when measured over a 4-h period in the yeast-fevered rat. The minimal effective dose of 'Ibufenac' for reducing thurfyl nicotinate ('Trafuril') erythema in man is 960 mg. The compound exhibits no glucocorticoid activity when tested in the mouse by a modification of the method described by Silber[4].

The acute oral LD_{50} in mice is approximately 1,800 mg/kg. Rats given 250 mg/kg daily for 6 months remained in good health and the only abnormality at necropsy was slight enlargement of the liver, kidneys and adrenal glands, which was reversible. Dogs were given daily oral doses of 40, 80 and 160 mg/kg for 6 months; some animals receiving 80 and 160 mg/kg showed simple renal hypertrophy, but kidney function was normal and there was no histological abnormality; gastric damage was found only at the highest dose.

Studies with the compound labelled with carbon-14 in the rat indicated that it is well absorbed from both the stomach and intestine. In human subjects, after doses of 640 mg, detectable levels of 'Ibufenac' were present in the blood within 30–60 min, rising to a maximum of 2–4 mg/100 ml. in 2–4 h. Small, but detectable, amounts were present 8 h after the dose.

The same two principal metabolites were found in the urine of the rat, rabbit, dog and man. Neither of these metabolites (extracted from the urine of subjects receiving 'Ibufenac') had analgesic or anti-inflammatory activity when tested by the oral route in animals. Preliminary metabolic studies in men and dogs indicate that the compound does not accumulate in the body.

S. S. ADAMS
E. E. CLIFFE
B. LESSEL
J. S. NICHOLSON

Research Department,
Boots Pure Drug Co., Ltd.,
Nottingham.

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HÆMATOLOGY

The Camel's Erythrocyte

THE camel's ability to live without water for long periods is outstanding[1,2,4,6]. It can lose about 30 per cent of its body-weight during dehydration in the desert and still move about[12]. A dehydrated camel can regain its losses of water (about 30 gallons) in about 10 min and resume its normal appearance; despite the large flow of water into the bloodstream its erythrocytes are not hæmolysed. In human beings the entry of 600 ml. of water into the circulation will produce hæmoglobinuria[11]. It is also known that cattle (in hot environments) deprived of water and then given large amounts of it may develop hæmoglobinuria. This, however, has never been observed in camels, according to statements made by camel raisers.

This unusual erythrocyte behaviour initiated the investigation of the rate of swelling and the osmotic resistance of the camel's erythrocyte to hypotonic solutions. Camels in Israel are of the single-humped 'dromedary' variety; they are found mainly in the hot and arid southern Negev. In the work recorded here, blood samples from 20 clinically healthy camels were compared with blood samples from 20 Holstein–Friesian adult healthy cows. Heparin served as anti-coagulant.

The rate of swelling of the erythrocytes in hypotonic buffered sodium chloride solutions and their osmotic resistance were carried out according to a modification of the Guest and Wing method[2]. The modification is based on the use of microhæmatocrit centrifuge and tubes[7,8]. One volume of packed erythrocytes is placed for one hour into test tubes containing four volumes of sodium chloride solution, with varying concentrations, and buffered to *p*H 7·3. The successive steps in the changing concentrations of the salt solutions used in the series were 0·05 in the range of 0·9–0·5 per cent and 0·025 per cent in the range of 0·5–0 per cent. Microhæmatocrit tubes were then filled with the diluted blood samples and centrifuged for 5 min at 12,000 r.p.m. The values for the volume of cells in the hæmatocrit tubes were measured and calculated in percentages on the basis of the first tube (0·9 per cent sodium chloride solution) the hæmatocrit value of which was taken as 100 per cent.

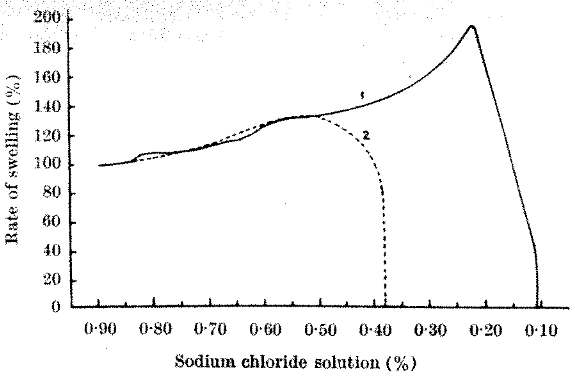

Fig. 1. Changes in volume of erythrocytes in hypotonic buffered salt solutions. 1, Normal adult camels; 2, normal adult cattle. (The initial hæmatocrit value counted as 100 per cent)

Fig. 1 and Table 1 show the osmotic behaviour of the camel's and cow's erythrocytes in hypotonic solutions. At first the swelling of all the cells raises the hæmatocrit values up to the point where the first trace of hæmolysis appears (minimal osmotic resistance), and then falls when the loss of cells by hæmolysis exceeds the increases in volume of the unhæmolysed cells. The camel's erythrocytes are by far more resistant to hypotonicity than the cow's and its minimal osmotic resistance was achieved, therefore, in a much lower concentration of sodium chloride solution (0·22 per cent sodium chloride, camel; 0·55 per cent, cow).

During the first decrements of the sodium chloride solution (down to 0·55 per cent) the camel's erythrocytes show the same rate of swelling as those of the cow. However, the maximal rate of erythrocyte swelling exceeds by far that of the cow (196 per cent of initial volume for the camel and 133 per cent for the cow (Fig. 1)).

Complete hæmolysis of the camel erythrocytes was achieved at the very low sodium chloride solution of 0·11 per cent, which deviates from the findings of Ponder[9], while in cattle complete hæmolysis occurred at a concentration of 0·36 per cent sodium chloride.

It can be observed from the curve (Fig. 1) that from a concentration of sodium chloride of 0·9 per cent down to 0·35 per cent a relative linear increase in erythrocytes volume occurs, but at a less-steep angle than that obtained for the perfect osmometer[7,10]. This deviation from the perfect osmometer curve of the cell swelling suggests that this may be the result of the excessive binding of water by intracellular components[1,5]. From a concentration of 0·35 per cent sodium chloride down to 0·22 another type of behaviour is observed, expressed in a steeper increase in the erythrocyte volume. This further angle increase in the curve of camel cell swelling attains the perfect osmometer.

Katchalsky *et al.*[3] attribute to the human erythrocyte membrane visco-elastic properties. When applying this to the animal erythrocytes one may presume that the elastic property of the camel red cell is smaller than that of the cattle and, therefore, stressing of the membrane does not cause counterstress as in a membrane with primarily elastic properties.

The cow erythrocytes, too, are far from exhibiting ideal osmometric behaviour. This may be seen from both their

Table 1. HEPARINIZED NORMAL CAMEL AND CATTLE BLOOD DILUTED WITH BUFFERED SOLUTIONS OF SODIUM CHLORIDE OF DIFFERENT CONCENTRATIONS

0·10	0·15	0·20	0·225	0·25	0·30	0·35	0·40	0·45	0·50	0·55	0·60	0·65	0·70	0·75	0·80	0·85	0·90	NaCl (%) Animal group
0	97 ±6·4	168 ±6·8	196 ±3·56	182 ±3·08	161 ±3·76	150 ±2·05	142 ±2·69	136 ±2·3	132·5 ±1·14	130 ±2·05	124 ±2·85	116 ±2·59	112 ±1·58	109·5 ±2·35	108 ±1·84	102 ±4·8	100	Camel
						0	105 ±11·7	121 ±7·05	131 ±6·9	133 ±6·44	126 ±6·36	120 ±7·1	115 ±7·05	111 ±7·08	106 ±4·2	101·2 ±2·67	100	Cattle

water-binding properties and membrane resistance, with their higher elastic component, as compared with the camel. The swelling of the corpuscle causes a counterstress in the membrane which in turn counterbalances the extent of the swelling correspondingly; at a certain amount of swelling, the elastic wall ruptures under the additional stress. This contrasts with the behaviour of the less elastic and more viscous properties of the cell membranes of the camel erythrocytes.

It would appear, therefore, that the capability of the camel blood to resist high dilutions is due to a number of causes, among these the increased binding capacity of the erythrocyte components for water and the large swelling capability shown by these corpuscles.

Investigations on the cell membrane components and its structure are being carried out at the present time.

I thank Mr. I. Horth and K. Lobel for their assistance.

K. Perk

Department of Animal Anatomy and Physiology,
Hebrew University,
Faculty of Agriculture,
Rehovot, Israel.

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IMMUNOLOGY

Immunological Changes and Therapeutic Effects of Convalescent Serum after Injury by Burns

Immunological changes after burns, induced by the breakdown of damaged skin tissues, were discussed half a century ago. Recently, reports from American and Russian authors seem to indicate the formation of antibodies against body-own burned skin, and also for the therapeutic value of convalescent serum. We have tried to confirm these findings.

Rabbits, guinea pigs, rats and mice were subjected to exact reproducible burns of third degree and 25 per cent extent in general anæsthesia. Sensibilization was performed by the intraperitoneal injection of extracts obtained from burned rabbit, guinea pig and rat skin, excised 2–96 h after a burn, or by implantation of excised burned skin into the peritoneal cavity of rats or guinea pigs. Convalescent serum from rabbits and rats was obtained by bleeding 3–4 weeks after the burn.

For testing the formation of antibodies, the following serological methods were used: Ouchterlony technique, Schultz–Dale technique, complement-binding-reaction, skin sensibilization test. For determination of the effects of convalescent serum, the following criteria were used: blood cell counts, plasma histamine-level, blood pressure, water content of the parenchymatous organs in rats, finally the survival time and rate after, if untreated, lethal burns in mice, and the mortality rate after injection of burned skin extracts.

In the Ouchterlony technique, all sera of sensitized guinea pigs gave positive reaction with extracts of burned skin from rabbits and with some extracts of burned skin from guinea pigs. All sera of sensitized rabbits reacted negative with extracts of burned rabbit skin. All sera of sensitized animals, both guinea pigs and rabbits, gave negative reaction with normal skin extracts, except one serum of a guinea pig.

In the Schultz–Dale preparation, after adding extracts of burned skin, the sensitized guinea pig uterus showed contractions of 30–75 per cent more than after addition of normal skin extracts. It is unlikely that histamine was responsible for this effect, since the burned skin extracts contained less histamine than those of normal skin.

Skin reactions could not be induced in sensitized guinea pigs by burned skin extracts. Complement-binding reactions gave negative results.

The determination of the effects of convalescent serum, injected in 2-ml. amounts intraperitoneally to rats subjected to burns as already described, in comparison with same amounts of normal rat serum or 0·9 per cent solution of sodium chloride, gave the following results.

Post-traumatic leucocytosis was reduced by normal and convalescent serum in the same degree; but not by sodium chloride. The histological picture in liver and kidney was not influenced by the application of the sera. The increase of water content of organs, well known after burns, was reduced by convalescent serum in the liver, with slight significance. Plasma histamine increase 1 h after burn was nearly completely inhibited by normal and convalescent serum, without significant difference. Fall in blood pressure, as measured from the rat tail, was not altered by the sera or by sodium chloride. Convalescent serum injected in guinea pigs did not show any increase of the survival time and the survival rate of these animals.

Survival time of lethally burned mice was prolonged by sera far more than by sodium chloride, but without any difference between the sera. Significant influence on blood pressure and on survival time and rate was only observed after injection of a protease inhibitor ('Trasylol ^{R}').

Our findings support the hypothesis of the development of immunological changes after burns, but they give no evidence for any protective action of convalescent serum in the therapy of post-burn disturbances. The interval between trauma and excision of burned skin, and also the method of extraction, seem to be of special importance for the results obtained in serological techniques.

L. Koslowski
B. Urbaschek
R. Versteyl

Surgical University Clinic,
and
Institute of Veterinary Hygiene,
Freiburg im Breisgau,
Germany.

Effect of Neonatal Thymectomy, Bursectomy and Thymo-bursectomy on Hæmagglutinin Production in Chickens

Since the demonstration by Glick *et al.*[1] that bursectomized chickens have a reduced ability to produce antibodies to *S. typhimurium*, a large body of evidence has shown the importance of the bursa of Fabricius in the development of antibody-forming capacity in birds. Mammals thymectomized in early life are unable to manufacture antibodies against certain antigens[2]. However, the role of thymus in the development of immunological potential in chickens is not clearly established. The work recorded here compares the relative roles of thymus and bursa in hæmagglutinin production in chickens.

Rhode Island Red chickens were thymectomized, bursectomized and thymo-bursectomized 2 days after hatching. One group of 9-week-old bursectomized chickens was grafted with bursa from newly hatched chickens implanted between parietal and visceral peritoneum. A second group was grafted with bursa from 8-week-old birds. An additional group of chickens was bursectomized at the age of 8 weeks. At 10 weeks all groups were

Table 1. ANTIBODY RESPONSE OF NORMAL, THYMECTOMIZED, BURSECTOMIZED AND THYMO-BURSECTOMIZED CHICKENS

Group	Age at time of operation	No. of chickens*	Grafting (age of bursa donor)	Mean peak agglutinin titre ($\log_2$)
Control	—	12/12	—	9·22 ± 0·79
Thymectomized†	2 days	7/7	—	7·92 ± 2·06
Thymo-bursectomized	2 days	6/8	—	2·92 ± 2·14
Bursectomized	8 weeks	9/9	—	10·12 ± 0·40
Bursectomized	2 days	10/16	—	3·61 ± 2·81
Bursectomized	2 days	17/17	2 days	8·21 ± 3·03
Bursectomized	2 days	15/19	8 weeks	6·12 ± 1·19

* Numerator, No. of chickens with hæmagglutinins; denominator, No. of chickens in group.

† Five birds had < 0·2 g, and two birds < 0·7 g, of residual thymus. Thymus weights in control chickens at 11 weeks were between 1·9 and 2·8 g.

injected with 1·5 ml. of a 10 per cent suspension of human O erythrocytes. Seven days later the birds were bled and hæmagglutinin determinations were carried out as described previously[3]. Since chicken antisera undergo *in vitro* changes on standing[4]; all titrations were made 5 days after bleeding. The cloaca and neck were examined *post mortem* for the presence of bursal and thymic residua.

The findings are summarized in Table 1. Birds surgically bursectomized or thymo-bursectomized at 2 days showed very low hæmagglutinin titres. Bursectomy at 8 weeks did not affect antibody-producing capacity, however. These results agree with those of Chang *et al.*[5], and Mueller *et al.*[6], who found that only early removal of the bursa impaired the antibody response. Totally or partially thymectomized chickens showed normal hæmagglutinin production. The bursal implants clearly enhanced the reactivity of bursectomized chickens, and new-born bursa was somewhat more effective than 8-week bursa. Histological examination of the methyl-green-pyronine stained sections of spleens has revealed marked proliferation of plasmocytic cells in normal and thymectomized chickens 7 days after immunization. Plasma cells were seen also in the spleens of bursectomized birds, but there was no proliferative response following antigenic stimulus.

Bursa-homografts were rejected by bursaless chickens with great uniformity. Eight days after grafting, that is, 3 days after the injection of heterologous erythrocytes, the periphery of the graft had been replaced by connective tissue, and the adjacent areas were infiltrated with mononuclear cells. Bursal follicles in the central part of the graft were still recognizable as such, but their cellular make-up had been totally destroyed (Fig. 1).

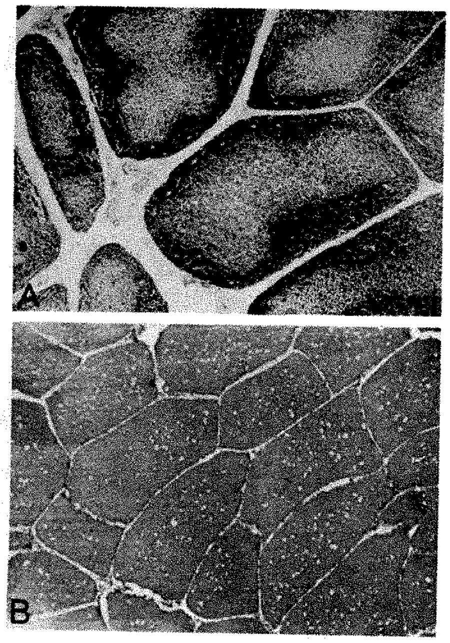

Fig. 1. *A*, Bursa of Fabricius from 8-week-old chicken. Normal appearing follicles. Basement membrane separates the cortex from the medulla; *B*, bursa-homograft at the eighth day. Cellular make-up of follicles is totally destroyed. (× *c*. 20)

Bursa of Fabricius may share in birds some of the functions[7-9] performed in mammals by the thymus alone[10,11]. The present work shows that neonatal thymectomy does not interfere with hæmagglutinin production in chickens. On the other hand, neonatal bursectomy suppresses antibody formation, but has no effect on homograft rejection. It is generally assumed that bursa acts as a source of lymphoid cells which are distributed to other tissues of the lymphoreticular system. Our observation that antibody-producing capacity is restored in bursectomized chickens by bursal homografts could be taken as evidence in favour of this supposition, but by no means rules out a hormone-like function of the bursa. In support of this latter contention Glick[12] has reported that injections of saline extract of acetone-dried bursæ enhances to some extent the antibody response to sheep erythrocytes in bursectomized chickens.

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KATARINA ISAKOVIC
BRANISLAV D. JANKOVIC
LJILJANA POPESKOVIC
DAFINA MILOŠEVIC

Microbiological Institute,
Faculty of Pharmacy, and
Immunology Unit, Institute of Biology,
University of Belgrade, Yugoslavia.

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HISTOCHEMISTRY

Blocking the Ninhydrin – Schiff Reaction with Copper Ions

THE ninhydrin–Schiff reaction of Yasuma and Ichikawa[1] has been widely used as a histochemical method for the detection of α-amino-acids[2,3] and recently it was applied quantitatively in cytochemistry by Rappay and Törk[4]. Whereas the detailed mechanism of the reaction has not been completely verified at the histochemical level, there is now fair agreement as to the specificity of the reaction for proteins[5]. The present experiments endeavour to show that colour production may be markedly affected by the purity of the reagents used.

Blood from adult individuals of *Amblystoma mexicanum* (axolotl) and *Pleurodeles waltlii* (a newt), obtained by decapitation and washed thrice with physiological saline, was smeared on slides, dried at room temperature, and fixed in Carnoy's fluid and 80 per cent cold acetone for 15 min. For some slides the fixatives contained 10^{-2} M of copper sulphate while others were placed in a 10^{-1} M copper sulphate solution for 30 min. Both untreated slides and those treated with copper sulphate were incubated for 16 h in 0·5 per cent ninhydrin ('Ninhydrinum', $C_9H_4O_3H_2O$, mol. weight: 178·15) dissolved (*a*) in absolute alcohol free from metallic salts; (*b*) in absolute alcohol saturated with copper sulphate; (*c*) in 96 per cent alcohol with copper sulphate added to it in final concentrations

Table 1. EFFECT OF BLOCKING THE REACTION WITH COPPER IONS

Erythrocyte	Copper sulphate (in molar concentration)									
	Added to fixative		Applied after fixation		In alcoholic ninhydrin solution (0·5%)					
					Absolute		96 per cent			
	0	10^{-2}	0	10^{-1}	0	Saturated	0	10^{-2}	10^{-4}	10^{-6}	10^{-8}
Nucleus	+ + +	+ + +	+ + +	+ + +	+ + +	–	+ + +	–	–	±	+ + +
Cytoplasm	+	+	+	+	+	–	+	–	–	–	+

+, Colour intensity; –, negative reaction; ±, questionable reaction.

ranging from 10^{-2} to 10^{-8} M. Treatment with ninhydrin was followed by immersion in Schiff's reagent for 30 min.

From Table 1 it can be seen that in a medium free from copper sulphate the ninhydrin–Schiff reaction gave an intense colour to nuclei and somewhat less staining of the cytoplasm of erythrocytes from the two amphibian species studied; further, that copper sulphate, whether added to the fixative or applied after fixation, was without influence on colour intensity. On the other hand, both nuclear and cytoplasmic stainability were remarkably dependent on the copper sulphate concentration in the ninhydrin solution. Apparently, concentrations of 10^{-2}–10^{-6} M copper sulphate were capable of completely suppressing the reaction.

The suppressive action of copper sulphate may be explained in several ways. One possibility is that copper ions attach to the reactive sites of amino-acids participating in the reaction, blocking the formation of aldehyde groups. Against this explanation is the fact that treatment with copper sulphate preceding incubation in ninhydrin had no influence on aldehyde-group formation and the subsequent colour reaction. Another possibility considered is that ninhydrin itself becomes in some way bound to copper sulphate. This seems improbable in view of the low concentration of copper sulphate present in the incubation mixture in relation to that of ninhydrin. The most likely explanation appears to be that the copper ions, as metallic salts, are responsible for altering the reaction mechanism, which generally would be an oxidative process. It merits mentioning that zinc sulphate also suppresses formation of the aldehyde groups necessary for the reaction to occur.

On the basis of the experimental evidence summarized here, the ninhydrin–Schiff reaction, particularly when used for quantitative purposes, calls for the use of high-purity reagents free from metallic salts.

GY. RAPPAY

Department of Morphology,
Institute of Experimental Medical Research,
Budapest, 9.

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New Data concerning Histochemical Structure of the Myocardium

TRANSVERSE striation of muscle fibres in the heart has been known for a long time; but two problems in relation to this peculiarity of myocardium are not as yet solved. The first problem is concerned with the histochemical nature of transverse striation and the second with the functional significance of the individual components of myofibrils.

This communication deals with the distribution of protein and glycogen in cardiac muscle fibres examined with the aid of polarizing and phase-contrast microscopes.

Investigations were provided on 10 intact dogs, the contracting hearts of which were fixed in Shabadash's fixator (calcium nitrate, 0·9 g; copper nitrate, 1·8 g; 96 per cent ethanol, 90 ml.; 40 per cent formalin, 10 ml.) injected into the coronary vessels. The serial paraffin sections (7μ thick) were stained for total protein using Danielli's tetrazonium method and for glycogen with the aid of periodic acid–Schiff reaction following Shabadash[1,2]. Incubation of the control sections in benzoilchloride and in diastase, respectively, prevented the positive reaction for protein and glycogen. In order to compare histochemical and histological structures of myofibrils the parallel sections were stained with hæmatoxylin and eosin and with iron hæmatoxylin. The slides were examined under the light microscope, polarizing microscope and in phase-contrast microscope.

It was established that ordinary muscles of myocardium in intact dogs have an uneven distribution of protein. Separate groups of fibres show more pronounced reaction for proteins than the others, where it is manifested less significantly. The same picture is observed in Purkinje fibres. In comparison with the ordinary muscle fibres of myocardium, the fibres of the conduction system contain more protein. This may be the reason for the absence of transverse striation in these fibres, although it could be readily seen in the polarized light. The transmission of Purkinje fibres into usual muscle fibres occurs gradually without any visible boundaries and is followed by decrease in the intensity of protein reaction. Usual light microscopy reveals in these instances manifested transverse striation of the cardiac muscles.

Histochemical investigations of the separate muscle fibres in polarized light as well as under the phase-contrast microscope have shown that the anisotropic disks are denser and correspondingly richer in protein, whereas isotropic disks contain small amounts of protein. In the central portion of these disks, however, which corresponds to the *Z*-line, the protein reaction is more manifested. In anisotropic disks it is possible to reveal three lines: two lateral lines are separated from each other by a pale zone with the third *M*-line in its centre (Figs. 1 and 2).

The intercalated disks are characterized by large, but not always constant, amounts of protein. Some of them react more actively and can be readily revealed against the background of the transverse striation, whereas the others are scarcely seen in the light microscope. Such intercalated disks, which are isotropic, may be found with the aid of a polarizing microscope even in those cases when the usual light or phase-contrast microscopy fails to reveal them. Furthermore, a polarizing microscope makes possible a study of structures of intercalated disks in unstained sections. This may be utilized as a new method for their detection (Figs. 3 and 4).

Correlations in the distribution of protein and glycogen in myocardium have demonstrated that these substances are localized predominantly in the same sites of Purkinje fibres of the heart and typical cardiac muscle fibres. At the same time, it is always possible to reveal essential differences in the form of their distribution in the microstructures of myofibrils. Glycogen in the sarcomeres of

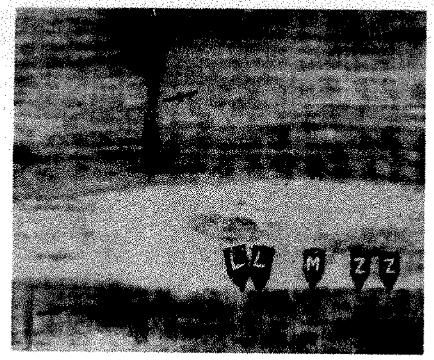

Fig. 1. Phase-contrast microscopy of cardiac muscle fibres of the dog. Reaction for protein. Arrows with letters indicate separate stripes of sarcomeres, simple arrows indicate intercalated disks. Objective 90, ocul. 10 (× c. 1,200)

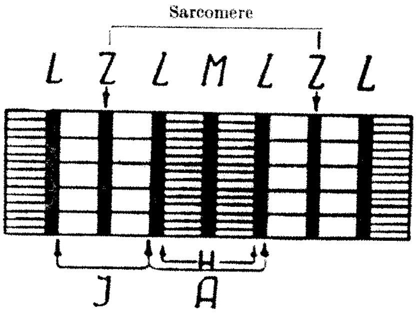

Fig. 2. A scheme of protein distribution in sarcomere. The dark areas correspond to the intensity of the reaction for protein

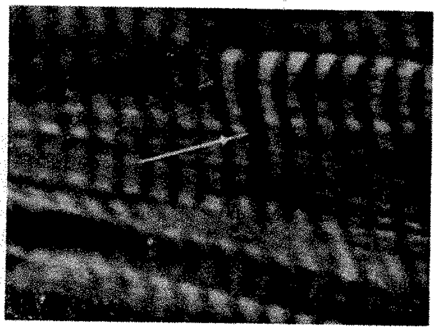

Fig. 3. Microphotograph of the dog's cardiac muscle fibres in polarized light (viewed with crossed polarizer and analyser). The intercalated disks are isotropic (indicated with arrow); the section is not stained. Objective 90; ocul. 10 (× c. 1,700)

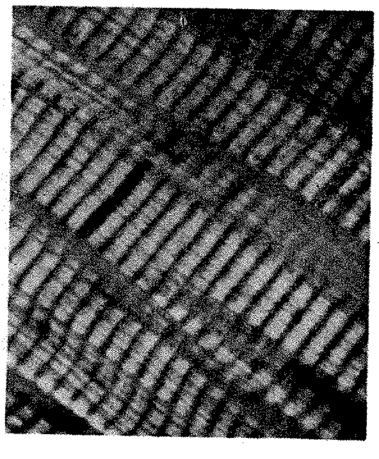

Fig. 4. Microphotograph in polarized light (viewed with crossed polarizer and analyser) of the dog's cardiac muscle fibres. Reaction for protein. Inserted disks and Z-stripes contain comparatively large amounts of protein. Objective 90; ocul. 10 (× c. 1,200)

individual muscle fibres is distributed differently. In some fibres it is predominantly accumulated, as well as protein, in A-disks and Z-lines (Fig. 5*a*); in other fibres it is located in I-disks (Fig. 5*b*). Different distributions of glycogen in sarcomeres are dependent on the functional states of muscle fibres during fixation. In the intercalated disks glycogen is localized essentially along the margins whereas protein has a diffuse distribution throughout the entire disk, leaving sometimes narrow and pale space in the centre. The latter seems anisotropic in polarized light (Figs. 1, 3, 4 and 5).

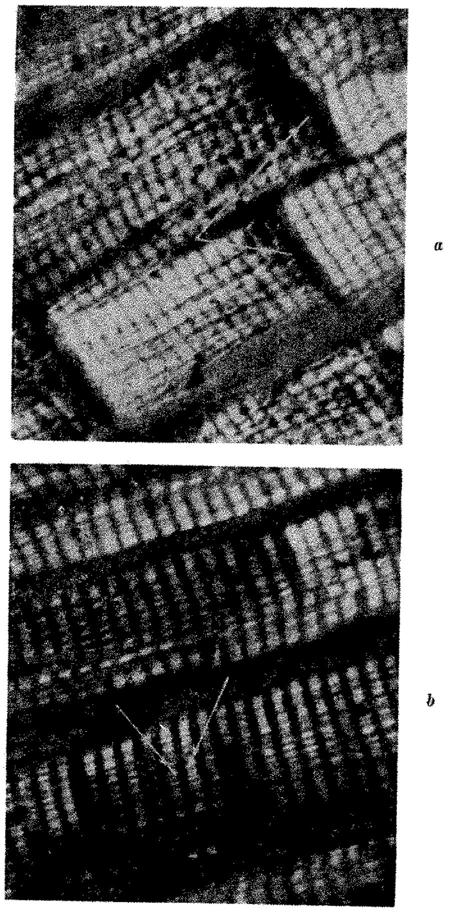

Fig. 5. Microphotograph in polarized light (viewed with crossed polarizer and analyser) showing unevenly distributed glycogen in the micro-structures of functionally different areas of myocardium. *a*, Contracted fibres. In the centre of the figure one can see two muscle fibres with different content of glycogen. The latter is manifested in Z-stripes and A-disks. The arrows indicate the distribution of glycogen at the margin of the intercalated disk. *b*, Enfeebled fibres. Glycogen is accumulated predominantly in I-disks; in Z-stripes and in A-disks it is contained in less significant amounts. The contents of glycogen in I-disks are shown by the double arrows. Objective 90; ocul. 10 (× c. 1,200)

Thus, these observations show that protein in myofibrils is distributed unevenly and is accumulated in the intercalated disks, Z-stripes and in three lines of anisotropic disks. The centre of these coincides with the M-line, whereas the two marginal ones separated by an H-zone do not have a special name. I propose to name them L-lines (the first letter of the Latin word, lateralis).

Unevenly distributed protein and glycogen[3] in microstructure of myofibrils may be considered as an essential cause of transverse striation in the muscle fibres of the heart.

Comparatively high contents of protein and glycogen in Z-lines and in the intercalated disks suggest active energetic participation of these structures in the contractile activity of myofibrils. This is confirmed by the literature, which presents information on the presence of various enzymatic systems in *Z*-lines and in the intercalated disks[4-6].

S. S. SAIDRASULOV

Laboratory of Pathological Physiology
and Experimental Therapy,
Institute of Cardiovascular Surgery,
Academy of Medical Sciences of the
U.S.S.R.,
Moscow.

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RADIOBIOLOGY

Relative Biological Efficiency of Negative μ-Mesons and Cobalt-60 γ-Rays

THE concept of the relative biological efficiency of different radiations is one that has considerable practical and theoretical interest, and an extensive literature exists which describes in detail the problems of the determination and interpretation of relative biological efficiencies[1]. Variations in the relative biological efficiency of different radiations are attributable to differences in their ion density or linear energy transfer, defined as the rate of energy loss in eV/μ of irradiated material. Recently an opportunity arose at this laboratory to determine the relative biological efficiency of a highly energetic radiation with low linear energy transfer characteristics—an almost pure beam of μ^- mesons. These have a mass of about $200m_e$, one negative charge, and an energy range in this experiment of 2–8 GeV (with a peak at 4–6 GeV).

The muons were generated in the Brookhaven alternating gradient synchroton. A beam of protons (energy ~ 28 GeV) was brought on to a beryllium target placed in the machine. Negative pions produced in the target between the energies of 7–9 GeV in the forward direction were bent through the magnet following the target and out of the machine at an angle of 9°. After passing through an entrance lens of 4 quadrupole magnets, the beam was confined by a series of 5 pairs of quadrupoles placed about 25 ft. apart[2]. The pions were concentrated in a spot of radius ~ 3 in., and the decay angle of the muons was small enough so that almost all the decay muons remained in the 6-in. radius which was available after the entrance. Roughly 10 per cent of the pions decayed before the beam was filtered through 7 ft. of concrete, which removed most of the pions and left the muons essentially unchanged except for about a 1-GeV loss of energy. With about 2×10^{11} protons striking the target an intensity of the order of 10^8 muons/pulse was obtained, the pulse rate being once every 2·5 sec.

Energy loss from muons occurs primarily through electromagnetic interactions-by direct ionization, and by the production of knock-on secondary electrons. The energy deposition in the *Tradescantia* tissues used in the present experiment could be calculated, since the elementary composition of the tissues[3], and the energy loss/gm/cm^2 in the different elements, were known. The average linear energy transfer was 195 eV/μ and on this basis a low relative biological efficiency would be predicted. The precise range of energy of the knock-on electrons, and hence of the range of linear energy transfers, was not known.

The biological test system used was that of somatic mutation induction in staminal hair cells of a diploid clone (02) derived from a variety of *Tradescantia occidentalis*[4,5]. This clone was heterozygous for genes controlling anthocyanin pigment, heterozygous cells being blue, homozygous recessive mutant cells red and easily distinguishable. A given inflorescence contained a large number of serially arranged buds—one flower emerging daily. Each flower had six stamens, and each stamen approximately 55 hairs. Staminal hairs are chains of single cells and their pattern of growth is as follows. A single cell in the filament becomes meristematic and becomes the terminal cell of the hair, continuing to divide until the mature hair (20–30 cells) is formed behind it. The subterminal cell always divides once after its formation, and occasionally an intermediate cell also divides. The mutations were almost certainly chromosomal deletions and recognition of a mutant phenotype was dependent on the loss of a particular fragment from that cell, that is, mutations could be expressed only after cell division. Since there were two cells at the tip of the hair and occasionally a third intercalary cell which were meristematic, the maximum number of cells per flower in which a mutation could occur and be expressed was 3×55 (hairs per filament) $\times$ 6 (filaments per flower) = 990. All the hairs do not develop simultaneously and the number of potentially mutant cells is low initially, increasing to a maximum of about 990, and then decreasing again. The pattern of mutation production was similar.

Eight inflorescences were exposed in air in the muon beam for each of the following periods of time—2, 4, 6, 8 and 10 h. Dosimetry was complicated by the fact that the beam was non-uniform over its entire area (a circle 12 in. diam.). To obtain accurate measurements individual phosphate glass dosimeters (rods 1 mm diam., 5 mm long)[6] and in some instances lithium fluoride thermal luminescent dosimeters also[7] were attached to each inflorescence. These dosimeters were calibrated in the muon beam against a Rossi type *W* tissue equivalent ionization chamber[8]. The small dosimeters had an accuracy of ± 5 per cent within the dose range used here. Muon doses for the 40 inflorescences (8 inflorescences, 5 exposure times) varied from 12 to 101 rads, and the dose rate fluctuated between 7·3 and 13·5 rads/h, the dose being administered as explained earlier in pulsed beams at 2·5-sec intervals.

Table 1. ESTIMATED RELATIVE BIOLOGICAL EFFICIENCY (RBE) VALUES FOR μ^- MESONS AND γ-RAYS

Day	α-coefficient (μ meson)	α-coefficient (γ)	RBE
1	0·14785	0·20128	0·73
2	0·17038	0·23147	0·74
3	0·17723	0·21877	0·81
4	0·14300	0·16000	0·89
		Mean RBE	0·79

As a standard for the muon treatments, inflorescences were exposed in air at a dose rate of 7·5 rads/h to four doses of cobalt-60 γ-rays: 30, 48·7, 71·2 and 120 rads (measured with the same tissue equivalent ionization chamber). Eight inflorescences per treatment were also used for the γ exposures. An estimate of the spontaneous mutation rate was obtained by scoring 72 unirradiated flowers.

Virtually no lethality was induced within these dose ranges, and there was no opportunity for cell selection or an elimination of mutant cells. Mutations were scored in the staminal hairs of flowers emerging on four successive days and the data relating yield per flower, to dose in rads, fitted to linear, $y = k + \alpha D$, and quadratic, $y = k + \alpha D + \beta D^2$, regressions. In all instances linear regressions better fitted the data, and the linear (α) coefficients for the muon and γ exposures for each of the four days are given in Table 1. The relative biological efficiency of muons: γ is given by the ratio of the α coefficients, and has a mean value for all days of 0·79. This value is essentially in agreement with what would have been predicted from a knowledge of the linear energy transfer characteristics of the μ mesons, and appears to be significantly lower than 1, though this would be difficult to prove conclusively with the present experimental system and methods of dosimetry.

An approximate estimate of the absolute 'mutation' rate per cell may be obtained from the data of that day when the number of meristematic cells per flower is at a maximum, that is, about 1,000. For the chronic γ exposures, the yield per flower for that day was 0·03527 + 0·23147 = 0·26674/rad, and the approximate rate per cell per rad was therefore $2·7 \times 10^{-4}$.

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D. R. Davies*
A. H. Sparrow
R. G. Woodley

Biology Department,

A. Maschke

Accelerator Division,
Brookhaven National Laboratory,
Upton,
New York.

* Present address: Wantage Research Laboratory, Wantage, Berks.

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Cæsium-137 Body Burden in Persons chosen at Random from Selected Areas in Norway

The global distribution of fall-out has not been uniform: due to nuclear weapon tests by far the largest fraction has been deposited on the northern hemisphere. These circumstances are partly a result of the geographical positions of the test sites; partly a result of meteorological conditions.

The amount of fall-out deposited is normally a function of rainfall. Local areas with high precipitation on the northern hemisphere have thus shown by far the largest contamination. This is the case in certain districts on the west coast of Norway having a yearly precipitation ranging up to 3,500 mm.

As a result of the fall-out, fission products have gained access to soil and vegetation, human food and consequently also to man himself.

One of the radionuclides in the fall-out which is of special interest in this connexion is cæsium-137 due to its long physical half-life, its large fission yield and its chemical similarity to potassium which is a very important body constituent.

In order to measure radioactivity in the human body, installation of a whole-body counter at Norsk Hydro's Institute for Cancer Research was begun in February 1962.

Early in the construction period while Norway was still covered by snow and hence the cattle fed on hay gathered before the nuclear tests were resumed in 1961, we started a systematic collection of milk samples from all Norwegian dairies. It was felt justified to assume that the amount of activity in the milk at that time would be down to a minimum of long-lived radionuclides, so that the milk survey would give an approximately stabilized picture of the geographical distribution of the fall-out. Since the milk was measured in a γ-spectrograph, cæsium-137 was the natural radionuclide to use as an indicator.

It has previously been shown that the cæsium-137 body-burden in man[1] to a large degree depends on milk consumption. It could thus be expected that the concentration of cæsium-137 in milk to some extent could be regarded as an indicator of cæsium-137-levels in people from the various districts.

In Table 1 the results of the milk survey are presented. The figures given are mean values for the cæsium-137 contents in regions I, II, III and IV, representing the eastern, western, middle and northern parts of Norway as indicated on the map. Also maximum and minimum values found in single samples from dairies in these regions are given.

Table 1

Region	No. of samples	^{137}Cs pc./l.* min.	max.	mean.	Production per month ($\times 10^6$ l.)	Total amount ^{137}Cs μc. per month	Population ($\times 10^6$)
I	96	0	120	20	35	737	1·75
II	108	5	505	115	30	3,510	1·08
III	42	5	175	45	14	641	0·33
IV	26	55	675	230	8	1,838	0·44

* 1 pc. = 1 μμc. = 10^{-12} c.

As can be seen from Table 1, the contamination in milk from regions II and IV is relatively high. From a body-burden point of view, both regions are thus of considerable interest. However, region IV is far more inconvenient than region II with regard to transport of people to Oslo for total body-burden measurements. We therefore decided to undertake a more detailed examination of the conditions inside this district. As a result of these investigations we directed our attention on Masfjorden, a small community near the coast, some miles north of Bergen. Masfjorden is one of the districts having the highest yearly precipitation in Norway.

A more detailed study of the milk from Masfjorden showed that its cæsium-137 content showed a first decided increase in June, rising all summer to a maximum of about 1,600 pc./l. by the end of September. Then the value showed a rapid decline.

As a first part of our cæsium-137 body-burden examination of people from the western part of Norway, we thought it would be of particular interest to start our investigations with a group from this district. A statistically selected assembly of 11 persons willing to take part in this programme travelled in February 1963 to Oslo to have their body-burden measured in the whole-body counter. Another group of 10 persons from the city of

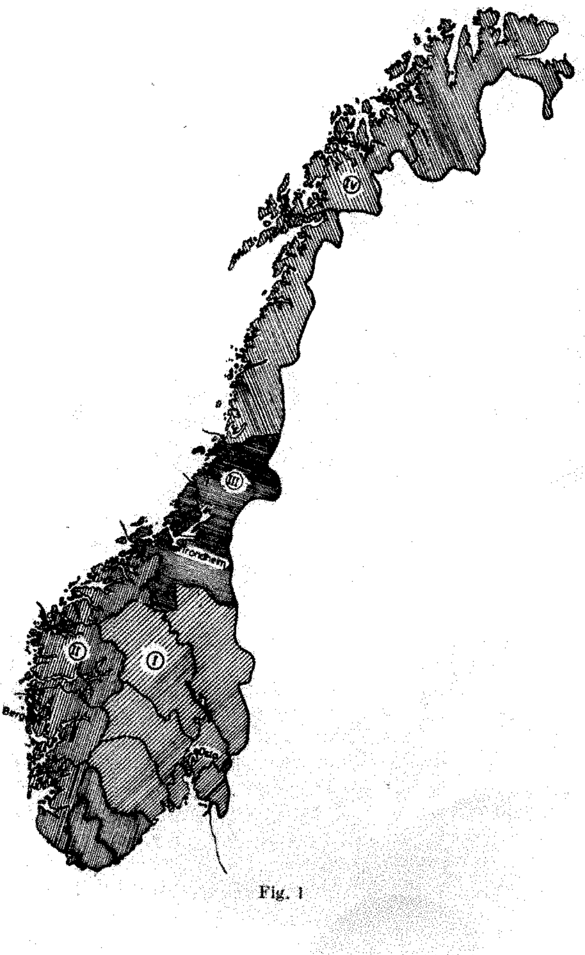

Fig. 1

Table 2

Locality	No of individuals	Body-burden nc ^{137}Cs min	max	mean	^{137}Cs/K pc /g min	max	mean
Masfjorden	11	27 6	162 5	91 8	211	1,395	784
Bergen	10	20 0	75 6	44 2	182	497	332

Bergen were measured at the same time The results of these measurements are shown in Table 2

It is interesting to note the great differences in the body-burdens measured in these two groups, despite the fact that their homes are not more than 30 miles apart This is probably a result of the fact that food consumed in Bergen is a mixture of products from a variety of places, many of them being localized to inland districts, whereas food consumed in Masfjorden to a large extent is of local origin

Two of the individuals from Masfjorden having nearly the same body-burden (162 5 nc and 153 0 nc) were of special interest, due to their highly different milk intake One of them was a heavy milk consumer (2 0 l /day) while the other stated that her milk consumption was only 0 25 l /day

There is thus no evidence of a direct correlation between milk consumption and the cæsium-137 body-burden, either for the people from Masfjorden or for those from Bergen It is therefore to be expected that even other products are of importance

KJELL MADSHUS
Norsk Hydro's Institute
for Cancer Research,
AKSEL STRØMME
The Norwegian Radium Hospital,
KRISTIAN KOREN
State Institute of Radiation Hygiene,
Norway

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BIOLOGY

Reduction of Radiation Mortality through Magnetic Pre-treatment

THE effect of whole body irradiation on rodents with lethal doses of X-rays or γ-rays is one of the most widely investigated phenomena Mortality depends on the total dose, intensity at which the dose is delivered, strain, sex and age of the animal Both serial histological examination and direct counts demonstrate the rapid loss of nucleated cells from the bone marrow The animals' failure to replenish the granulocytes, platelets and erythrocytes from the circulating blood is primarily responsible for the death, as a consequence of septicæmia, hæmorrhage and anæmia

In a previous communication[1] it was shown that leukocytosis setting in after treatment of animals in magnetic fields[2] can be used to compensate partially the radiation syndrome In 17 further experiments a total of 920 mice of different age and strain were treated in homogeneous and inhomogeneous magnetic fields Various time-intervals were interposed between the termination of magnetic treatment and irradiation, also treatment after irradiation was tried In all experiments the permanent magnets, cages and dummy magnets, described elsewhere[3], were used In most instances 30 mice were treated in 10 permanent magnets for a period of 14–20 days and the same number of mice housed during the same time in ten dummy magnets After treatment all were removed for a specified number of days to standard plastic cages, whereupon all of them were exposed to irradiation with γ-rays in the Cobalt Therapy Unit of the Cook County Hospital in Chicago, Illinois The total administered dose was always 800 r , delivered at an intensity 33–100 r /min It produced in the control group a mortality of 30–100 per cent

After irradiation the animals were placed in standard plastic cages, 3 treated and 3 controls to the same cage, to compensate for possible cage effects The body weight was measured daily, the dead removed within 4 h after death, even during the night They were immediately autopsied, liver and spleen weight measured and from the general inspection of the organs the possible cause of death noted In four experiments blood samples were taken from the tail vein on several occasions after irradiation Leukocytes and erythrocytes were counted with a Coulter electronic particle counter

These investigations revealed many interesting features, but in most instances they can be considered only as preliminary results which need further confirmation However, two significant results emerged

A magnetic pre-treatment of mice in a homogeneous magnetic field of 4,200 œrsteds strength and less than 3 days of delay between the termination of treatment and irradiation decreases the death rate by 24–30 per cent The results of five such experiments, performed under the described conditions, are shown in Table 1 The difference in mortality rate is significant on a probability-level (*P L*) of 1 800

Table 1

Mortality (%) Magnet	Dummy	Relative mortality difference (%)
70 0	90 4	−22 6
66 7	100 0	−33 3
45 0	70 0	−35 7
60 0	76 7	−21 8
53 4	66 7	−19 9
		−26 7 ± 3 2

t, 8 3, *n*, 4, *P L* , 1 800

The leukocytosis following the magnetic treatment does not compensate the leukopenia caused through irradiation it merely diminishes it slightly For example, on the 10th day after irradiation in one experiment the leukocyte count of the treated group was 13 per cent of normal, while that of the controls was 9 per cent Nevertheless, it seems to be that even this small difference is adequate to prevent death in some cases The lowering of the death rate is most pronounced from the 7th to the 14th day after irradiation, that is, during the intestinal and bone marrow mode of the radiation syndrome This interval coincides with the time when the maximum of the leukocytosis, caused by the magnetic treatment, is observed

Treatments in inhomogeneous fields were performed at a somewhat lower field strength (3,600 œrsteds), but with a vertical gradient of 650 œrsteds/cm, produced with the help of special pole caps It corresponds to an average paramagnetic strength of 2 3 mega-œrsteds per square cm In some experiments the animals were changed daily from a north pole-up field to a south pole-up field. This is equivalent to a change of the field vector direction by 180° relative to the animal It should decrease all reversible effects caused by a homogeneous field, but the exchange leaves unaltered the direction of the vertical gradient vector relative to the animal and hence should not affect reversible paramagnetic effects

In four experiments in which the mortality of the controls was between 25 and 45 per cent, the magnetically treated animals had a higher mortality rate (relative mortality difference $+19 \pm 9$ per cent) In three experiments, with a mortality of the controls between 65 and 95 per cent, the mortality of the treated was lower (relative mortality difference $-21\ 3 \pm 6\ 5$ per cent) The lowering of the death rate was most pronounced from the 10th to the 20th day after irradiation, it covers, therefore, mainly the late mode of the radiation syndrome, a mode in which anæmia plays a major part

Treatment in inhomogeneous fields affects not only the leukocyte count, as does homogeneous fields, but also the erythrocyte count and could therefore affect irradiation anæmia But due to the simultaneous variation of two blood elements (probably also platelets), the interpretation of the mortality curve in experiments with inhomogeneous fields is too complex to be attempted at present

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MADELEINE F BARNOTHY

College of Pharmacy,
University of Illinois,
Chicago

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Distribution of Cyanole and Neutral Red in the Giant Axon of the Squid

PREVIOUS work on the permeability of frog muscles[1] and giant axons of Cephalopoda[2,3] to vital dyes has shown that basic and acid dyes rapidly penetrate the cell and its intracellular content may be less or greater than in the medium, depending on the outside concentration and type of the dye For giant axons in the steady state the coefficient of distribution ($Q = \frac{C_{in}}{C_{out}}$, where C_{in} and C_{out} represent respectively intra- and extra-cellular concentration) for the acid dye (cyanole) was found to be 0 12 with the external dye concentration varying from 0 1 to 1 0 per cent (ref 3) At the same time the coefficient of distribution of the basic dye (neutral red) changed from 5 5 to 0 65 on a corresponding increase in C_{out} from 0 004 to 0 16 per cent (ref 2)

The purpose of the work recorded here was to investigate the Q values for axoplasm itself and compare them with the previously obtained values for the whole axons Therefore the content of the dye was determined both in the whole axons and pipetted axoplasm

Experiments were performed on the axons of *Loligo* sp , 200–300μ in diameter Isolated axons were soaked in cyanole or neutral red solution for 20 min As has already been shown[2], a period of 10–20 min is enough to reach equilibrium Then the axons were washed up (5–10 sec) in sea-water and blotted The amount of dye in ethanol acid extracts of axons was determined with the aid of a visual microcolorimeter For measuring the dye content in axoplasm the stained fibres were cut, axoplasm was then extracted with a micropipette and also transferred to the ethanol acid mixture The amount of dye was calculated per unit of the fibre or axoplasm volume.

The results obtained are given in Table 1

Table 1

Dye	Concentration in the axon (C_{in}) (mg %)	Concentration in the axoplasm (C_{ap}) (mg %)	$\frac{C_{in}}{C_{ap}}$	$\frac{C_{ap}}{C_{out}}$
1% Cyanole	120 ± 5	82 ± 16	1 46	0 082
0 01% Neutral red	63 ± 5	35 ± 5	1 80	3 5

As can be seen, an increase as well as a decrease in the bulk dye concentration in axons compared with the medium depends on the concentration of dye in the axoplasm In accordance with the previous studies[1–3], the observed distribution of dyes can be considered as a result of two factors (1) decreased solubility of protoplasm for the substances with the size of molecules as big as that of dyes, (2) adsorption or chemical binding of dyes by protoplasmic components Hence the total content of the dye in axoplasm may be expressed with the formula given by Troshin[1] $C_{in} = K \times C_{out} + A$, where K is the solubility coefficient of the dye in protoplasm as compared with the medium, thus $K \times C_{out}$ gives the dissolved portion of the dye, A is adsorbed or chemically bound dye per unit volume

The analysis of cyanole distribution shows[3] that, by varying C_{out}, A is practically equal to 0, whereas for neutral red the bound dye fraction is big enough[2] This is also confirmed by special experiments made to investigate the efflux of dyes from axons When cyanole-stained axon is put in sea-water, the dye leaves the fibre very soon, and the axon becomes completely colourless within 5 min Similar experiments with neutral red show that the colour is retained for a much longer time Thus, after 30 min soaking in sea-water, the neutral red concentration in axoplasm was still 14 ± 4 mg per cent, that is, 40 per cent of the dye still remained in the axon The same slow efflux of cyanole was observed only in killed or damaged axons

The experiments show that in the case of cyanole the dissolved fraction of the dye gave only $0\ 12 \times C_{out}$ while for neutral red[2] $K = 0\ 25$

The values obtained for K, considerably less than unity, probably indicate that not the whole protoplasmic water is available for the substances with the size of molecules more than 3 Å In any event, a steady-state distribution of dyes with the Q value less than unity cannot be explained by the Donnan effect[2,3], analogous Q values were obtained for the distribution of non-electrolytes in muscles[1,4,5]

Thus we can conclude that the difference in the steady-state concentration of the dye in axon as compared with medium (decrease as well as increase) may be explained by the specific properties of axoplasm, and for this explanation no membrane pump hypothesis is needed

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N N NIKOLSKY
S A KROLENKO

Institute of Cytology,
Academy of Sciences of the U S S R ,
Leningrad

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Dispersal Rates of Several Ungulates introduced into New Zealand

BETWEEN 1851 and 1923 several species of ungulates were introduced into New Zealand During this period there were also many liberations into unstocked areas of game animals taken from recently established populations Dates and localities of liberations have been summarized[1,2] and the present or recent distributions of most species have been published[2–5]

Table 1 gives mean rates of dispersal over periods exceeding 50 years, taken from populations that were continuously expanding their ranges over these periods The rate is measured as the distance between the point of liberation and the farthest known breeding colony, divided by the number of years that have elapsed

Table 1 RATES OF DISPERSAL

Species	Locality	Miles a year
Chamois (*Rupicapra rupicapra*)	South Island	5 4
Thar (*Hemitragus jemlaicus*)	South Island	1 1
Red deer (*Cervus elaphus*)	Nelson	1 0
	Fiordland	1 0
Sika deer (*Cervus nippon*)	North Island	1 0
Whitetail deer (*Odocoileus virginianus*)	Stewart Island	0 6
Fallow deer (*Dama dama*)	Wanganui	0 5
Rusa deer (*Rusa timorensis*)	North Island[9]	0 5
Sambar deer (*Rusa unicolor*)	Manawatu	0 4
Wapiti (*Cervus canadensis*)	Fiordland	0 4

There have been more than 100 liberations of red deer[6] and the populations developed from these have coalesced so that the pattern of dispersal is now completely obscured But the distributions had been roughly mapped before this occurred[7], and a rate of dispersal can be calculated for the Nelson herd between 1851 and 1924 This is probably one of the few red deer herds the distribution of which was not strongly modified by secondary liberations near its periphery The rate of spread in Fiordland has been published[6], it was calculated from evidence derived from the vegetation and from hunters' reports.

No research has been undertaken to determine whether dispersal in New Zealand is largely independent of a population's density, or whether the rate fluctuates with changes in the population and its environment. Present work on sika deer, chamois, and thar should eventually answer this question.

GRAEME CAUGHLEY

Forest Research Institute,
New Zealand Forest Service,
Rotorua, New Zealand.

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Significance of Allopreening in the Red Avadavat and its Development in Other Birds

ALLOPREENING, that is, the habit of preening a neighbouring individual's feathers, is characteristic of many gregarious species of birds. Recent work on the Estrildine finch, the red avadavat (*Amandava amandava*), has shown that the amount of mutual grooming does not vary with the presence of peripheral dirt on the regions to which most of the allopreening is restricted, that is, the head. It thus seems likely that allopreening is more than simple co-operation in toilet behaviour between individuals which cannot effectively clean or arrange the feathers on their own heads as suggested by Morris[1].

Preening is a subsidiary resting response, and in species in which the individuals are forced together, nibbling each others' feathers is a common activity, as in guillemots (*Uria aalge*) or young chickens (*Gallus gallus*). Birds seem to possess an innate response to feathers as primates have to fur[2]. Ritualized allopreening invitation postures are found in many highly gregarious 'contact' species and in 'contact' species where the members of a pair are closely associated throughout the year. In *A. amandava* there is specialized behaviour for eliciting grooming by a neighbour in the form of mandibulation nudging, and head proffering.

When two red avadavats which are unfamiliar with each other approach and clump, the clumper is invariably pecked first, but in response to this, an allopreening invitation posture is taken up which results in an amelioration of the aggressive behaviour and the assumption of mutual preening[3]. Between strangers, then, allopreening serves to reduce aggression between the clumping individuals, but when they are fully familiar and their relationship is characterized by an absence of fear and aggression, allopreening serves to strengthen the social bonds between the birds, as suggested for social grooming in primates[2].

The allopreening invitation posture is rarely used as a simple appeasement gesture in the face of an all-out attack in the manner suggested by Morris[1], but is only given if the submissive individual is in strong conflict between approaching and fleeing, and is usually at the same time sexually attracted to the attacker. Otherwise, the immediate response to attack is flight.

J. H. SPARKS

Department of Zoology,
Queen Mary College,
University of London.

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Fertilization of Hamster Eggs *in vitro*

FERTILIZATION of rabbit eggs *in vitro* has been reported by many workers in recent years and Austin[1] concluded that "success seems most likely to have been attained by Smith (1951), Dauzier *et al.* (1954), Dauzier and Thibault (1959), Thibault *et al.* (1954), Thibault and Dauzier (1960), Moricard (1954 *a*, *b*), and, particularly, Chang (1959)". So far as is known, no authentic successful experiments to fertilize rodent eggs *in vitro* have been reported[1-4]. This communication describes a repeatable technique to fertilize the eggs of the golden hamster (*Mesocricetus auratus*) *in vitro*, and the results obtained by this technique.

Female hamsters that came into heat between 6 p.m. and 9 p.m. were killed at 6-7 a.m. the next morning. Since most of the females ovulate about 8 h after the onset of heat[5], these females were considered to have ovulated at about 2-5 a.m. Thus, the eggs were considered to be about 1-5 h after ovulation at the time of recovery. The oviducts of an animal were isolated, placed on a piece of filter paper, and most of the blood adhering to the oviducts was rubbed off. The oviducts were then put on the centre of a watch glass (4 cm in diameter) and were immediately covered with warm (30°-37° C) mineral oil (Squibb). Under a dissecting microscope and on a warm stage, the distended portion of the ampulla was incised with needles and the eggs in cumulus oophorus were pressed out with some of the tubal fluid. The oviducts were discarded and blood and tissue debris, if any remained in the watch glass, were removed with a pipette. A small drop of sperm suspension, about 1-3 times the amount of the egg clot, was then added through the needle (25-27 gauge) of a tuberculin syringe to the eggs that were under the mineral oil. The eggs and the sperm suspension were thoroughly mixed with a needle. The watch glass was then kept in an incubator at 36° C.

The sperm samples were collected either from the uterus of a female mated with proved male about 0.5 h, or 4-5 h, previously, or from the caudal epididymis of a fertile male. Usually, a drop of dense sperm mass was put on the centre of a watch glass (6 cm in diameter) and covered with 2-4 ml. of a solution containing 10 parts of tissue culture medium, '*TC* 199' (Difco Laboratories), 3 parts of M/8 glycine, and 100 units/ml. of penicillin *G*. Under the microscope, the dispersion of sperms could be seen, the motility assessed to choose the best sample, and a suspension of desired concentration of sperms taken through the needle into a tuberculin syringe.

All the glassware and instruments were washed with double distilled water, sterilized, and kept at 30°-37° C before and during use. Aseptic precautions were maintained during the whole operation.

Seven to ten hours after insemination, the eggs in the watch glass were transferred to a slide, compressed gently between a coverslip and the slide, and examined under a phase-contrast microscope for evidence of fertilization. The eggs were considered to be fertilized only when enlarged sperm heads or male pronuclei were found in the vitellus. Particular attention was directed to the tail of fertilizing sperm in or around the vitellus (Fig. 1). Occasionally, one or more sperms with intact (unswollen) heads were found in the perivitelline space or on the surface of the vitellus. Such sperms were recorded as supplementary sperms.

When 171 eggs from 21 females were inseminated with sperms recovered from the uterus of females mated 4-5 h previously, 113 (66 per cent) were fertilized. Of these fertilized eggs, 76 (67 per cent) were monospermic and 37 (33 per cent) were polyspermic (24 dispermic, 7 trispermic, 5 tetraspermic, and 1 pentospermic, as shown by the number of pronuclei and that of sperm tails in or around the vitellus). Seventeen monospermic eggs and 14 polyspermic eggs had 1-5 supplementary sperms.

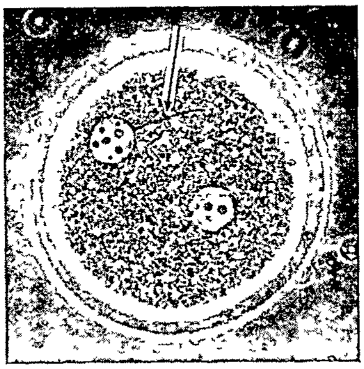

Fig. 1. A hamster egg fertilized *in vitro*. Phase-contrast, arrow indicates the tail of the fertilizing sperm

Supplementary sperms (1–3) were also found in 7 unfertilized eggs.

When 116 eggs from 15 females were inseminated with sperms recovered from the uterus of females mated 0.5 h previously, 36 eggs (31 per cent) were fertilized. Of these 36 eggs, 31 (86 per cent) were monospermic and 5 (14 per cent) were polyspermic (4 dispermic and 1 trispermic). Supplementary sperms (1–3) were observed in 6 monospermic, 4 polyspermic, and 2 unfertilized eggs.

Insemination of 152 eggs from 17 females with epididymal sperms resulted in the fertilization of 26 eggs (17 per cent). Of these 26 fertilized eggs, 20 (77 per cent) were monospermic and 6 (23 per cent) were polyspermic (5 dispermic and 1 tetraspermic). Supplementary sperms (1–5) were found in 5 monospermic, 4 polyspermic, and 3 unfertilized eggs.

These results clearly show that sperms that have been in the female tract for a longer period (4–5 h) can fertilize a higher proportion of eggs (66 per cent) than sperms in the female tract for a short period (31 per cent) and those recovered from the epididymis (17 per cent). This indicates the possibility that capacitation[6–8] of hamster sperm may have occurred in the female tract.

When the hamster eggs are normally fertilized *in vivo*, the zona reaction[9] to block the entry of the second sperm is very strong and polyspermy is rare[10,11]. Under the present condition, on the contrary, the proportion of polyspermic eggs was extremely high (14–33 per cent). Whether this is due to a high concentration of sperm at insemination, fast ageing of eggs *in vitro* as has been shown in the polyspermy of aged eggs *in vivo*[12], or other factors, is still not known at present. Except in a few cases where degenerative changes were observed, most of the eggs fertilized by a single sperm appeared to be quite normal at the pronuclear stage. Whether they can develop further in culture or can develop into young by transplantation into recipient animals is under investigation.

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R. YANAGIMACHI
M. C. CHANG

Worcester Foundation for
Experimental Biology,
Shrewsbury, Massachusetts

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Distribution of Nuclei in a Heterocaryon of *Penicillium expansum*

THE overall frequency of diploid nuclei in a balanced heterocaryon has been estimated by Pontecorvo[1] to be of the order of one in 10^6 or 10^7. Thus, a colony containing 1×10^9 conidia would include 100–1,000 diploid conidia.

In experiments with *Penicillium expansum*, I established a heterocaryon between M54, a brown strain requiring riboflavin and pyridoxine (br_3 ri_1 pyr_1), and M67, a white acriflavine-resistant strain requiring biotin and nicotinic acid (w_3 acr_1 bi_1 nic_2)[2]. In attempts to recover diploids from this heterocaryon, 317 million conidia were plated out without success. In contrast, in a similar experiment between strains M53 (ri_1 bi_4 w_2) and M54 (ri_1 pyr_1 br_3), 179 diploids were recovered from a plating involving only 19 million conidia.

This can be explained if only one or few nuclei are responsible for initiating the entire conidiophore in *Penicillium*. In such a situation, all or most of the conidiophores would be homocaryotic, and in a population involving 1×10^9 conidia the expected 100–1,000 diploids would be in a single head. The experimental result would be the recovery of either a large number of diploid nuclei or none at all. To verify this in *P. expansum*, the conidiophores of a heterocaryon M54+M67 were processed and classified as either homocaryotic or heterocaryotic.

The conidiophores are quite small and closely packed, and it was not possible to classify heads visually based on colour of the conidia. Whole conidiophores were, therefore, picked off with a needle of drawn glass and the spores streaked on complete medium. Following sporulation, the genetic nature of the conidiophores was assessed on the basis of colony colour. Analysis of 463 heads in this manner gave the following results: brown, 173; white, 227, variegated, 63. Thus, 86 per cent of heads in the experimental heterocaryon were homocaryotic brown or white and only 14 per cent were heterocaryotic, giving rise to variegated colonies.

A nuclear ratio in the heterocaryon significantly removed from 1 : 1 could influence considerably the number of homocaryotic heads of one or other type. The nuclear ratio in the heterocaryon was therefore estimated by making a suspension of a 100 million conidia and, by dilution series, arriving at a concentration of approximately 100 conidia per ml. This suspension was plated on complete medium at the rate of 1 ml. per plate and the resulting colonies classified according to colour. Analysis of colonies gave 385 white and 362 brown. In the particular heterocaryon being investigated, the nuclear ratio as based on conidium analysis closely approximated 1 : 1.

In the heterocaryon studied only one nucleus, or a few like nuclei, segregated into the large majority of the young conidiophores. Such nuclei were then removed from contention so far as diploidization was concerned and a population of 1×10^9 conidia loses its numerical significance. In effect, we are sampling a million significant nuclei segregating into the conidiophores and thence being multiplied by a factor of approximately a thousand times by the conidiophore apparatus. If the frequency of diploid nuclei in the somatic hyphæ is one in 10^6, and we presume for argument that all conidiophores are homocaryotic, then we would expect on the average only one diploid conidiophore in a stand of one million conidiophores, that is, 1×10^9 conidia. If we consider further that the frequency of diploid nuclei in the vegetative hyphæ is one in 10^7, and the likely possibility that the distribution of diploid nuclei in somatic hyphæ is non-random, then the problem is compounded. To some extent, the situation is compensated for by the fact that not all conidiophores are homocaryotic, but it can be appreciated, nevertheless, that a population of 1×10^9 conidia may contain no diploids at all

In connexion with the study of parasexual phenomena in fungi imperfecti, then, it would be an advantage to know if the conidium bearing fructification is derived from one nucleus or from many nuclei In the latter situation diploidization may still occur in the reproductive parts, resulting in a better distribution of diploids

In an examination of *Colletotrichum lagenarium*, Dutta and Garber[3] failed to recover a diploid from platings involving 100 million conidia from several heterocaryons In *Colletotrichum* each acervulus may produce 10,000–100,000 conidia If an acervulus has its origin in one or few nuclei, many acervuli will be homocaryotic and a sample of 100 million too small perhaps to locate a diploid Even in a heterocaryotic acervulus, a mosaic pattern of development might permit considerable multiplication of nuclei without the possibility of diploidization

As a corollary it might be stated that abundant sporulation is not in itself an advantage in the study of genetics of imperfect fungi, and even when a fungus has a relatively low sporulation capacity (1/1,000 that of *Penicillium*), it may still be studied adequately with respect to parasexual phenomena

GEORGE L BARRON

Department of Botany,
Ontario Agricultural College,
Guelph, Ontario

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Rapid Changes in Transpiration in Plants

IN the recent discussion in *Nature* under this heading[1–3], Rufelt[1,3] has adopted Ivanoff's[4] hypothesis that the transient increase in transpiration rate observed when a leaf is detached in air is caused "by a sudden release of the water stress in the conducting elements, which means that it is caused by an increase in the water supply to the leaf"[1] He has further supposed that the increase in transpiration obtained after bathing the roots of intact plants with mannitol solution[5], or 1 M sodium chloride[1], is also due to the release of water stress in the plant, making the subsidiary hypothesis that the mannitol or sodium chloride causes an increase in the water permeability of the roots[1]. He concludes that this last effect is peculiar to the roots[1], and since he writes of "the permeability barrier in the root"[3] he must postulate that the transpiration stream here passes through the cells, though in the whole of the rest of the plant he supposes it to move in the cell walls only, except when it is in the xylem He would appear to have obtained no data for the behaviour of the stomata in his experiments; his discussion of the supposed absence of 'mechanical effects' and the different permeabilities of guard cells and epidermal cells therefore seems too great an extrapolation from his results—transpiration rate can only be taken as a measure of stomatal diffusive conductance when there is a constant potential difference across the stomata

Heath[2] supposed that the observed increase in transpiration rate, in the experiment with sodium chloride, was due to 'passive' stomatal opening (Rufelt's 'mechanical effect') brought about by rapid wilting He suggested that the 'Ivanoff effect' must be mainly due to the same cause and that the contribution of the release of tension in the xylem sap must be very small

We have investigated the effects, on stomatal movement, transpiration rate and leaf water content of wheat plants, of bathing the roots with 1 M sodium chloride (Table 1). Potted plants of wheat var Thatcher growing in John Innes No 1 compost were used Stomatal movement was followed with a 'Wheatstone bridge porometer'[6], and Log_{10} leaf resistance to viscous air flow (in 'Gregory and Pearse units'[6]) is tabulated, transpiration rates were followed with the 'differential transpiration porometer'[7] for the same place on the leaf and the data are tabulated as mm galvanometer deflexion Leaf water contents were estimated from six leaf blades, similar to that used for the porometers, for each pot on each of three occasions—the first when stomatal apertures and transpiration were steady, the second when both had increased as a result of sodium chloride treatment and the third when they had decreased again Stomatal opening began within 20–60 sec of flooding the soil with 1 M sodium chloride and a rise of transpiration showed in the next reading of the transpiration porometer (2–4 min) The data are strongly suggestive of stomatal control of transpiration and show a fall in leaf turgor within a few minutes of applying the sodium chloride

Table 1 EFFECTS OF TREATMENT OF ROOT SYSTEMS OF WHEAT PLANTS WITH 1 M SODIUM CHLORIDE

Pot No		Time* (min from supply of NaCl to roots)	Stomata $\text{Log}_{10} R$	Transpiration deflexion (mm)	Change of transpiration Increases and decreases in deflexion (mm)	Leaf water content (% of dry wt (6 leaf blades))	Relative turgor
			Before NaCl treatment				
1	Old leaf	−2	$\bar{1}$ 36	25	—	389	100
2	Old leaf	−2	$\bar{2}$ 13	52	—	877	100
3	Young leaf	−2	$\bar{3}$ 52	60	—	879	100
4	Young leaf	−2	$\bar{4}$ 82	90	—	615	100
5	Young leaf	−2	$\bar{4}$ 62	100	—	570	100
			After NaCl treatment				
1	Old leaf	+4	$\bar{2}$ 96	40	+15	381	98 0
2	Old leaf	+4	$\bar{3}$ 98	65	+13	823	94 0
3	Young leaf	+5	$\bar{3}$ 01	80	+20	815	93 0
4	Young leaf	+15	$\bar{4}$ 70	110	+20	580	94 5
5	Young leaf	+8	$\bar{4}$ 55	115	+15	540	95 0
1	Old leaf	+26	$\bar{1}$ 36	30	+5	294	76 0
2	Old leaf	+14	$\bar{2}$ 26	40	−12	796	91 0
3	Young leaf	+30	$\bar{3}$ 55	50	−10	807	92 0
4	Young leaf	+30	$\bar{4}$ 82	80	−10	525	85 0
5	Young leaf	+30	$\bar{4}$ 64	100	0	510	89 5

* These are the actual times for the $\text{Log}_{10} R$ values, the corresponding values for transpiration and for leaf water content were obtained slightly earlier

On Rufelt's hypothesis of "an increase in the water supply to the leaf", the leaf water content must increase even if the water travels entirely in the cell walls, not only will the cell walls themselves hold more water but also as they will hold it less strongly (increased water potential) more of it will pass into the vacuoles It is not very clear whether Rufelt considers that the increase in transpiration is entirely due to this increase of water potential, in which case leaf turgor must remain higher so long as the higher rate of transpiration persists, or whether he supposes that stomatal opening caused by increased guard cell turgor also contributes In the latter case, leaf turgor could fall again before the transpiration rate The hypothesis put forward by Heath demands in this type of experiment a continuously falling leaf water content and would be disproved by an observed increase Both our data here and those in Rufelt's Table 1 (ref 1) are consistent with Heath's hypothesis We suggest that Dr Rufelt should attempt to detect an increase

We have also carried out a series of experiments with detached leaves of *Xanthium pennsylvanicum*, kept first in water and then transferred to mannitol solutions Stomatal behaviour and transpiration rates were followed as in the wheat experiments (Table 2)

Stomatal aperture and transpiration rate increased together, within a few minutes of applying the mannitol solutions and more rapidly with the higher concentrations Here there can have been no release of tension, as in the 'Ivanoff effect', nor were there any roots the permeability of which might have been affected An effect on the water permeability of the leaf cells bordering the xylem could be postulated but this would not accord with the suggestion that the water travels in the cell walls We prefer the view that such cells lost turgor owing to the osmotic pressure of the mannitol solution in the xylem, that this

Table 2 EFFECTS OF SUBSTITUTING MANNITOL SOLUTIONS FOR THE WATER BATHING THE PETIOLES OF DETACHED LEAVES OF *Xanthium pennsylvanicum*

Leaf No	Time (min from mannitol application)	Mannitol concentration (M)	Stomata (Log$_{10}$ R)	Transpiration deflexion (mm)
		Before mannitol treatment		
1	−1	—	$\bar{2}$ 46	160
2	−1	—	$\bar{3}$ 61	70
3	−1	—	$\bar{3}$ 21	140
4	−1	—	$\bar{3}$ 50	180
5	−1	—	$\bar{4}$ 86	180
6	−1	—	$\bar{4}$ 91	170
7	−1	—	$\bar{4}$ 98	110
8	−1	—	$\bar{4}$ 84	120
		After mannitol treatment		
1	+2½	0 4	$\bar{2}$ 26	180
2	+2½	0 4	$\bar{3}$ 21	80
3	+3	0 2	$\bar{4}$ 98	175
4	+3	0 2	$\bar{4}$ 72	240
5	+3	0 1	$\bar{4}$ 77	150
6	+3	0 1	$\bar{4}$ 46	200
7	+5	0 05	$\bar{4}$ 84	125
8	+5	0 05	$\bar{4}$ 68	130

resulted in a change in the suction pressure gradient to the epidermal cells and the loss of turgor of the latter caused 'passive' opening of the stomata, resulting in increased transpiration

In a third type of experiment we have observed the stomatal changes that follow the cutting of the stem of a *Xanthium* plant in air ('Ivanoff effect') Within 2–5 sec of excision a minute movement of the meniscus in the Wheatstone bridge porometer (observed with a lens or a reading microscope) indicates a very small closing movement of the stomata This we interpret as the 'passive' closure due to the release of tension in the xylem sap There is then a delay of a further 15 sec, after which the usual opening movement begins We suggest that this delay is the time necessary for the water in the xylem to be used up in transpiration and the leaf water content to begin to fall Quantitative estimates of the amounts of water involved are of course highly desirable and we hope to attempt these in the future

This work was carried out while one of us (H M) held a University of Reading visiting fellowship

H MEIDNER*
O V S HEATH

University of Reading
Horticultural Research Laboratories,
Shinfield Grange,
Shinfield, Berks

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* Present address Department of Botany, University of Natal, Pietermaritzburg

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FIRST, I would like to correct a vagueness in my contributions to this discussion I certainly think that the changes in transpiration are caused by stomatal movements I think this is very evident from an earlier paper of mine[1] Certainly I have myself no experimental proof in my papers hitherto published but a flood of evidence can be found in the literature[2–6] and theoretical considerations point in the same direction[7] Secondly, I do not agree with the authors when they assume that I postulate that the transpiration stream passes through the cells in the root because I postulate a permeability barrier In earlier experiments[1] the root permeability and cell permeability were found to be probably not identical Where the permeability barrier is localized is still a matter for discussion[8]

Thirdly, the three series of experiments published do not disprove either the hypothesis of Ivanoff or my interpretations The main point is that the stomata are thought to be influenced by the water potential of the cell wall and not by the water potential of the whole leaf In short-term experiments these may differ Consequently, changes in water content of the total leaf such as those obtained by Heath and Meidner in the first series of experiments are not illustrative of the changes determining the stomatal movements The increase which Heath and Meidner suggest that I should try to detect cannot be shown in determinations of water contents of leaves One possibility would be to demonstrate it as an increase in water transport upwards in a plant and as such it has in fact been shown by Allerup[9–11] It might be added that the reason why I have assumed that the easily movable water is localized in the cell walls is that this has been suggested by Strugger and others[12,13]

The results of the second series of experiments are very similar to those obtained by Haines[14] and Humphries[15] These authors interpreted the increases in transpiration as caused by changes in water permeability However, one circumstance makes the present experiments difficult to interpret An osmotic suction from the mannitol solution requires a semipermeable structure If there is one, its permeability can be changed

The third series of experiments is a recapitulation of those of Allerup[10] which he has interpreted according to Ivanoff's hypothesis

It is evident that most of the experiments can be interpreted according to both the hypotheses Nevertheless, I still cannot find that the experiments described in my communication[16] by Figs 1, 2 and 3 have been explained by Heath's hypothesis However, I think we all make extrapolations from our results and would like to postpone further discussions as to who is right and who is wrong until some more pertinent experiments have been performed

HENRY RUFELT

Institute of Physiological Botany,
University of Uppsala.

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Effect of Chloramphenicol on the Uptake of Salts and Water by Intact Castor Oil Plants

THE antibiotic chloramphenicol has been found to inhibit accumulation of salt by slices of red beet tissue and carrot tissue without significantly affecting respiration[1]. This supports the hypothesis that the mechanism of salt uptake is closely related to protein synthesis put forward by Steward and Millar[2], as chloramphenicol is a specific inhibitor of protein synthesis

Experiments have been carried out to determine the effect of chloramphenicol on the salt uptake of intact plants Castor oil plants (*Ricinus communis*) were grown singly in aerated culture solution in 500 ml polythene growth vessels The culture solution was similar to that described by Stout and Arnon[3] except that it was diluted ten times and iron was present as chelate The concentrations of potassium, calcium and nitrate were 0 79 mM, 0 50 mM and 0 38 mM respectively. The solution was renewed daily during the growth of the plants In each experiment the uptake of the foregoing three ions and water by a single six-week old plant was followed first from complete culture solution, then from culture solution to which chloramphenicol (2 g/l) had been added and finally again from culture solution alone Each experiment was of 12-h duration and the medium was drained from the vessel for analysis and replaced with a fresh volume (250 ml) after a given period, usually 1 h. Uptake of water was determined from the decrease in volume and uptake of potassium, calcium and nitrate from the decrease in the amounts of these ions in the medium over the given period The cations were determined with an EEL flame photometer and nitrate colorimetrically by the phenoldisulphonic acid method The temperature was kept constant at 25° C and a light intensity of about 750 ft - candles was given throughout each experiment

Table 1 EFFECT OF CHLORAMPHENICOL ON THE UPTAKE OF POTASSIUM, CALCIUM, NITRATE (μg equiv /h) AND WATER (g/h) BY TWO INTACT *Ricinus communis* PLANTS AT 25° C

Time (h)	Medium	Plant 1 K	Plant 1 Ca	Plant 1 NO_3	Plant 1 Water	Plant 2 K	Plant 2 Ca	Plant 2 NO_3	Plant 2 Water
1	Culture	45	30	68	10	65	4	139	6
2	Solution	59	42	117	10	85	18	139	6
3		54	42	117	10	80	28	146	6
4	Culture	22	18	79	9	21	10	48	6
5	Solution	−8	20	78	10	−3	2	−13	6
6	+ Chloramphenicol 2 g/l	−6	26	32	10	−5	4	40	8
7		1	44	46	10	2	10	93	6
8	Culture	16	40	4	10	35	12	101	5
9	Solution	24	38	99	9	45	20	104	5
11		42	34	56	7	—	—	—	—
12		25	48	67	8	46	22	82	5

The results from two plants are shown in Table 1 Chloramphenicol had a marked effect on the uptake of salt but the uptake of water was unaffected In both experiments uptake of potassium was stopped completely with chloramphenicol around the roots for 3 h. Uptake of calcium and nitrate was partially reduced for plant 1 and stopped completely for plant 2 These results, therefore, support those obtained with disks and indicate that uptake of salt in the intact root is closely related to protein metabolism There was a recovery in uptake of salt on the removal of chloramphenicol from the medium in all cases although the initial levels were not always regained, probably because of the evening fall off in uptake through diurnal rhythm These experiments were started at 9 a m and finished at 9 p m , and uptake of salt is at its maximum at mid-day and minimum at mid-night in these plants In work to be published shortly[4], it has been shown that most of, and sometimes all, the potassium taken up by these plants is transferred to the vessels and so it appears that the mechanism of salt transport across the root is, like the accumulation of salt by the individual cell, affected by chloramphenicol The complete stoppage of uptake of salt by chloramphenicol leaving the water flux unaffected strongly suggests that passive mass flow of these ions in solution direct from medium to xylem is not occurring in these plants

I thank Prof P E Weatherley for his advice

D J. F BOWLING

Department of Botany,
University of Aberdeen

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VETERINARY SCIENCE

Prophylactic Activity of Suramin Complexes in 'Surra' (*Trypanosoma evansi*)

SURAMIN, being acidic in reaction, forms insoluble precipitates—called suramin complexes—with other trypanocides which are basic compounds like antrycide dimethyl sulphate, 'Berenil' (di-(amidinophenyl)-triazene(-*N*-1 3)diaceturate), pentamidine, etc Such combinations have been reported to have markedly reduced toxicity and considerably enhanced prophylactic activity Williamson and Desowitz[1] reported maximum decrease of five- and three-fold in the toxicity of suramin complexes of antrycide and 'Berenil' respectively They found that suramin–antrycide complex protected cattle against *Trypanosoma vivax* and *T congolense* for at least 95-199 days at dose rates of 10–40 mg/kg of the antrycide content Williamson[2] reported that rats were protected against *T congolense* for 53 and 11 weeks with suramin complex of antrycide (201 mg/kg) and 'Berenil' (129 mg/kg body-weight) respectively as compared with 1½ weeks, 18 weeks and <4 weeks protection afforded by suramin (62 5 mg/kg body-weight), antrycide dimethyl sulphate (7 8 mg/kg body-weight) and 'Berenil' (50 mg /kg body-weight) respectively Since then complexes of suramin have been widely experimented with in animal trypanosomiases in Africa Suramin complexes of antrycide dimethyl sulphate and 'Berenil' were prepared after Williamson[2] and tried for their prophylactic activity against *T evansi*

Rats and mice were inoculated subcutaneously with antrycide–suramin complex, prepared fresh at 50 mg/kg body-weight of the complex They were challenged in pairs with an intraperitoneal inoculum of about 2,000 trypanosomes (*T evansi*) freshly collected from a rat showing intense parasitæmia, blood of which was diluted in citrate–saline Rats received the first challenge 70 days after treatment, afterwards at fortnightly intervals up to 254th day, then at 20-day intervals until the protection broke down Mice were challenged 45 days after treatment at weekly intervals up to the 170th day and then at 20-day intervals Suramin–'Berenil' complex at 50 mg/kg body-weight was injected in mice only They were challenged in pairs on the 57th day, 77th day and on the 81st day after the protective inoculation

Along with each batch of challenge, an untreated rat and one untreated mouse were infected with the same dose of trypanosomes as used for infecting the protected animals These animals served as untreated infected controls The survivors were observed until the end of the experiment, and the batches immediately preceding those in which trypanosomes broke through were examined for at least 30 days after being infected Tail blood of the challenged and control animals was examined fresh for trypanosomes daily for the first 15 days and afterwards at 3–4 day intervals Their tails were pricked with separate pins to prevent accidental re-infections Trypanosomes appeared in the tail blood of control rats and mice within 4 days of the infection and all of them died during the next 4 days

The first break-through in the protection given by suramin–antrycide occurred in one of the two mice challenged 190 days after treatment, and in one of the two rats challenged on the 294th day of treatment The immediately preceding batch of mice and rats challenged on 170th day and 274th day respectively stayed negative At the time of break-through in mice, there were 10 treated mice in stock which were simultaneously challenged Five of them developed parasitæmia within 10 days whereas the rest of the 5 mice remained free of the parasites for 45 days after the challenge, when they were discontinued

Protection with suramin–'Berenil' complex first broke in the pair of mice challenged on the 81st day of the protective inoculation The stock of 22 treated mice were infected, 18 developed parasitæmia whereas 4 mice were yet negative for the parasites on the 48th day when the study was concluded

Suramin–antrycide complex thus conferred solid protection against *T evansi* for 274 days in rats and 170 days in mice Therefore, the protection obtained with the suramin–antrycide complex is considerably longer than that reported for either of the constituents, namely, 63 days with antrycide prosalt under challenge of 5,000 trypanosomes[3], for 82 days with antrycide methosulphate and for > 60 days with suramin under challenge with 10,000 trypanosomes[4]. Suramin–'Berenil' complex completely protected mice up to 57 days, and only one of the two mice challenged on the 77th day after treatment

The encouraging result obtained with suramin–antrycide complex in the investigation recorded here suggests further investigations with it under greater challenges, as also trial with it in large animals

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B S Gill
M N. Malhotra

Indian Veterinary Research Institute,
Izatnagar, U P ,
India.

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Mechanism of Early Sporidesmin Intoxication in Sheep

In a recent publication[1] on the effect of sporidesmin intoxication in sheep, attention was directed to the fact, based on histopathological evidence, that enterohepatic disturbances precede biliary obstruction. Furthermore, the total liver lipids were found to be increased in the intoxicated group of animals

This report outlines a more detailed investigation (to be published elsewhere) into the nature of these liver lipids In addition, the mechanism of the characteristic lipid changes induced by sporidesmin intoxication is considered in the light of the available information

From a group of eight weaned lambs four animals received an oral dose of sporidesmin (1 mg/kg body-weight) All animals were killed two days after dosing The livers were excised immediately, sliced into strips and frozen in liquid nitrogen They were then stored at −20° C until required for analyses The total lipids were extracted by means of a modification of the Bligh and Dyer procedure[2] Eighteen lipid components were isolated from each liver lipid extract by means of a combination of DEAE, silicic acid-silicate, and silicic acid column chromatography Identification was based on reverse-phase paper chromatography, infra-red spectrometry and chemical analyses During the course of this investigation it has become evident that the liver triglyceride-levels of the intoxicated group of animals were increased about 100 per cent above the triglyceride-levels of the normal group No significant changes were found in the other liver lipid components

There is adequate evidence that the liver is the main source of circulating plasma triglycerides[3–8] Although fatty infiltration of the liver can be induced by a wide variety of toxic substances and metabolites, the lipids accumulating in the liver are predominantly triglycerides[9–13] Oral administration of hepatotoxins, such as carbon tetrachloride, also results in a rapid increase of liver triglycerides This has been interpreted as the result of a malfunction of a hepatic triglyceride secretory mechanism[14] Many hepatotoxins appear to inhibit or destroy one or more steps in the conversion and secretion of liver triglycerides to plasma lipoproteins The initial conversion of plasma non-esterified fatty acids to liver triglycerides appears to be unaffected[14,15] Whereas the liver triglyceride-levels rapidly increase as the result of the action of hepatotoxins, the plasma triglyceride-levels, in general, become markedly decreased[14] In addition, because of their slower rates of metabolic turnover, the plasma phospholipid and cholesterol-levels remain unaffected in the initial stages

It appears then that, on the basis of the present investigation and of previous work[1,16], the effect of early sporidesmin intoxication (that is, two days after dosing) on the liver resembles the effect of other hepatotoxins

Thus in the clinical picture, described by Mortimer and Taylor[17], the early first-phase symptoms of the diphasic response strongly suggest that a metabolic disturbance in the liver triglyceride secretory mechanism is one of the contributing factors The phospholipid- and cholesterol-levels remain relatively unaffected in both liver and plasma, whereas the liver triglyceride-levels increase. However, if the early pathological situation induced by sporidesmin intoxication inhibited exit of triglycerides from the liver into the plasma, as well as their exit from the plasma into the adipose tissue, a hypotriglyceridæmia might not be observed even though the hepatic secretory mechanism is inhibited or destroyed Further work is in progress to determine the mechanisms which operate during sporidesmin intoxication

J Andrew Peters

Department of Scientific and
Industrial Research,
Fats Research Laboratory,
Wellington,
New Zealand.

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Correlation between the Reaction of Horses to a Pyrogen and their Immunological Responses in Antitetanic Serum Production

In a series of observations on about 5,000 rabbits, it was found that they could be divided into 3 groups according to their low-, medium- or high-temperature reactions to intravenous injection of a standard dose of sodium nucleinate (Merck and Co Inc , U S A., sodium salts of the acids from yeasts)[1,2] In later experiments there was found to be a good correlation between the thermal reactions of individual rabbits to the pyrogen and the intensity of anaphylactic reactions, the measurement of the rise in temperature being determined during the anaphylactic reactions[3]

Similar relationships were demonstrated between the thermal reactions to the pyrogen of individual rabbits and the degree of production of lytic antibodies (anti-sheep hæmolysins)[4,5], the mean hæmolytic titres being 1 1534. 1 2957 and 1 4317 respectively for rabbits with low, medium and high temperature reactions

It is recognized that there is considerable variation in both man and other animals in respect of the readiness with which individual members of a group can be immunized[6] To our knowledge, however, there have been no reports of investigations of a possible relationship between the temperature reactions of animals to pyrogens and the readiness with which they can be immunized

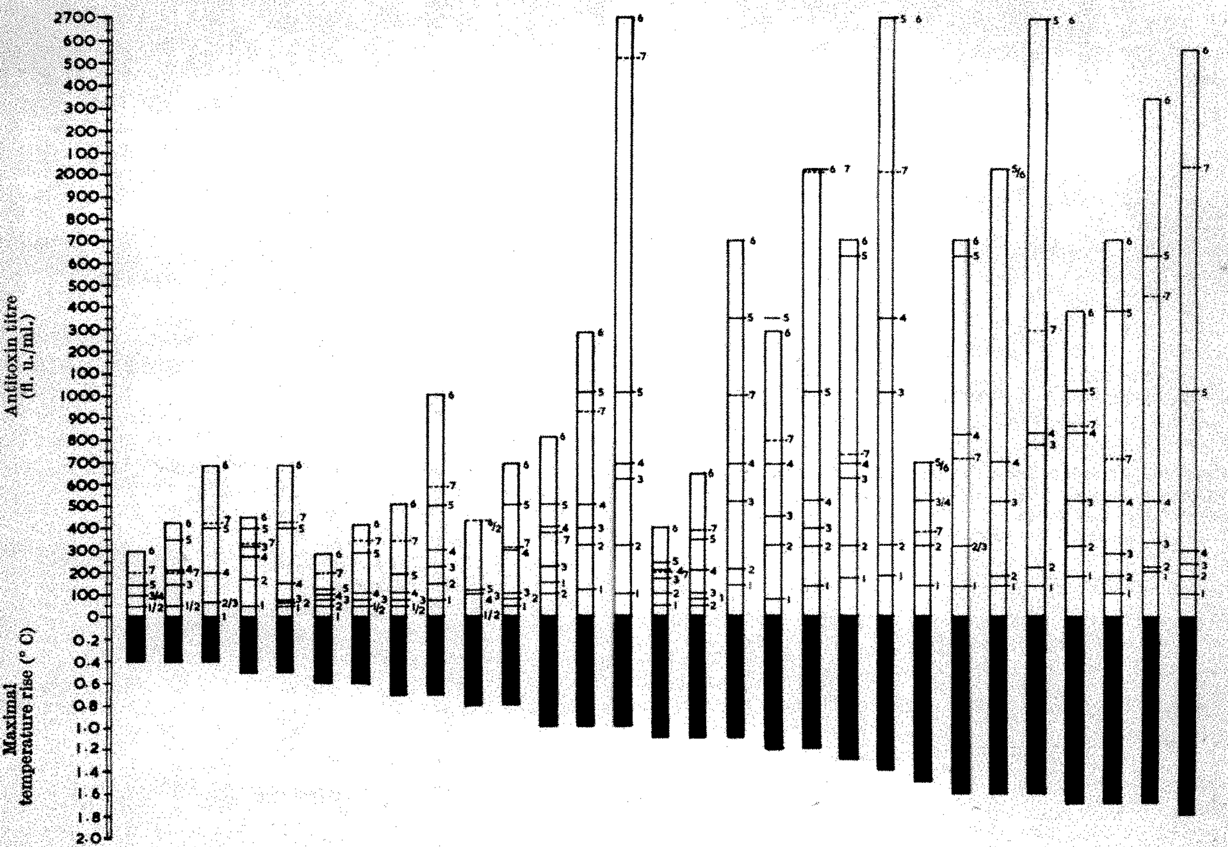

Fig. 1. Individual responses of 29 horses to pyrogen (black columns) and to antitetanic immunization (white columns). ——, 1, 2, 3, 4, 5 = titres before fourth, fifth, sixth, seventh and eighth injections; 6, that is, column height = final titre (bulk bleeding); - - -, 7, titre on second bleeding after bulk bleeding

The results presented here were obtained from investigation of the relative individual production of tetanus antitoxin by 29 horses, by examination of their sera in the course of their first series of immunizing injections, these horses having been tested for their thermal reaction to a standard dose of the pyrogen (10 mg sodium nucleinate/kg body-weight) injected intravenously as a 10 per cent solution in pyrogen-free distilled water.

The procedure adopted in the test for reaction of the horses to the pyrogen was to take the temperature of the animals on three occasions during the hour prior to the injection of the sodium nucleinate, and the figure recorded for the normal temperature was the mean of the three readings. Following the injection of the pyrogen, the temperatures of the animals were recorded every hour for 5 h and the maximal rise in temperature was taken as the figure indicating the response.

Afterwards, all 29 horses were given the series of injections for antitetanic immunization by the usual procedure adopted at the Pasteur Institute, Bucharest, which consists of a first injection of 15 ml. of anatoxin adsorbed on aluminium hydroxide followed by a series of 7 injections at 5-day intervals, starting 30 days later, with increasing doses of anatoxin and toxin adsorbed on aluminium hydroxide.

Serum samples were collected immediately before the fourth and later injections and 10 days after the last injection. The flocculation method (Ramon), with ammonium sulphate precipitated anatoxin as antigen, was used for the titration of the sera and their titre was recorded as flocculent units per ml. of serum (fl.u./ml.).

As will be seen from Fig. 1, the results show, apart from one or two exceptions in horses with medium temperature reactions, a good correlation between the thermal reaction of the individual horses to the pyrogen and the antibody-levels of their sera, and this suggests that the assessment of the reaction of horses to the pyrogen would be of value in the selection of the most suitable donors for the production of antitetanic serum.

The conclusion is supported by the statistical analysis, which was designed to establish the degree of correlation

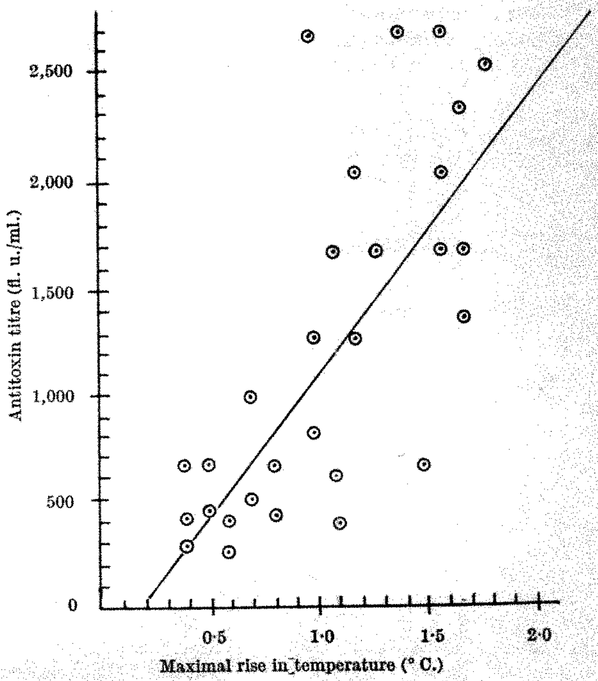

Fig. 2. Regression line indicating the correlation between the response to the pyrogen and the antitoxin titre

and best functional relationship expressing the interdependence of both responses.

The 29 response pairs were plotted on linear, semi-log and logarithmic scales and the linear was found to give the most adequate fit.

The correlation coefficient of 0·72 was highly significant (P 0·01) with a 0·05 confidence interval of 0·48–0·86. The equation for the regression line (Fig. 2) of Y (the antitoxin titre in fl.u./ml.) on X (the response to pyrogen in degrees C) is:

$$Y = 1{,}340\ X - 252$$

This equation can be used to determine the probable antitoxin titre on the basis of pyrogen response.

Further work is planned in the hope that, if similar correlations can be confirmed, it will be possible to use the thermal response, to the same or other pyrogens, as a guide for the selection of animals for the production of other therapeutic sera or for the selection of homogeneous groups of animals for, as an example, potency tests of vaccines.

L. Chiosa
N. Muntiu
C. Constantinescu
Gh. Prelipceanu
I. Boncioat

Pasteur Institute for
Veterinary Research and Bioproducts
and
Institute for State Control of
Drugs and Pharmaceutical Research,
Bucharest, Rumania.

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ENTOMOLOGY

Some Differences in the Passage of Food through the Intestines of the Desert and Migratory Locusts

By means of an X-ray technique[1] a study of the passage of radiopaque foods through the intestine of the migratory locust, *Locusta migratoria migratoriodes* R. and F., and the desert locust, *Schistocerca gregaria* (Forsk), has been made as a preliminary to work on the absorption of stomach poisons. Two observations of immediate interest are described here.

Albrecht[2] has described the *S* bend in the colon of *Locusta* as variable. In the work recorded here it was found that this region of the intestine first separates fæcal pellets from the midgut contents by a constriction, and then by bending breaks the peritrophic membrane between them (the various stages are shown diagrammatically in Fig. 1). At defæcation the *S* bend straightens out by the elongation of the abdomen, and the contents of the posterior region of the midgut move backwards as far as the anus (Fig. 1*b*). The centre of the colon then constricts to isolate the fæcal pellet (Fig. 1*c*), and almost simultaneously the *S* bend reforms (Fig. 1*d* and 1*e*). The process is complete in about 15 sec at 30° C. The *S* bend is present in both fed and unfed *Locusta*. The colon in *Schistocerca* also forms an *S* bend in a well-fed insect, but in a hungry insect the *S* bend is reduced, the colon remaining almost straight.

Locusta starved for 3–4 days retain an *S* bend without there being any food in the intestine, but starved *Schisto-*

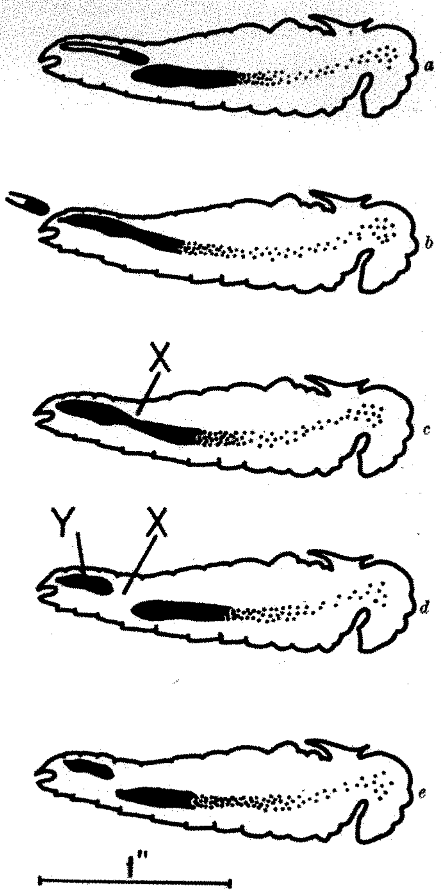

Fig. 1. Function of the *S* bend in the colon of *Locusta*. Tracings of radiographs of the gut contents of a locust that has received a barium meal are shown. The density of the contents is proportional to the amount of radiopaque substance present. The stages represented are: (*a*) appearance of the gut contents just before defæcation; (*b*) appearance at defæcation; (*c*) lumen of the central region of the colon is just beginning to contract (*X*); (*d*) lumen in the central region of the colon is occluded and a fæcal pellet has been isolated (*Y*); (*e*) *S* bend beginning to reform. (Compare the relative positions of the anterior edge of the fæcal pellet with the posterior edge of the food in the midgut in *d*, *e* and *a*)

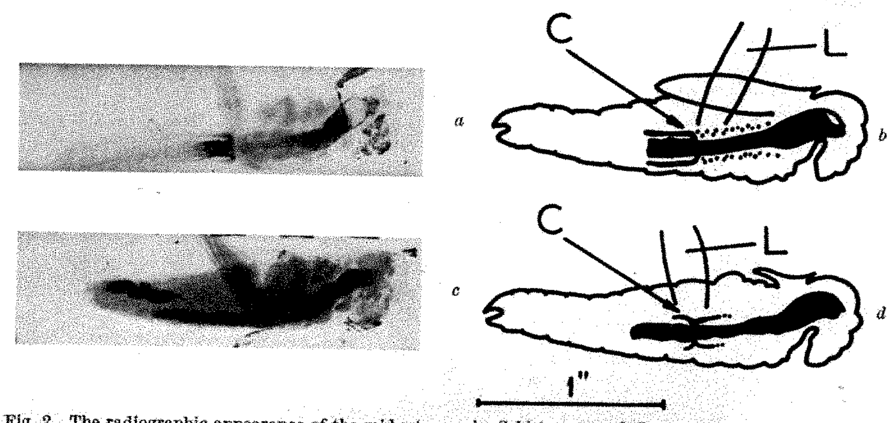

Fig. 2. The radiographic appearance of the midgut cæca in *Schistocerca* and *Locusta* that have received a barium meal: *a* and *b*, the radiographic appearance of the midgut cæca of *Schistocerca* (*C* in diagram); *c* and *d*, the radiographic appearance of the midgut cæca of *Locusta* (*C* in diagram). The stippled regions represent cæcal tissue that does not contain any visible radiopaque substance. *L* is the outline of the jumping leg

cerca do not retain the *S* bend, although, as Monchadsky and Skoblo[3] noted, some food residues do remain in the intestine. The disappearance of the *S* bend in *Schistocerca* may account for the normal retention of residual food through inability to produce discrete fæcal pellets If the locust is poisoned by means of a benzene hexachloride sandwich[4] the entire contents of the intestine may be ejected, at first as discrete pellets and finally as a continuous rod, which suggests that the quantity of food in the intestine normally controls the formation of the *S* bend

Opening into the anterior end of the midgut are six cæca, each cæcum consists of an anterior and a posterior arm In *Locusta* intestinal contents pass into both arms (Figs. 2*c* and *d*) and sections cut through the cæca show a peritrophic membrane extending into them In *Schistocerca* it is found that only the posterior arms of the cæca contain the intestinal contents (Figs 2*a* and *b*), the anterior arms remaining unfilled, a peritrophic membrane is found in the posterior arm The significance of this difference is at present unknown

DEREK GOODHUE*

Department of Zoology,
Imperial College,
London, S W 7

* Present address Trinity College, Dublin

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Feeding Adult Mosquitoes on Solid Sugars

THAT mosquitoes may feed on flowers and sources of food other than blood has been observed many times Notable among these observations are those of Wesenberg-Lund[1], Haeger[2], West and Jenkins[3], and Sandholm and Price[4] Mosquitoes which visit flowers in greatest numbers are various species of *Aedes* According to most of the observations recorded, the food consumed is liquid or semi-liquid The only reference to indicate that mosquitoes may be able to feed on dry food was found in a paper by Downs and Arizmendi[5] on colonization of Mexican anophelines These two authors substituted powdered sugar for honey solutions which they normally supplied to the colonies and noted that females more readily took blood after having fed on powdered sugar They did not further elaborate on this part of their work, and there was no mention of how the powdered sugar was presented to the mosquitoes

Interest in the ability of mosquitoes to feed on solid sugars was raised by a recent chance observation made by me during the course of other experiments A female *Culex tarsalis* Coquillett was observed to probe the nearly dry remains of an insect smashed on the windshield of an automobile This female probed so persistently for about 0 5 h that an experiment was conceived to test the ability of mosquitoes to feed on dry or nearly dry material

In an initial test, glass slides were coated with syrupy sucrose solutions to which had been added ordinary food colours *C tarsalis* readily ingested this sticky material and the colour of the syrup soon became apparent in their bodies The slides were then air-dried for several days at room temperature, until the sugar completely crystallized This is a variation of the technique used by Bartlett[6] to examine dry sugar ingestion by parasitic Hymenoptera Mosquitoes were placed in glass vials, the open ends of which were covered with the prepared slides placed sugar-coated side down, or towards the mosquitoes Observations on feeding were made by placing the assembly under a dissecting microscope so that the manipulations of the mouth parts could be seen through the glass Cover-slips were substituted for the slides when observations were made at greater magnifications with a compound microscope

The technique described here has enabled me to observe many male and female mosquitoes feeding on solidified, dry sugar A secretion flows from the mouth parts of the mosquito, and the sugar is ingested in solution Individuals have been observed to feed for as long as 30 min without apparent diminution of the flow of secretion The coloured sugar solution appeared to move from the periphery of the labella, across its surface and into a central groove The food colour could be seen to accumulate within the abdomen during the feeding process Dissection showed that the coloured material accumulated in the œsophageal diverticula

The removal of the sugar during feeding left clear spots on the slides or cover glasses Forty or fifty mosquitoes, placed in a small cage, were able to remove nearly all the dry sugar from a heavily coated slide in 2–3 days A source of water was available in the cage at all times There appeared to be no regurgitation in the mosquitoes under test since previously ingested food colours were not noticeable in the secretion when the mosquitoes were later fed on uncoloured sugar crystals This is not regarded as conclusive evidence, however

It has been customary to describe mosquitoes as having piercing mouth parts According to these observations mosquitoes have sponging mouth parts as well The manner in which the mosquitoes dissolve and ingest dry sugar from the glass slides resembles the manner in which houseflies were observed to ingest sugar from slides prepared in the same way

The species of mosquitoes so far tested for ability to feed on crystallized sugar include the following *C tarsalis*, *Culex pipiens* L , *Culiseta inornata* (Williston), *Culiseta incidens* (Thomson), *Anopheles freeborni* Aitken, *Aedes sierrensis* (Ludlow), *Aedes aegypti* (L), and *Aedes taeniorhynchus* (Wiedemann)

Many individuals of the different species of *Culex* and *Culiseta* which have been tested readily fed on the dry sugars, while species of *Aedes* and *Anopheles* which were tested were not avid feeders on this material

The fact that there is a flow of secretion, or secretions, through the tips of the mouth parts during solid sugar feeding may have practical application in certain kinds of diagnostic and nutritional studies The fluids which flow to the tips of the mouth parts for dissolving food may be collected and examined for pathogens Burtt[7], for example, has examined the saliva of tsetse flies for the presence of trypanosomes He collected the saliva by interposing microscope slides between the flies and the laboratory animals which they attempted to bite Dr M M J Lavoipierre, Hooper Foundation, University of California College of Medicine, San Francisco, is at present trying the dry sugar feeding method to study the release of filarial worms from mosquitoes I have initiated an investigation of the feeding habits of field mosquitoes in relation to the kinds of substrates on which they rest

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DONALD A. ELIASON

University of California,
Davis,
California

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MICROBIOLOGY

Influence of Time of Addition of Antibiotic on the *in vitro* Life of Rumen Holotrich Protozoa

EXTENDED maintenance *in vitro* of rumen oligotrich protozoa has been reported by Coleman[1] Similar results have not been achieved with the rumen holotrich protozoa[2]. Coleman[1] suggested that to accomplish maintenance of the oligotrich protozoa satisfactorily, it was desirable to control the numbers of bacteria present rather than to eliminate them One of the principal differences between oligotrich and holotrich protozoa is that the former ingests and then digests starch granules, while holotrichs utilize mainly soluble carbohydrates[3] In view of this fact, it would seem possible that competition between bacteria and protozoa for available substrate may be greater in the case of holotrichs than with oligotrichs

Recent experiments in this laboratory have shown that when streptomycin plus penicillin are added to a suspension of rumen organisms prior to the addition of nutrients a marked inhibition of gas production occurs over that observed when the two are added simultaneously[4] Consequently, it seemed possible that adding antibiotics to holotrich cultures prior to the time nutrients are added to the cultures may provide more favourable growth conditions for the holotrichs An experiment to test such a possible effect was carried out

Rumen contents were collected from four sheep which had been fed a basal pelleted ration plus corn Inoculum was obtained by a method similar to that described by Oxford[5] The volume used was sufficient to provide a concentration of 20×10^3 holotrichs per ml of culture media *Isotricha* spp were predominant Cultures were maintained both in 16 mm × 150 mm culture tubes and in tissue culture flasks having an area of 15 cm^2 Seven different media were used (Table 1); the final volume for each culture was 2 ml The incubation temperature was 39° C Depleted media were removed each day by withdrawing the supernatant fluid after the cultures had been placed in an ice-bath for 3 min Fresh media containing nutrients were then added The cultures were gassed at all times with oxygen-free carbon dioxide Streptomycin, at a final concentration of 20 µg/ml of media, was added to half the cultures 1 h before the media was changed As this streptomycin was removed with the depleted media, streptomycin (20 µg/ml) was added to all cultures when the media were changed

The cultures were observed each day under a stereoscopic microscope and the maximum life of the culture recorded The results are shown in Table 2 Analysis of these results showed that the addition of antibiotic prior to the addition of nutrients significantly ($P < 0\ 001$) extended the life of holotrichs *in vitro*

In this experiment particulate material was not removed from the cultures, nor were they divided The maximum life obtained was 26 days (media 6) It will be of interest to determine whether division and/or removal of particulate material from the cultures will extend culture life longer It has been found in this laboratory that oligotrich cultures maintained for extended periods on media similar to that described by Coleman[1] have a maximum life of approximately 30 days if debris is not removed and the culture is not divided

Table 1 COMPOSITION OF MEDIA*

	1 /100 ml	2 /100 ml	3 /100 ml	4 /100 ml	5 /100 ml	6 /100 ml	7 /100 ml
Basal media	†	†	†	†	†	†	‡
Glucose	0 05 g	0 05 g	0 05 g	0 05 g	0 05 g	0 05 g	0 05 g
Casein hydrolysate§	2 ml	2 ml	2 ml	2 ml	—	—	—
Clarified rumen fluid¶	—	—	20 ml	20 ml	—	20 ml	—
Distilled water	40	40	20	20	40	20	—
Solka floc‖	—	50 mg	—	50 mg	—	—	—
Grass**	—	—	—	—	50 mg	50 mg	—

* Prepared under sterile conditions as described by Bryant and Robinson (ref 6)

† Based on the media described by Bryant *et al* (ref 8)

‡ Media described by Coleman (ref 1)

§ Enzymatic 'vitamin free' casein hydrolysate (NBC)

¶ Clarified rumen fluid as described by Bryant and Robinson (ref 6), but autoclaved 20 min

‖ Ball-milled 3 days.

** Ground in a Wiley mill using a 60-mesh screen

Table 2 MAXIMUM CULTURE LIFE (DAYS)

Treatment		Media 1	2	3	4	5	6	7
Culture flasks	Antibiotic prior to nutrients	13	12	16	16	12	26	4
	Antibiotic with nutrients	8	11	6	10	12	18	3
Culture tubes	Antibiotic prior to nutrients	7	11	11	11	9	20	4
	Antibiotic with nutrients	7	7	6	6	7	11	3

The results presented here suggest that the inability to maintain holotrichs *in vitro* may be due, in part at least, to ineffective control of bacterial competition for available substrate It will be of interest to determine whether the rumen oligotrich protozoa can be more readily maintained using a similar technique

Viable bacterial counts[6] were made on the 17th day, from culture flasks containing media 6, and these indicated that the concentration of bacteria was not markedly affected by the different antibiotic treatments However, Gram stains and motility tests on bacteria isolated from the foregoing cultures suggested that the composition of the bacterial population was markedly affected, for example, organisms isolated from roll tubes containing approximately 30 colonies showed that 9 out of 9 of the organisms from the culture receiving antibiotic before nutrients were non-motile while 7 out of 10 of the organisms from the cultures not receiving antibiotic early were motile[7]

Work is now in progress to identify presumptively the isolated bacteria and to maintain holotrichs *in vitro* for extended time periods It will be of interest to determine whether the relationships indicated here are cause or effect relationships

D B PURSER
H H WEISER

Institute of Nutrition and Food Technology,
Ohio State University, Columbus

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Proteolytic Activity of Growth Media Filtrates from Non-pathogenic Species of *Verticillium*

THE production of proteolytic enzymes by micro-organisms is widely recognized, and several have been obtained from fungi In an attempt to identify the enzymatic activities of the filtrates of the growth media of members of the genus *Verticillium*, it was noted that filtrates of some non-pathogenic species of this group of fungi are able to lyse purified proteins and ovalbumin blocks The number of investigations dealing with the production of proteases in micro-organisms is low[1] Recently our interest has centred on the genus *Verticillium* as a part of our investigations on the lytic activity of a non-pathogenic strain of *Verticillium hemileiae* on uredospores of various rusts[2] The results reported here are a consequence of some studies on the effectiveness of certain species of *Verticillium* controlling rust, as well as their *in vivo* effect on the pathogenicity of these organisms To our knowledge, no results have been reported of the quantitative or qualitative aspects of proteolytic enzyme production by *Verticillium*

Cultures of *Verticillium* species were obtained from Estaçao Agronomica National of Oeiras (Portugal), University College of Swansea, Oregon State University and some were isolated by us Forty strains of the following species were considered in the survey *Verticillium albo-atrum*, *V tricorpus*, *V dahliae*, *V nigrescens*, *V lateritium*, and *V hemileiae*. The cultures, received in solid culture media, were transferred to a modified liquid 'Czapek Dox' media When *Verticillium hemileiae* is grown in a medium containing a protein substrate, very active proteolytic enzymes are elaborated into the medium These proteinases rapidly degrade the incorporated protein Aliquots of 100 ml of media were distributed in Erlenmeyer flasks of 250 ml capacity and autoclaved A sample of culture was transferred to each flask Cultures were incubated at room temperature (25° C) and grew luxuriously in all cases After specific time (15–20 days), the contents of flasks were centrifuged at 5,000g for 15 min or filtered The proteolytic activity was readily demonstrable in most of the cases using these filtrates The amount of proteinase produced was related to the amount of growth. Partial purification was accomplished using solid ammonium sulphate, the precipitate obtained between 60 and 80 per cent saturation being collected in a refrigerated centrifuge and dialysed This preparation could be stored for several months at 5° C with little loss of activity Commercially available bovine fibrinogen (Armour), trypsine (B D H), ovalbumin and casein (Light and Co) were used for the tests

Test procedures were selected which would allow us to compare the enzymatic activity of each strain. The presence of substrate specific proteolytic enzymes was detected by the use of methods which measured gelatin liquefaction, casein digestion and lysis of egg albumen clots After incubation at 27° C for 6 h, the degree of digestion of casein and egg albumen was examined by standard techniques[3] A variety of procedures have been developed to detect gelatin hydrolysis by fungi In the present study, the procedure of Pisano *et al* [4] was utilized to measure gelatinase activity by the strains of *Verticillium* The method measures the liquefaction of a purified calfskin gelatin in a tube Casein digestion was measured by the method of Dworschack *et al* [5] The amount of substrate, sodium caseinate, remaining after digestion was determined by precipitation with 5 per cent trichloroacetic acid For further investigations proteinase activity on casein substrate (1 per cent) was measured at pH 5 7 and 35° C following the method described by McDonald[6] The egg-white lytic ability of *Verticillium* species was determined using small cubic pieces of the coagulated protein in the growth media Erlenmeyer flasks containing the culture were incubated for two weeks at 28° C under static conditions The end-point was recognized by the digestion and disappearance of the solid albumen It was found that high gelatin liquefaction ability was associated with high egg-white digesting abilities, suggesting no differences in the character and composition of the proteolytic enzyme formed by the individual fungal strains

Growth of the organism was measured at various temperatures The results suggest that optimum temperature for growth and enzyme production was between 25° C and 28° C The stimulation effect of various carbohydrates and salts on the proteolytic enzyme formation on the organism growing in a minimal synthetic medium is being investigated

Attempts to obtain proteinase from non-proliferating cells of *Verticillium hemileiae* by incubating heavy suspensions in liquid media for several hours were unsuccessful Consequently a study was made of the effects of time and temperature of incubation and of composition of the media to determine if enzyme production could be effected independently and the results will be reported elsewhere

Extracts from non-pathogenic strains (in *Verticillium hemileiae*, and in much lower proportion from *V lateritium*) were found to show good activity against bovine fibrinogen, egg albumen and casein The *V. hemileiae* was also able to liquefy gelatin All the other species of *Verticillium* tested did not show any activity on the same proteins and were not able to digest gelatin It was also noted that optimum activity of the proteolytic strains was reached at one definite point during growth which corresponded to initial formation of spores

Dependence of proteinase production on the presence of protein has been shown for some organisms[7,8] but not for others[9,10] *V hemileiae* seems to require the presence of protein in the growth medium for proteinase production Synthetic peptides have not been tested as enzyme substrates The enzyme is greatly inhibited by thiol groups, cysteine, glutathione mercaptoacetate and mercaptoethanol acting as potent inhibitors Sodium acetate stimulated while chelating agents greatly reduced enzymatic activity This inhibition could not be relieved by the addition of various metal ions Nutritional experiments indicate that the enzyme is not formed or only in very low proportion when the organism is grown in mineral salts medium containing sucrose as carbon source

Many organisms have been shown to elaborate proteinases in direct relation to the amount of growth[11], and *Verticillium hemileiae* can be added to this group Excellent correlation was found between the amount of proteolytic activity exhibited by the *Verticillium* species and the lytic effect observed on the rust

As a result of this work, we can conclude that all the pathogenic species of *Verticillium* tested are pectolytic and non-proteolytic, in contrast with the non-pathogenic species which show high proteolytic activity but lack pectolytic activity[13,14].

J A Leal
Isabel Garcia Acha
J. R Villanueva

Instituto 'Jaime Ferrán' de Microbiologia,
Velazquez 138, Madrid, 6

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VIROLOGY

Effect of Basic Dyes on Host-cell Reactivation of Ultra-violet-irradiated Phage

Strains of bacteria can be divided into two classes depending on their ability to effect dark repair of ultra-violet-induced damage to phage DNA[1] Those strains which manifest this ability are *hcr*$^+$ (host-cell reactivation positive) and those which show a reduced ability are *hcr*$^-$ (ref 2) Since post-irradiation exposure of bacteria to basic dyes reduces the ability to repair damage to their own DNA, presumably by hindering replication[3], a similar effect of these dyes on irradiated phage DNA might be expected Under these conditions, *hcr*$^+$ strains would then resemble *hcr*$^-$ strains This possibility has been confirmed

All experiments were performed with phage $T1$ The bacterial hosts were *Escherichia coli* strains B, B/r—a radio-resistant mutant, and strains B_{s-1} and B_{s-2}—two radio-sensitive mutants[4] Strains B, B/r and B_{s-2} are *hcr*$^+$ and strain B_{s-1} is *hcr*$^-$ (refs 5,6) Assays for plaque formation were performed by the agar layer technique, with Difco nutrient broth agar containing 0 5 per cent sodium chloride as both layers. Crystal violet was added to the plating media before autoclaving, and acriflavine after autoclaving Two-tenths of 1 ml of overnight aerated broth cultures containing about 10^9 organisms per ml were used for plating Irradiation of $T1$ and subsequent dilution prior to plating were carried out in 2 per cent CH_3COONH_4 The concentration irradiated was 1 5 × 10^8 phages per ml Plaque counts were made after 2 h incubation of plates at 37° C followed by overnight incubation at room temperature The apparatus used for irradiation has been described previously[7] Fractionated doses at a rate of 8 1 ergs/mm²/sec were given All experiments were performed under dim light to minimize both photodynamic action of the dyes[8] and photoreactivation[9]

Since basic dyes suppress the production of infectious particles by unirradiated phage[10], preliminary experiments were done to determine the minimum inhibitory concentrations for phage $T1$ Reduction in plaque formation was found when the concentrations of the dyes in the plating agar were more than 5 μg acriflavine per ml and more than 8 μg crystal violet per ml (the former value in agreement with Foster's value of 7 5 μg/ml (ref 10)) The concentrations used afterwards were kept below these minima Preliminary tests of adsorption of $T1$ in phosphate buffer prior to plating indicated that both unirradiated and irradiated phages were more than 95 per cent adsorbed to all the bacterial hosts, regardless of the presence or absence of 1 μg acriflavine/ml of buffer In addition, the plaque count in all cases was not affected by a 15-min suspension of $T1$ in buffer containing 1 μg acriflavine/ml Thus it seems certain that in later experiments, where the phage was plated on dye-containing agar, adsorption to the host was normal and no inactivation occurred prior to adsorption

If dye-free agar is used, all the *hcr*⁺ strains give the same survival curve for irradiated $T1$ (Fig 1) The decrease in slope at high doses has been interpreted[2] as due to repair of a portion of the ultra-violet-induced damage by a fraction of the host cells This fraction of cells is given by the extrapolated intercept of the final portion of the curve on the survival axis and is 30 per cent, in agreement with previous reports[2] (Starvation of cells increases the fraction to 100 per cent[11]) In contrast, when the *hcr*⁻ strain B_{s-1} is used as host, the $T1$ survival curve is nearly exponential and shows a very much greater rate of inactivation than that corresponding to the final portion of the curve obtained with *hcr*⁺ hosts

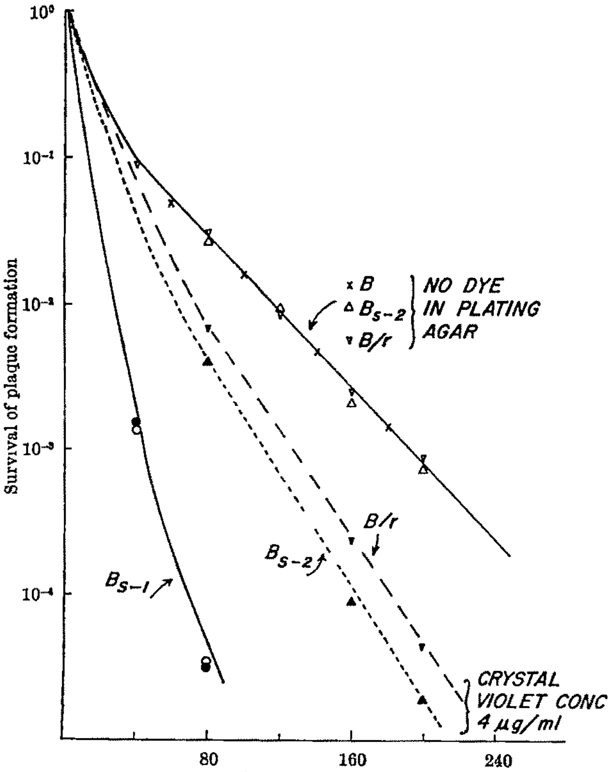

Fig 1 Effect of crystal violet on survival of ultra-violet-irradiated phage $T1$ in different host strains Curve for strain B_{s-1} open circles, no crystal violet in plating agar, solid circles, 4 μg crystal violet/ml of plating agar

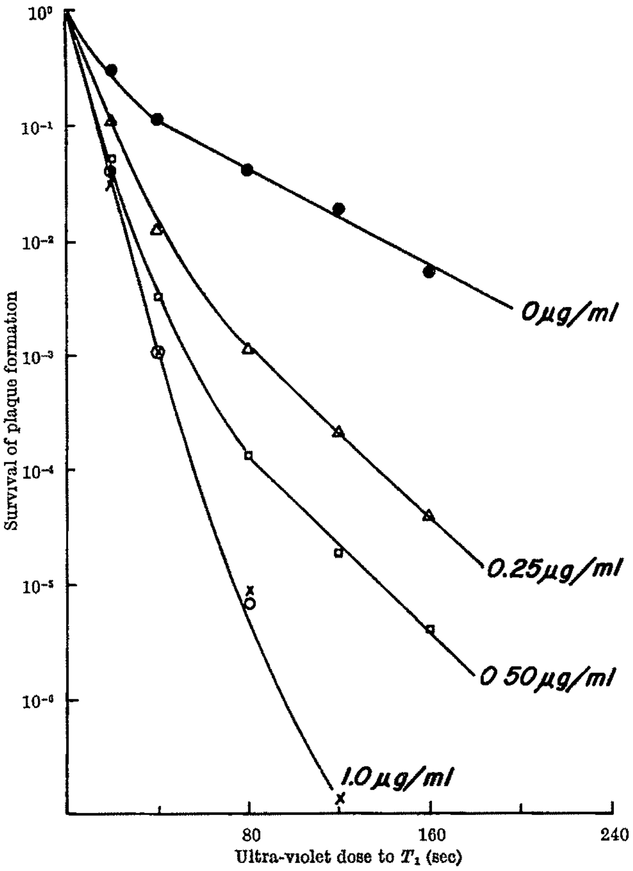

Fig 2 Effect of varying concentrations of acriflavine in the plating agar on the survival of $T1$ with strain B_{s-2} as host On the curve for 1 0 μg/ml, open circles are for B_{s-1} as host The crosses are for strain B_{s-1} without acriflavine in the plating agar

When the plating agar contains dye, the rate of inactivation increases markedly for the *hcr*⁺ hosts but there is virtually no effect for the *hcr*⁻ host (Figs 1 and 2) In the case of crystal violet at a concentration of 4 μg/ml, the effect is slightly more marked when B_{s-2} is used as host than when B/r is used Of the two dyes, acriflavine is more effective At a concentration of 1 μg acriflavine/ml, which corresponds to about one-third of the molarity for 4 μg crystal violet/ml, the phage survival curve, using B_{s-2} as host, coincides with the curve obtained by using the *hcr*⁻ B_{s-1} strain A greater efficiency of acriflavine compared with crystal violet has also been observed for the photodynamic inactivation of pneumococcal transforming DNA[12] and of the T phages[8] and for the retardation of mutation frequency decline and delay of mutation fixation in *E coli* after ultra-violet irradiation[3]

The explanation originally proposed by Garen and Zinder for the mechanism of dark repair of ultra-violet-induced damage to phage was genetic exchange between homologous regions of phage and bacterial DNA[1] This hypothesis was attractive because the phages for which this type of repair is observed—P_{22}, λ and T-odd (except $T5$)—are either temperate themselves or have mutants capable of establishing a lysogenic relationship of variable duration with their hosts[13] In addition, the base ratios for the DNA of these phages is the same as that for the host DNA[14] However, more recent evidence has been interpreted to the effect that the mechanism of repair is enzymatic[2,15]

Table 1 Effect of Dyes on Reactivating Fraction of Bacteria

Dye	Concentration (μg/ml)	Reactivating fraction of strain B_{s-1}	B/r
Crystal violet	0	$3\ 0 \times 10^{-1}$	$3\ 0 \times 10^{-1}$
	4	$1\ 4 \times 10^{-1}$	$2\ 2 \times 10^{-1}$
Acriflavine	0	$3\ 0 \times 10^{-1}$	—
	0 25	$3\ 0 \times 10^{-2}$	—
	0 50	$4\ 0 \times 10^{-3}$	—
	1 0	$(< 4\ 0 \times 10^{-3}\ ?)$	—

In view of these different hypotheses, one feature of the present results is of interest Figs 1 and 2 show clearly that the dyes increase the slope of the final portion of the *T*1 survival curve According to present interpretation, this means a decrease in the fraction of the damage which can be repaired Presumably the dyes reduce the accessibility of damaged regions to the repair enzyme However, besides this effect, the fraction of cells which can repair also decreases (Table 1) This suggests that bacterial DNA is also necessary for repair and that its action is impaired by the dyes Since the repair enzyme is apparently constitutive and present at the time of phage infection[2], the role of the bacterial DNA cannot be simply to produce it in response to infection by irradiated phage Instead, the bacterial DNA must be acting directly. Therefore it is possible that the two hypotheses regarding the nature of dark repair are not mutually exclusive This would mean that repair by genetic exchange between phage and bacterial DNA occurs and is mediated by a 'recombinase' enzyme Since all *hcr*⁻ strains reported so far show an increased sensitivity to ultra-violet radiation[2,5,6,16], the same mechanism of dark repair may rescue ultra-violet-irradiated cells as well as ultra-violet-irradiated phages

Since completing this work, we have learnt that the same possibility regarding dark repair has been derived from investigations of the recovery of diploid but not haploid yeast cells exposed to ionizing radiation The reader is therefore referred to these reports[17,18]

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Rose R. Feiner
Ruth F Hill

Radiological Research Laboratory,
Department of Radiology,
Columbia University, New York

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GENETICS

Stabilizing Selection and "Balanced Combinations of Polygenes"

In a recent paper, Gibson and Thoday[1] suggest that their analysis of an experiment with *Drosophila* populations submitted to long-term 'disruptive' and 'stabilizing' selection on the number of sternopleural chætæ confirms the ideas of Mather[2-5] that stabilizing selection can generate what is called "balanced combinations of polygenes". As Mather's theories and Gibson and Thoday's findings are important for our understanding of the maintenance of genetic variability in natural populations it seems worth while to make a closer comparison

Mather's theses can be summarized in the following points (1) Quantitative characters (as, for example, chætæ number in *Drosophila*) are under the control of a large number of genes (polygenes) each with only a small effect compared to total variability and to the effects of non-genetic variation Therefore, their presence and properties can only be assessed by statistical methods and they are not amenable to classical Mendelian analysis (2) Polygenes have no pleiotropic action (3) In chromosomes of wild populations, the polygenes are organized in combinations of alternating plus and minus genes (4) These 'balanced combinations' are built up under the influence of natural selection acting against individuals with values deviating from the optimal mean value of the character The selective disadvantage of these individuals must be caused by the deviation in the quantitative character itself as the polygenes have no pleiotropic action

Now Gibson and Thoday's results demonstrate (1) Variability in sternopleural chætæ number is at least partially under the control of genes with large effects the presence of which is shown by essentially classical Mendelian methods (2) These genes have important pleiotropic actions which are revealed by the lethality of certain combinations of the genes concerned (3) The maintenance of the genes in 'balanced combination' is not caused by the action of natural selection for an optimal mean value of sternopleural chætæ number but is brought about by the pleiotropic action of the chætæ number genes which causes recessive or dominant lethality of the − − combinations and + + combinations respectively

It will be noted that the findings of Gibson and Thoday are at variance with Mather's ideas on essential points Their model, however, shows some similarities with a situation described by Reeve and Robertson[6] where interacting recessive lethals seemed to be responsible for variation in body size

A most important point is the maintenance in repulsion linkage of the genes affecting chætæ number by their viability interactions instead of by natural selection towards an optimal value of the character itself It seems relevant here to point out that the concept of 'polygenic balance' depends largely on experiments with chætæ number in *Drosophila* Now extensive experiments have shown that differences in this character do not have much selective value[7] So polygenic balance for genes influencing this character must be based on a random distribution of the plus and minus genes along the chromosomes or on action of natural selection via the pleiotropic effects of these genes on characters which are more closely related to fitness The latter situation would result in a spurious cross-section of fitness for chætæ number[8]

Mather[2] has pointed out that if polygenic balance for a character is caused by the action of natural selection for an optimal mean value of this character, the distribution of polygenes in the chromosomes must be totally different from that of modifiers for mutant expression Therefore, a different response to artificial selection on naturally variable characters (as, for example, chætæ number in *Drosophila*) and on the expression of mutants has been postulated by Mather However, selection on the expression of the mutant ci^D (cubitus interruptus dominant)[9] in *Drosophila* shows the type of selection response which was thought by Mather[10] to be typical for the unravelling of 'balanced combinations of polygenes' Since the interruption of the 4th wing vein in ci^D, which was used as a quantitative character, does not exist in the normal wild-type fly, the distribution of its modifiers cannot be caused by natural selection acting on this character The similarity of response finds an easy explanation if genes governing a quantitative character are distributed at random along the chromosomes A random distribution will be found if the distribution of genes along the chromosomes in general is a random one Also if the distribution of polygenes, for example, bristle number, is governed by the action of natural selection on more important pleiotropic effects on various other characters the distribution of these genes will be at random relative to their action on bristle number

W SCHARLOO

Genetisch Laboratorium der Rijksuniversiteit,
Leyden, The Netherlands

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Transmission and Segregation of a Non-chromosomal Factor controlling Streptomycin Resistance in Diploid *Chlamydomonas*

CHROMOSOMAL and non-chromosomal mutations to streptomycin resistance have been described in *Chlamydomonas reinhardi*[1–3] The chromosomal mutants, *sr*-1, are resistant to low levels of streptomycin (50–100 μg/ml), possess a Mendelian pattern of inheritance, are closely linked or allelic, and lie in the left arm of linkage group IX The non-chromosomal mutants, *sr*-2, are resistant to high levels of streptomycin (500 μg/ml) and exhibit a uniparental pattern of inheritance When a mating is made between wild-type cells (*ss*) and cells of an *sr*-2 mutant, all the progeny in each tetrad are, with rare exceptions[1,4], endowed with the resistance-level of the mating type plus (*mt*$^+$) parent. The purpose of the present communication is to show that this pattern breaks down when diploids are made between *sr*-2 and *ss* cells and that a large proportion of the diploid clones segregate both resistant and sensitive cells during vegetative growth

The method for obtaining diploid cells in *C reinhardi* was recently devised by Ebersold[5], and only a brief description of the procedure will be presented here since it will be described in detail elsewhere[5,6] In the present experiments, reciprocal matings were made between two non-allelic arginine-requiring mutants according to the method of Ebersold and Levine[7] The two mutants, *arg*-1 and *arg*-2, complement in diploids[5,6] and are located six map units apart in linkage group I (ref. 7) The *arg*-2 parent always carried the *sr*-2 mutation while the *arg*-1 parent was always *ss*. In each experiment the cells were mated for 1 h and then the mating mixture was plated directly on minimal medium supplemented with sodium acetate[3] The plates were incubated in the light at 27° C for several days after which visible colonies had appeared on them

Since the original mating mixture had contained normal zygotes as well as diploid cells, the colonies included prototrophic haploid recombinants produced by germinated zygotes in addition to diploid cells Usually, the diploid colonies were larger than the colonies formed by the wild-type recombinants and they could be selected on this basis for further testing The most likely explanation of this phenomenon seems to be that the diploids begin to divide soon after being plated while the zygotes giving rise to wild-type recombinants probably do not begin to germinate for at least 36 h After a number of presumed diploid colonies had been isolated, they were tested for mating type and streptomycin resistance The *mt* gene lies in linkage group VI (ref 8) and assorts independently of the *arg* mutants so that one half of the wild-type recombinants are *mt*$^+$ and the other half are *mt*$^-$ Diploids, on the other hand, are always *mt*$^-$ in phenotype probably because *mt*$^-$ is dominant to *mt*$^+$ (refs 5 and 6) Therefore, the mating type test could be used to estimate the proportion of diploids and wild-type recombinants isolated in a given experiment Fortunately, all colonies tested proved to be *mt*$^-$ in the experiments to be discussed In a number of cases presumptive diploids were tested further by crossing them to haploid wild-type *mt*$^+$ cells and screening the resulting tetrads for arginine-requiring progeny The two arginine-requiring mutants can be distinguished by testing them on ornithine-containing medium since *arg*-1 will grow on either ornithine, citrulline, or arginine whereas *arg*-2 will only utilize arginine[7] These crosses showed that all the presumptive diploids carried both the markers

Table 1 TRANSMISSION AND SEGREGATION OF STREPTOMYCIN RESISTANCE AND SENSITIVITY IN DIPLOIDS DERIVED FROM RECIPROCAL CROSSES BETWEEN *sr*-2 AND *ss* CELLS

Cross	*sr*-2 *mt*$^+$ × *ss* *mt*$^-$			*ss* *mt*$^+$ × *sr*-2 *mt*$^-$		
Experiment	1	2	3	1	2	3
Percentage of *ss* cells in diploid clone						
100	5 55 (3/54)	1 24 (1/82)	1 85 (3/179)	24 50 (22/90)	44 50 (12/27)	6 70 (6/90)
100–80	0 00 (0/37)	1 86 (1/53)	0 00 (0/28)	0 00 (0/12)	15 10 (3/11)	4 91 (3/57)
80–60	5 10 (2/37)	3 72 (2/53)	3 50 (1/28)	12 50 (2/12)	15 10 (3/11)	8 25 (5/57)
60–40	7 65 (3/37)	13 00 (7/53)	7 02 (2/28)	12 50 (2/12)	15 10 (3/11)	16 30 (10/57)
40–20	15 30 (6/37)	7 45 (4/53)	10 50 (3/28)	12 50 (2/12)	10 00 (2/11)	34 40 (21/57)
20–0	20 40 (8/37)	22 30 (12/53)	38 60 (11/28)	31 90 (5/12)	0 00 (0/11)	26 20 (16/57)
0	45 80 (18/37)	50 40 (27/53)	38 60 (11/28)	6 25 (1/12)	0 00 (0/11)	3 26 (2/57)

Figures not in parentheses indicate the percentage diploids in a given cross belonging to each of the categories listed Figures in parentheses show the actual number of diploids in a given category and the total number of diploids examined In all crosses the *sr*-2 parent carried the *arg*-2 marker and the *ss* parent carried the *arg*-1 marker

Two important points emerge in the experimental results (Table 1) First, 50–> 90 per cent of the diploids arising from reciprocal crosses between *sr*-2 and *ss* cells produce mixed clones containing both resistant and sensitive cells This phenomenon cannot be explained by the assumption that the diploid nucleus is unstable since it has been found that three unlinked chromosomal markers do not segregate in a similar manner when the diploids are grown under non-selective conditions[6] Secondly, although 50 per cent or more of the diploid clones are mixtures of *sr*-2 and *ss* cells, a certain amount of the polarity characteristic of an ordinary cross is retained Thus, in a cross of *sr*-2 *mt*$^-$ × *ss* *mt*$^+$ up to 50 per cent of the diploid clones may be composed entirely of *ss* cells while less than 10 per cent are composed only of *sr*-2 cells In the reciprocal cross the reverse is true

If it is assumed that *sr*-2 and *ss* represent alternate mutational states of the same factor, then in a normal cross the transmission of the factor present in the *mt*$^-$ parent is usually blocked while the transmission of the factor present in the *mt*$^+$ parent is never blocked Diploids probably arise because some function necessary for normal zygote formation is never realized Whatever this function may be, the function which results in the uniparental transmission of the *sr*-2 factor is also impaired or missing in most newly formed diploid cells As a result, the majority of diploid cells contain both *ss* and *sr*-2 determinants and these determinants segregate during subsequent mitotic divisions

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NICHOLAS W GILLHAM *

Biological Laboratories,
Harvard University,
Cambridge, Mass

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* Present address Biological Laboratories, Harvard University, Cambridge 38, Mass

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 21

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Dr J Metivier and Mr B J Heywood "Vamidothion—a New Systemic Insecticide"

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W C 2), at 6 30 p m —Mr R C Foss "Feedback" (Chairman's Address)

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 8 15 p m —Mr Tom Harrisson "Inside Borneo"

Monday, October 21—Tuesday, October 22

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at the Royal Commonwealth Society, Craven Street, London, W C 2), at 11 15 a m on Monday and 10 a m on Tuesday—Symposium on "Residual Porosity in Powder Metallurgy"

Tuesday, October 22

Society of Chemical Industry, Agriculture Group (at 14 Belgrave Square, London, S W 1), at 10 30 a m —Meeting on "Ruminant Digestion"

University of London (at the Institute of Education, Malet Street, London, W C 1), at 5 15 p m —Dr W D Wall "Learning to Think" *

Institution of Civil Engineers (joint meeting with the British Section of the Société des Ingénieurs Civils de France, at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr J Aubert 'Rhône–Rhine Navigation"

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Mr J F Coales, O B E 'Predictive Control" (Group Chairman's Address)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m — Dr F J Prime "The Mechanical Properties of the Human Being" (Second of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Dr Jerry S Olson (Oak Ridge National Laboratory) "Movement of Radioactive Isotopes in Natural Environments" (Further lectures on October 23 and 25)*

Institution of Mechanical Engineers, Process Engineering Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "The Applications of Mechanical Seals in Process Engineering"

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Dr R R Smith "Improvements in Toughened Polystyrene and A B S"

Wednesday, October 23

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Dr R C G Williams "Electronics—the Expanding Frontier" (Chairman's Address) *

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Mr A Harrison "Methods of Distinguishing Sea Targets from Clutter"

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Mr R C Bond "A Commentary on the Change from Steam Traction on Great Britain's Railways, and some Thoughts on the Future" (Presidential Address)

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W C 1), at 6 15 p m —Prof Sir Lindor Brown, C B E , F R S "Reading the Literature of Science" (Presidential Address).

Oil and Colour Chemists' Association, London Section (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Dr T G Green and Mr M Thomas "Organic Coatings for Metal Containers"

Society for Analytical Chemistry, Biological Methods Group (in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W 1), at 7 p m —Meeting on "Assay of Virus Vaccines" Mr A J Beale "Manufacture and Control of Poliomyelitis Vaccine", Mr F Perkins "The Control Testing of Vaccines"

Society for Visiting Scientists (at the English Speaking Union, Dartmouth House, 37 Charles Street, London, W 1), at 7 30 p m —Discussion on "Science and the City" Chairman Baron Fleck of Saltcoats K B E , F R S Speakers Dr H M Finniston, Mr E P Hawthorne and Dr J V Dunworth

Thursday, October 24

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr W G Chaloner 'Fossil Hayfever" *

Chemical Society (in the Lecture Theatre, Middlesex Hospital Medical School, Cleveland Street, London, W 1), at 2 15 p m —Symposium on 'Peptide Chemistry"

Institution of Civil Engineers, Engineering Management Group (at Great George Street, Westminster, London, S W 1), at 5 30 p m — Informal Discussion on "The Use of the Critical Path Method" introduced by Mr D J Armstrong

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m — Dr L B Strang "Changes in the Lungs at Birth" (Third of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Institute of Refrigeration (at the National College for Heating Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road London, S E 1), at 6 p m —Prof S A Andersen "Cooling and Dehumidification of Moist Air in Cold Stores"

Society of Instrument Technology (at the Royal Institution, Albemarle Street, London, W 1), at 6 p m —Prof Dennis Gabor, F R S "Technology, Life and Leisure" (Thomson Lecture)

Royal Institute of Chemistry, London Section—Kent Sub-Section (at the Medway College of Technology, Horstead, Maidstone Road, Chatham), at 7 p m —Prof J C Tatlow "Aromatic Fluorocarbon Chemistry"

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at Highbury Technical College, Dovercourt Road, Cosham, Portsmouth), at 7 30 p m —Mr A Gasiunas and Mr W P Lewis "Hydraulic Jet Propulsion—a Theoretical and Experimental Investigation into the Propulsion of Seacraft by Water Jets"

Friday, October 25

Association of Applied Biologists, and British Ecological Society (at the British Museum (Natural History), Cromwell Road, London, S W 7), at 10 50 a m —Symposium on "Dispersal as a Factor in the Distribution and Regulation of Animal Populations, and its Economic Significance"

Royal Astronomical Society (at Burlington House, Piccadilly, London, W 1), at 4 15 p m —Sir Harold Jeffreys, F R S "How Soft is the Earth ?"*

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "The Impact of Fouling on Heat Transfer Design"

Society of Chemical Industry, Pesticides Group (joint meeting with the Manchester Section, at the Literary and Philosophical Society, 36 George Street, Manchester 1), at 6 30 p m —Mr G Edmund-Jones "Crop Protection in Cotton".

Royal Institution (at 21 Albemarle Street, London, W 1), at 9 p m — Prof K W Cross "A Baby's First Breaths"

Saturday, October 26

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Dr F Hall "Animal Communication" *

Monday, October 28

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 5 p m —Dr G Melvyn Howe "A National Atlas of Disease Mortality in the U K"

British Society for the History of Science (in the Council Room of the Science Museum, Exhibition Road, London, S W 7), at 5 30 p m — Dr J L Russell "Kepler's Planetary Laws 1609–66"

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Solid State Nanosecond Techniques" opened by Mr A F Boff, Dr R E Hayes, Dr P Orman and Mr A R Owens

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Ir R A Grijseels "Self-Checking in the SATCO Air Traffic Control System"

Society of Chemical Industry, London Section (at Shell Centre London, S E 1), at 6 30 p m —Scientific Film Evening

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Experimental Officer (if under 22 years of age a G C E in four subjects including English and Mathematics at "O" level and two scientific subjects, preferably chemistry and biology at "A" level, if over 22 a pass degree, including chemistry, or an equivalent qualification), to assist in chemical and plant physiological research on storage of fresh foods —The Secretary Low Temperature Research Station, Agricultural Research Council, Downing Street, Cambridge (October 26)

Lecturers and Assistant Lecturers in the Department of Psychology—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C 1 (October 26)

Lecturer or Assistant Lecturer in Statistics—The Registrar, The University, Keele, Staffs (October 31)

Post-doctoral Research Fellow in the School of Natural Philosophy, St Salvator's College, St Andrews, for work on relaxation time measurements in solids by spin-echo techniques—The Secretary, The University, St Andrews, Scotland (October 31)

Lecturer in Electrical Engineering at the University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, November 1)

Assistant Lecturer (preferably with industrial and research experience, and prepared to become interested in research in paper technology) in the Department of Paper Science—The Registrar, Manchester College of Science and Technology, Manchester 1 (November 2)

Director (with many years' experience in theoretical and applied statistics and related disciplines at the post-doctoral level and adequate administrative experience) of the Institute of Statistics, University of Ghana —The Registrar, University of Ghana, P O Box 25, Legon, Accra, or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (November 6)

Assistant Director of Research in the Statistical Laboratory, to engage in, and supervise, research in mathematical statistics, to undertake consulting work, especially in connexion with the physical sciences, and to help with the course for the Diploma in Mathematical Statistics— The Secretary, Appointments Committee, Faculty of Mathematics, Arts School, Benet Street, Cambridge (November 9)

Assistant Lecturer (preferably with experience or special interest in biochemistry and nutrition) in the Agricultural Chemistry Section of the Department of Agriculture—The Registrar, The University, Leeds 2 (November 11)

Chair of Chemistry—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C 1 (November 15)

Junior Lecturer (preferably interested in cytological or physiological aspects of vertebrate reproduction) **in Zoology**—The Secretary, The Medical College of St Bartholomew's Hospital, West Smithfield, London, E C 1 (November 18)

Chair of Mechanical Engineering at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W C 1 (November 21)

Chair of Control Engineering within the School of Engineering Science—The Secretary and Registrar, The University College of North Wales, Bangor, North Wales (November 25)

Chair of Microbiology—The Registrar, University College of South Wales and Monmouthshire, Cardiff (November 29)

Scientific Officer (Palæontologist) (with a B Sc (Hon) or M Sc with zoology and/or geology as leading subjects) with the New Zealand Geological Survey, Lower Hutt, to undertake research on the systematics and history of Tertiary molluscan fauna of New Zealand and thus to contribute to their use in stratigraphy—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S W 1, quoting Ref No B 13/18/18/2758 (December 18)

Assistant Experimental Officer (with at least a pass degree in agriculture or equivalent) **in the Department of Grass/Animal Ecology**, to assist in the investigation of factors affecting the health and productivity of sheep—The Secretary, The Grassland Research Institute, Hurley, Maidenhead, Berks

Assistant Lecturer, Grade B (with a good honours degree in zoology, and preferably some industrial and/or teaching experience), **to teach Zoology** to G C E "A" level and B Sc General Degree level—The Principal, South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex

Assistant Lecturers and **Lecturers in Electrical Engineering** with opportunities to specialize in any branch—The Secretary, Northampton College of Advanced Technology, St John Street, London, E C 1

Geneticist (with a good honours degree), to work on developmental genetics and gene action in *Drosophila*—The Secretary, Poultry Research Centre, Agricultural Research Council, King's Buildings, West Mains Road, Edinburgh 9

Histology Technician, for a research programme concerned with the effects of radiation on tissues—The Secretary, The Medical College of St Bartholomew's Hospital, London, E C 1

Laboratory Assistant (with experience of animal house maintenance and work in experimental physiology)—The Secretary, Royal College of Advanced Technology, Salford, Lancs, quoting Ref CH/13

Lecturer in Veterinary Physiology—The Registrar, The University, Liverpool, quoting Ref No CV/424

Lecturer or **Assistant Lecturer** (graduate in microbiology or in chemistry/biochemistry with training or experience in microbiology)—The Principal, National College of Food Technology, St George's Avenue, Weybridge, Surrey

Lecturer (with at least two years' experience with a digital computer, a sound knowledge of the construction or modification of processors, and of the application of computers to the solution of technological problems) **in the Computer Centre**—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref 31/G

Research Assistant (physicist or mathematician, preferably post-doctoral, with an interest in observational science) at the Imperial College Field Station near Ascot, for a D S I R sponsored study of cloud- and rain-systems—The Meteorology Department, Imperial College of Science and Technology, London, S W 7

Research Fellow in Endocrinology in the Department of Anatomy, to study the influence of environmental and other factors on seasonal changes in the ferret—Dr W P Dallas Ross, The Department of Anatomy, The Medical School, Birmingham 15

Research Fellow (with a higher degree or other substantial research experience) **in either the Department of Electrical Engineering or the Department of Electronics**, for work on electrical materials, machines and apparatus, or on microwave and quantum electronics (including lasers)—The Registrar, The University, Southampton

Senior Research Fellow (with an honours degree) **at the Advanced School of Automobile Engineering**, to carry out research, under the sponsorship of the Nuffield Foundation, into vibration in relation to vehicle comfort—The Registrar, The College of Aeronautics, Cranfield, Bletchley, Bucks

Senior Technician (male over 21, with G C E "A" level chemistry and physics or equivalent, and several years' experience in a biochemical or organic chemical laboratory) **for a Biochemical Laboratory** engaged in cancer research—The Secretary, Institute of Nuclear Medicine, Middlesex Hospital Medical School, London, W 1

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Spicers, Ltd Typing in Style (Paper at Work, No 3—a Series of Spicers Guides) Pp 16 (London Spicers, Ltd , 1963) [208

Family Planning Association 32nd Annual Report 1962/63 Pp 22 (London Family Planning Association, 1963) [208

Commonwealth Mycological Institute Mycological Papers, No 91 Rust Fungi II On Oceraceae, Actinidiaceae, Adoxaceae and Aizoaceae By Geoffrey F Laundon Pp 19+2 plates 7*s* 6*d* Mycological Papers, No 92 Key to the Species of *Phytophthora* de Bary By Grace M Waterhouse Pp 22 7*s* 6*d* (Kew Commonwealth Mycological Institute, 1963) (208

Royal Institute of Chemistry Monographs for Teachers, No 7 Principles of Catalysis By Dr G C Bond Pp 52 (London Royal Institute of Chemistry, 1963) 6*s* [208

Transactions of the Royal Society of Edinburgh Vol 65, No 10 Some Specimens of *Lyginorachis papilio* Kidston Associated with Stems of *Pitys* By Albert G Long. Pp 211–224+plates 1–4 10*s* 6*d* Vol 65, No 11 A Late Glacial Site at Loch Droma, Ross and Cromarty By W Kirk and H Godwin Pp 225–250+plates 1 and 2 10*s* 6*d* Vol 65, No 12 Petrology and Petrogenesis of some Garnetiferous Peridotites. By Dr J M O'Hara and Dr E L P Mercy Pp 251–314 25*s* Vol 65, No 13 Liguria Nappe Northern Appennines By Sir Edward Bailey and Dr W J McCallien Pp 315–334 12*s* 6*d* Vol 65, No 14 The Functional Morphology and Feeding Mechanisms of the Chydorid Cladoceran *Eurycercus lamellatus* (O F Muller) By Geoffrey Fryer Pp 335–382 17*s* 6*d* Vol 65, No 15 The Periostracum of *Mytilus edulis* By J F Dunachie Pp 383–410+plate 1 10*s* 6*d* (Edinburgh Royal Society of Edinburgh, 1963) [208

The Institution of Chemical Engineers Regulations for the Admission of Student, Graduate, and Corporate Members, and for the Examination of the Institute Pp iii+28 (London The Institution of Chemical Engineers, 1963) [208

British Chemicals and Their Manufacturers 1963 The Directory of the Association of British Chemical Manufacturers Pp 230 (London Association of British Chemical Manufacturers, 1963) [218

The Wildfowl Trust Fourteenth Annual Report, 1961–62 Edited by Hugh Boyd Illustrated by Peter Scott Pp 180+42 photographs (Slimbridge The Wildfowl Trust, 1963) 17*s* 6*d* net [218

Other Countries

National Academy of Sciences—National Research Council Publication No 1087 United States IGY Bibliography 1953–1960—an Annotated Bibliography of United States Contributions to the IGY and IGC (1957–1959) Compiled by Frank M Marson and Janet R Terner (A joint project of the Library of Congress, The National Science Foundation, The National Academy of Sciences) IGY General Report No 18, April 1963 Pp viii+392 (Washington, D C National Academy of Sciences—National Research Council 1963) 3 dollars [128

Ceylon Administration Report of the Director of National Museums for 1960–61 Pp 16 Rs -/45 Administration Report of the Director of National Museums for 1961–62 Pp 49 Rs 1/35 (Colombo Government Publications Bureau, 1962 and 1963) [128

Occasional Papers of the California Academy of Sciences No 38, (July 10, 1963) Oil Seepages on the Arctic Coastal Plain, Alaska By G Dallas Hanna Pp 18+1 plate No 39, (July 10, 1963) The Genus *Calotarsa* with special reference to *C Insignis* Aldrich (Diptera Platypezidae) By Edward L Kessel Pp 24+1 plate No 40, (July 10, 1963) A New Species of Giant Lima from Off Southern California (Mollusca Pelecypoda) By Leo G Hartlein Pp 6 No 41, (July 10, 1963) Pastures of the Sea By W A. Clemens Pp 8 (San Francisco California Academy of Sciences, 1963) [128

Organization for Economic Co-operation and Development—European Nuclear Energy Agency Fourth Annual Report of O E C D "Dragon" High Temperature Reactor Project Pp 160 (Paris Organization for Economic Co-operation and Development—European Nuclear Energy Agency, 1963) [128

University of California Publications in Geological Sciences Vol 45 Paleontology and Nonmarine Stratigraphy of the Cuyama Valley, Badlands, California Part 1 Geology, Faunal Interpretations, and Systematic Descriptions of Chiroptera, Insectivora, and Rodentia By Gideon T James Pp iv+171 (8 plates) (Berkeley and Los Angeles University of California Press, London Cambridge University Press, 1963) 3 50 dollars [128

Report and Accounts of the National Botanic Gardens of South Africa, (Kirstenbosch, Newlands, Cape, Karoo Garden, Worcester, and Harold Porter Botanic Reserve, Betty's Bay), for the financial year ended 31 March, 1963 Pp 32 (Kirstenbosch, Newlands, Cape National Botanic Gardens of South Africa, 1963) [128

United States Department of Health, Education and Welfare Public Health Service The MEDLARS Story at the National Library of Medicine Pp vii+74 (Bethesda, Maryland U S Department of Health, Education and Welfare, Public Health Service, 1963) [128

Publications of the Dominion Observatory, Ottawa Vol 22, No 11 Bibliography of Seismology, Items 14535–15345, January–June, 1962 By J H Hodgson and F E Langill Pp 289–327 (Ottawa Queen's Printer, 1962) 1 dollar [128

Bibliographien des Deutschen Wetterdienstes Nr 13 Agrarmeteorogische Bibliographie, 1961 Bearbeitet von Maximilian Schneider Pp xiii+253 (Offenbach a M Selbstverlag des Deutschen Wetterdienstes, 1963) [128

FAO–WHO–OIE Animal Health Yearbook, 1962 Pp viii+399 (Rome Food and Agriculture Organization of the United Nations, 1963) 15*s* , 3 dollars, 10 50 Fr francs [128

Canada Department of Mines and Technical Surveys Geological Survey of Canada Geological Maps—Preliminary Series Map 8–1963 Upsalquitch Forks, New Brunswick Map 11–1963 Riding Mountain, Manitoba Map 20–1963 Sachigo River, Ontario and Manitoba (Ottawa Director, Geological Survey of Canada, 1963) [208

United States Department of the Interior Geological Survey Geophysical Abstracts, No 197, June 1963 By James W Clarke, Dorothy B Vitaliano, Virginia S Neuschel, and others Pp iii+477–572 35 cents Index to Geophysical Abstracts, 188–191, 1962 By James W Clarke, Dorothy B Vitaliano, Virginia S Neuschel, and others Pp 651–820 40 cents (Washington, D C Government Printing Office, 1963) [208

Canada Ministère du Nord Canadien et des Ressources Nationales Musée National du Canada Bulletin No 182 Saint-Pierre et Miquelon Une Mission Folklorique aux îles Par Carmen Roy Pp 192 (Ottawa Imprimeur de la Reine et Contrôleur de la Papeterie, 1963) 3 dollars [208

Food and Agriculture Organization of the United Nations Freedom from Hunger Campaign. Basic Study No 3 Animal Disease and Human Health Pp vii+50 (Rome Food and Agriculture Organization of the United Nations , London H M Stationery Office, 1963) 2*s* 6*d* , 0 50 dollars [208

Canada Department of Northern Affairs and National Resources National Museum of Canada Anthropology Papers, No 3 An Archaeological Survey Along the North Shore of Lake Superior By J V Wright Pp 9 Bulletin No 184, (Anthropological Series, No 58) The Donaldson Site By J V Wright and J E Anderson Pp viii+113 (26 plates). 1.50 dollars (Ottawa Queen's Printer, 1963) [208

Uganda Government Annual Report of the Forest Department for the year ended 30th June, 1962 Pp vii+82 (Entebbe Government Printer, 1963) Sh 6–50 [208

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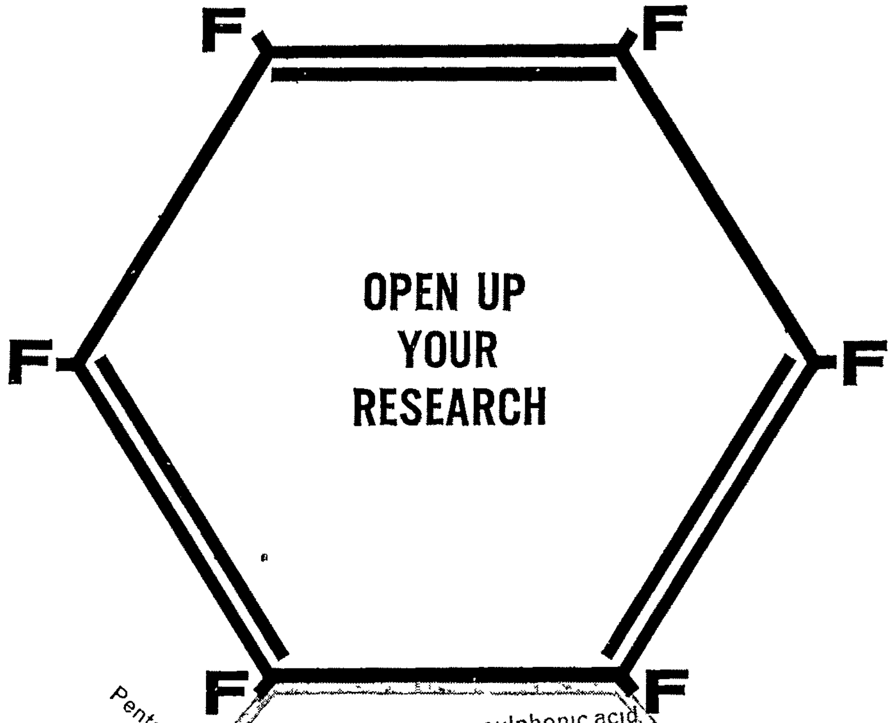

Pentafluorobenzene sulphonic acid
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Pentafluorobenzene
Bromopentafluorobenzene
Decafluorobiphenyl
Pentafluorophenylhydrazine
Methylpentafluorobenzene
Methoxypentafluorobenzene
2,3,5,6-Tetrafluorophenol
Pentafluorothiophenol
1,2,3,4-Tetrafluorobenzene
2,3,5,6-Tetrafluoroaniline
Octafluorotoluene
Pentafluorophenol
Iodopentafluorobenzene
Pentafluoroaniline
3-(Pentafluorophenyl) propene-1
1,2-Dibromotetrafluorobenzene
Pentafluorobenzoic acid
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APPOINTMENTS VACANT

ROYAL PERTH HOSPITAL
WESTERN AUSTRALIA
HOSPITAL MICROBIOLOGIST

Applications for the above post are invited from medical graduates with suitable experience in Microbiology The Hospital Department of Microbiology is under the control of the Director of Hospital and University Services in Microbiology

The successful candidate will be required to assist the Director (Professor N F Stanley) in the administration of the routine laboratory and clinical services of the Hospital Department of Microbiology, to act in the absence of the Director, and to carry out special investigations from time to time

Royal Perth Hospital is the main teaching hospital of the Medical School of the University of Western Australia The Hospital Department of Microbiology is well equipped, adequately staffed for the performance of all modern microbiological techniques, functions in close association with the University Department of Microbiology in the adjacent School of Medicine and the Virus Research and Diagnostic Laboratory of the Department of Public Health

SALARY Within the range of £A3,800 to £A4,350 per annum, commencing rate according to qualifications and experience Salary will commence from the date the appointee takes up duty

CONDITIONS OF SERVICE Include annual leave, long service leave (three months on full pay after each seven years service) and sick leave Study leave may be granted as a privilege for some special purpose approved by the Board Contribution to the State Superannuation Scheme is required, or alternatively, the Hospital would consider subsidizing the appointed officer's existing commitments under an approved scheme or endowment policy

TRANSPORT EXPENSES A travel grant equivalent to minimum First Class sea passage will be provided for successful applicant and family (under 16 years of age), together with an allowance of £S200 for the cost of removal of personal effects, etc (excluding furniture and motor vehicle), subject to a bond to remain in the Hospital's service for a minimum period of three years

A memorandum of Information including further details of the Department of Microbiology is available upon request

Further information concerning Royal Perth Hospital and the State of Western Australia may be obtained from the Agent General for Western Australia, Savoy House, 115 Strand, London, W C 2

Applications in writing should include date of birth, qualifications, experience, present and previous appointments, a list of publications, a recent photograph and the names and addresses of three referees

CLOSING DATE November 30, 1963

Joseph Griffith,
ADMINISTRATOR

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF ANATOMY
RESEARCH FELLOWSHIP IN THE PHYSIOLOGY OF REPRODUCTION

Applications are invited for a Research Fellowship in the Department of Anatomy (Professor Sir Solly Zuckerman, C B , F R S) to study the physiology of reproduction and in particular to investigate the control of fertility in primates and lower mammals The salary will be determined according to qualifications and experience F S S U and family allowance will be paid Initially the appointment will be for a period of two or three years, which may be extended

Further particulars may be obtained from the Assistant Registrar, the Medical School, Birmingham, 15, to whom applications, naming two referees, should be sent as soon as possible

AUSTRALIA — NEW SOUTH WALES DEPARTMENT OF AGRICULTURE

CAREERS FOR PROFESSIONAL OFFICERS

Applications are invited for the following positions in the New South Wales Department of Agriculture, Australia Commencing rate in each case according to qualifications and experience.

ECONOMICS RESEARCH OFFICERS

(ref 234/A) Salary £A1,403 (three-year graduate), range £A2,123 Honours graduate or a four-year graduate with an appropriate degree commencing salary £A1,473 Progression to £A2,393 subject to specified conditions Appointee required to undertake research in Agricultural Economics, Farm Management or the Marketing of Farm Products Should possess a degree in Agricultural Economics, Agricultural Science, Economics or equivalent qualifications Headquarters Initially Sydney, but appointee may be required to serve in any part of New South Wales

CHEMIST (TOBACCO RESEARCH)

(ref 234/B) Division of Science Services Salary £A1,405 (three-year graduate), range £A2,305, four-year graduate commencing salary £A1,505 Progression to £A2,655 subject to special conditions Degree in Agricultural Science or Science and postgraduate experience in organic chemistry essential Appointee required to undertake research, in association with other tobacco research workers, into problems related to the production, manufacture and smoking quality of tobacco and may be given a period of training in this specialized field Headquarters initially will be at the Department's Science Services laboratories at Rydalmere, near Sydney, and facilities will be available for field trials Final location will probably be in a country tobacco growing area

AGRONOMISTS (ref 234/C) Minimum salary (graduates) £A1,473 (non-graduates) £A1,023 with progression to £A2,123 subject to meeting requirements Opportunities exist for further promotion to £A2,453 and beyond Vacancies exist in pastures, weeds and crop research, general agronomy and extension with field crops and vegetables Qualifications B Sc Agr , B Rural Sc , or Diploma of the Hawkesbury or Wagga Agricultural Colleges or equivalent Must be prepared to serve in any part of the State

VETERINARY RESEARCH OFFICERS (ref 234/G) Veterinary Research Station, Glenfield Salary £A1,613 with range to £A2,343 subject to meeting requirements Opportunities exist for further progression to £A2,618 and beyond Qualifications B V Sc or equivalent essential, postgraduate experience in a veterinary diagnostic or research laboratory desirable Duties Examination of specimens in connection with stock diseases—one appointment will be in the field of bacteriology and pathology and the other in virology Opportunity to conduct research in these fields available

SPECIAL LIVESTOCK OFFICER

(Beef Cattle, Horses and Goats) (ref 234/H) Salary £A2,273 range £A2,453 Degree in Veterinary Science, Agricultural or Rural Science essential The position holds prospects for advancement Appointee may be required to serve in any part of the State

CHEMIST (SOIL SURVEYOR)

(ref 234/L) Salary £A1,405 (three-year graduate) range £A2,305 Honours graduate or a four-year graduate commencing salary £A1,505 Progression to £A2,655 subject to satisfying certain conditions Applicants should possess B Sc (Agr), B Sc , or equivalent degree qualification Duties would principally comprise carrying out soil surveys in any part of New South Wales, preparation of data and draft maps from surveys, laboratory work, and such other duties as may be required Headquarters at Division of Science Services, Rydalmere -

FRUIT RESEARCH OFFICERS

(ref 234/D) Minimum Salary (graduates) £A1,473, (non-graduates) £A1,023 with range to £A2,123 subject to meeting requirements Opportunities exist for further promotion to £A2,453 and beyond Vacancies at Sydney, Alstonville Tropical Fruit Research Station (near Lismore) and the Lower Murray Research Station at Dareton Duties include investigations into fruit growing problems associated with tropical, sub-tropical and deciduous fruits Degree in Agricultural Science or equivalent essential

RESEARCH AGRONOMIST (Weed Control) (ref 234/E) Yanco Agricultural Research Station Salary £A1,473 with range to £A2,123 subject to meeting requirements Opportunities exist for further progression to £A2,453 and beyond Appointees will be required to undertake research on the control of weeds occurring mainly under irrigation in the south-western districts of New South Wales Considerable scope for the appointee to develop research with weeds in rice, vegetables, irrigated pastures or irrigation ditches Degree in Agricultural Science or its equivalent essential

VETERINARY OFFICERS

Division of Animal Industry (ref 234/J) Salary £A1,613 with range to £A2,343 subject to meeting requirements Opportunities exist for further progression to £A2,618 and beyond Degree in Veterinary Science or equivalent essential Duties Investigations in stock and poultry disease outbreaks , take regulatory action in cases of scheduled diseases Appointee must be prepared to serve anywhere in the State

SENIOR RESEARCH OFFICERS

(ref 234/K) Plant Nutrition and Cereal Chemistry, Tamworth Agricultural Research Station Salary £A2,455 range £A2,655. Prospects of progression to £A2,755 subject to meeting certain requirements (If not possible to appoint an experienced officer consideration may be given to appointing a Research Officer with commencing rate up to £A1,905 with prospects of further progression subject to meeting requirements) Duties Plant Nutrition—the study of environment on uptake and utilization of nutrient elements by the major crops and pasture species grown in the northern part of New South Wales Cereal Chemistry—original research in cereal chemistry and routine quality evaluation of cereal samples from breeding and experiment plots Qualifications appropriate postgraduate experience desirable The recently established Research Station, situated seven miles from the City of Tamworth serves as a centre of agricultural research for a large section of the north-west of New South Wales Excellent facilities for research Appointees will be expected to provide research leadership and, where necessary, in-Service training for junior officers in their particular fields

LECTURER IN MICROBIOLOGY

(ref 234/F) Hawkesbury Agricultural College, Richmond Salary £A1,403 with range £A2,123 subject to meeting requirements Opportunities exist for further progression to £A2,393 and beyond Applicants must possess appropriate degree of a recognized University and have ability to lecture and conduct examinations and give practical instruction and demonstrate, where necessary , required to lecture in Microbiology in Agriculture, Food Technology and Dairy Technology Diploma Courses, and to share disciplinary and other College duties with other Lecturers Cottage at moderate rental available within four miles of College

Please write for application forms to the New South Wales Government Offices, 56 Strand, London, W.C.2, quoting the reference number of the appointment in which you are interested

THE CLOSING DATE FOR APPLICATIONS IS NOVEMBER 11, 1963.

CHEMIST OR BIOCHEMIST REQUIRED IN Department of Chemical Pathology for work on metabolism of steroid hormones involving microchemical and radiochemical techniques Some experience in chemical work preferred but recent graduate would be trained Opportunity for study for higher degree —Apply Secretary, St Mary's Hospital Medical School, London, W 2

ENTERIC REFERENCE LABORATORY Technician wanted to assist in M R C supported research project, mainly concerned with bacterial polysaccharides Experience in chromatographic techniques an advantage N H S scales of pay according to qualifications and experience —Applications to the Director, Central Public Health Laboratory, Colindale Avenue, N W 9

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The Laboratory will be similar to the good academic research laboratories as regards intellectual atmosphere, equipment and general facilities The publication of scientific work and communication with researchers elsewhere will be encouraged as much as possible.

In the physics department there are vacancies for two types of graduate

(a) Research workers experienced in the fundamental study of polymer melt flow or solid state physical properties They should be interested not only in the pure physics of polymers but also in the way properties are dependent upon chemical structure Candidates most likely to be suited to this work are Honours graduates in physics or physical chemistry

(b) Physicists or technologists interested in correlating the behaviour of polymers in laboratory experiments with their performance in practical processes Examples of this work are the analysis of (a) screw extrusion in relation to polymer visco-elasticity, or (b) the mechanical strength of a moulding in terms of the inherent strength of the polymer and the effect of thermal and shear history Candidates for posts in this field should be graduates in physics or related subjects and although some experience of such work would be an advantage, a more important qualification is an intelligent enthusiasm for the analysis of technological processing

Applications quoting ref P/P26 should be made to The Laboratory Head, I C I Ltd , Petrochemical and Polymer Laboratory, P O Box 11, The Heath, Runcorn, Cheshire

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UNIVERSITY OF SHEFFIELD

Applications are invited for two posts as Research Assistant to take part in programmes designed to study (1) human chromosome abnormality and associated disorder , and (2) chromosome behaviour in normal and abnormal karyotypes Suitable for graduates with a first degree in the Biological Sciences or Chemistry Initial salary up to £1,100 per annum, according to qualifications and experience

Please apply to the Secretary, Genetics Department, the University, Sheffield, 10, giving full details of qualifications and experience and the names of two referees

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UNIVERSITY OF NOTTINGHAM

Applications are invited for a Lectureship in Physics Candidates should have research experience in nuclear magnetic resonance Salary scale £1,250 to £2,150 per annum, initial salary according to qualifications and experience, plus membership of the Universities pension scheme and children's allowance

Conditions of appointment and application form, which should be returned by Wednesday, November 27, 1963, from the Registrar

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UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF ANATOMY

RESEARCH FELLOW IN ENDOCRINOLOGY

Applications are invited for a Research Fellowship in the Department of Anatomy (Professor Sir Solly Zuckerman, C B , F R S) to study the influence of environmental and other factors on seasonal changes in the ferret The salary will depend on qualifications and experience and will include F S S U and family allowance benefits

Further particulars may be obtained from Dr W P Dallas Ross, the Department of Anatomy, the Medical School, Birmingham, 15, to whom applications, naming two referees, should be sent as soon as possible

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SENIOR II TECHNICIAN REQUIRED FOR work on tumour destroying virus in tissue culture General tissue culture experience necessary human cell culture experience an advantage Salary £945 to £1,445 per annum according to age and experience —Written applications should reach Dr J G Bennette Courtauld Institute, Middlesex Hospital, London, W 1 before November 1, 1963

UNIVERSITY OF CAPE TOWN

SENIOR LECTURER OR LECTURER IN MATHEMATICAL STATISTICS

Applications are invited from persons with special qualifications in Mathematical Statistics for the post of Senior Lecturer or Lecturer in the Department of Mathematics The present salary scales are Senior Lecturer, R 3,520 by R 120 to R 4,120 (£1,760 by £60 to £2,060) per annum Lecturer, R 2,680 by R 120 to R 3,520 (£1,340 by £60 to £1,760) per annum The salary scales are under review, and it is hoped that improved scales will be introduced from the beginning of 1964 The initial salary will be dependent upon the qualifications and experience of the successful applicant, who would be expected to commence his duties as soon as reasonably possible

Two copies of the application must be sent to the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (from whom memoranda giving the general conditions of appointment, travel allowance on appointment, and detailed information about the department and its programme of teaching and, research in Mathematical Statistics should be obtained), not later than November 11, 1963 A third copy of the application should be sent direct by airmail to the Registrar, University of Cape Town, Private Bag Rondebosch, South Africa, to reach him by the same date The University reserves the right to appoint a person other than one of the applicants or else to make no appointment

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NATIONAL RESEARCH COUNCIL OF CANADA

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1964-65

Approximately 150 Fellowships will be awarded for 1964-65 by the National Research Council Of these, 40 will be tenable in National Research Council laboratories, 15 in laboratories of the Department of Agriculture, 4 in laboratories of the Department of Forestry, 15 in laboratories of the Department of Mines and Technical Surveys 4 in the laboratories of the Department of National Health and Welfare, 2 in the Fisheries Research Board of Canada laboratories, 2 in the Department of Transport—Meteorological Service of Canada laboratories, 8 in the laboratories of Atomic Energy of Canada Limited, and 60 in laboratories of Canadian universities

Applicants should be not more than 35 years of age and should possess a Ph D degree from a recognized university, or expect to obtain such a degree before taking up an award (Candidates having equivalent research experience may be considered) There are no restrictions regarding nationality of applicants, but successful candidates must meet all Canadian immigration requirements

The annual stipend, which is free of income tax, is $4,000 for single Fellows and $5,000 for male Fellows who are married

The fields in which Fellowships are available in the laboratories of the National Research Council, the Department of Agriculture, the Department of Forestry and the Department of Mines and Technical Surveys are described in greater detail in booklets that may be obtained on request, together with application forms Applicants who are interested in Fellowships in the universities may write directly to these institutions, or to the National Research Council for information The fields of interest should be stated in the initial inquiry in order that appropriate information may be supplied The closing date of the competition is January 15, 1964

Inquiries should be addressed to

Awards Officer,
National Research Council,
OTTAWA 2, Canada

or to :

Chief Scientific Liaison Officer,
National Research Council of Canada,
Africa House,
Kingsway,
LONDON, W C 2, England

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSICS

Applications are invited for two posts of Research Assistant to Professor N H March tenable from January 1, 1964 One is a senior post for work on the angular distribution of charge in the unit cell of transition metals Applicants should have a Ph D in Theoretical Physics or equivalent research qualifications Initial salary according to age and experience not less than £1 000 a year The other post is a junior one for work on the density matrix theory of impurities and imperfections in metals Applicants will be encouraged to read for a higher degree, and financial support at least equal to that of a D S I R postgraduate studentship will be available

Applications should be sent to the Registrar The University, Sheffield, 10, by November 9, 1963

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(3) A physicist to study semiconductor laser materials using resonance techniques

(4) A physicist to investigate crystalline laser materials using infra-red spectroscopic techniques

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Principal J D Mounfield, M Sc Tech, Ph D, F R I C

Applications are invited from graduates in microbiology or in chemistry/biochemistry with training or experience in microbiology, for a teaching post at the College Facilities for research are available Appointments may be made at Lecturer or Assistant Lecturer level Salary scales Lecturer, £1,670 by £45 to £1,895 per annum, Assistant Lecturer, £930 to £1,550 per annum Starting salary dependent on qualifications and experience

Application forms (obtainable from the College), should be sent to the Principal as soon as possible

---

HORTICULTURE ADVISORY OFFICERS National Agricultural Advisory Service (Ministry of Agriculture, Fisheries and Food) Six or more pensionable posts for men or women at least 20 on October 1, 1963 with degree in horticulture (or closely related subject), N D H, or equivalent qualification, and at least one year s full-time practical experience National salary scales Grade IV, £769 to £1,064, Grade III, £1,157 to £1,821 Starting salary may be above minimum in both cases Promotion prospects—Write Civil Service Commission, 23 Savile Row, London, W 1, for application form, quoting 5766/63/7 Closing date November 12, 1963 (1)

UNIVERSITY OF MANCHESTER

Applications are invited for the posts of Lecturer and Assistant Lecturer in the Department of Physics in the Faculty of Science The research interests of applicants should lie in the High Energy Physics field, in which the Department has an active programme Duties to commence as soon as possible Salary scales Lecturer, £1,250 to £2,150 per annum, initial salary according to qualifications and experience, Assistant Lecturer, £1,000 to £1,150 per annum Membership of the F S S U and Children's Allowance Scheme

Applications should be sent not later than November 15, 1963, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained Applications by airmail letter in the first instance will be received from overseas candidates, who should state the names and addresses of at least three persons to whom reference can be made

---

POST-DOCTORATE FELLOWSHIPS UNDER the National Research Council of Canada, in the following fields—Physical Chemistry gas kinetics, combustion reactions by mass spectrometry, enzyme kinetics, radiation, induced polymerization, molecular structure by infra-red spectroscopy Organic Chemistry steroid hormones, synthesis and conformational problems Natural quinones, isolation and structure For particulars write to Paul A Giguère Director, Chemistry Department, Laval University, Québec 10, Canada

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AUSTRALIAN NATIONAL UNIVERSITY
LECTURER IN BOTANY (GENETICS)

Applications are invited for appointment to a new post of Lecturer in Botany (Genetics) in the School of General Studies of the University The appointee will be required to take up duty as early as possible in 1964 An interest and experience in biochemical and/or physiological genetics is desired The salary range for lecturer is at present £A1,750 by £A100 to £A2,45 per annum Salary on appointment will be i accordance with qualifications and experience A cost of living allowance, at present £A31 per annum, will be paid to an appointee within the range, except at the maximum Salaries are under review

Further information and the forms which should accompany an application may be obtained from the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House Pall Mall, London, S W 1 Applications close i Australia and London on November 25, 1963

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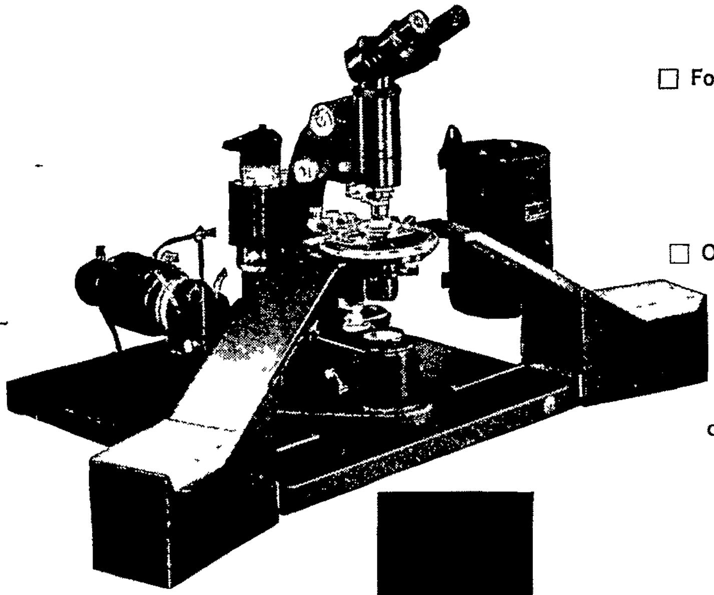
Applications are invited for a newly created lectureship in Physiology at a salary within the range R 2,040 to R 3,480 (£1,020 to £1,740) per annum, depending on experience and qualifications A medical degree or a higher degree in physiology or biochemistry is desirable Applicants should be prepared to assume duties on or soon after January 1, 1964 Applicants should state age, qualifications, experience, publications, and research interests, and give the names of two referees whom the University may consult

Two copies of the application must be sent to the Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (from whom memoranda giving the general conditions of appointment, travel allowance on appointment, and further information about the department should be obtained), not later than November 11, 1963 A third copy of the application should be sent direct by airmail to the Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town South Africa, to reach him by the same date The University reserves the right to appoint a person other than one of the applicants or else to make no appointment

---

THE MEDICAL COLLEGE OF ST BARtholomew's Hospital, West Smithfield, E C 1 Applications are invited for the post of Junior Lecturer in Zoology tenable as from January 1, 1964, or as soon thereafter as possible The successful applicant will be required to assist in the teaching of medical students and to carry out research Preference will be given to a person interested in cytological or physiological aspects of vertebrate reproduction The salary will be on the scale £1,000 by £50 to £1,150 plus £60 London Allowance, together with Children's allowance and membership of the F S S U—Forms of application, which should be returned not later than November 18 may be obtained from the Secretary of the Medical College



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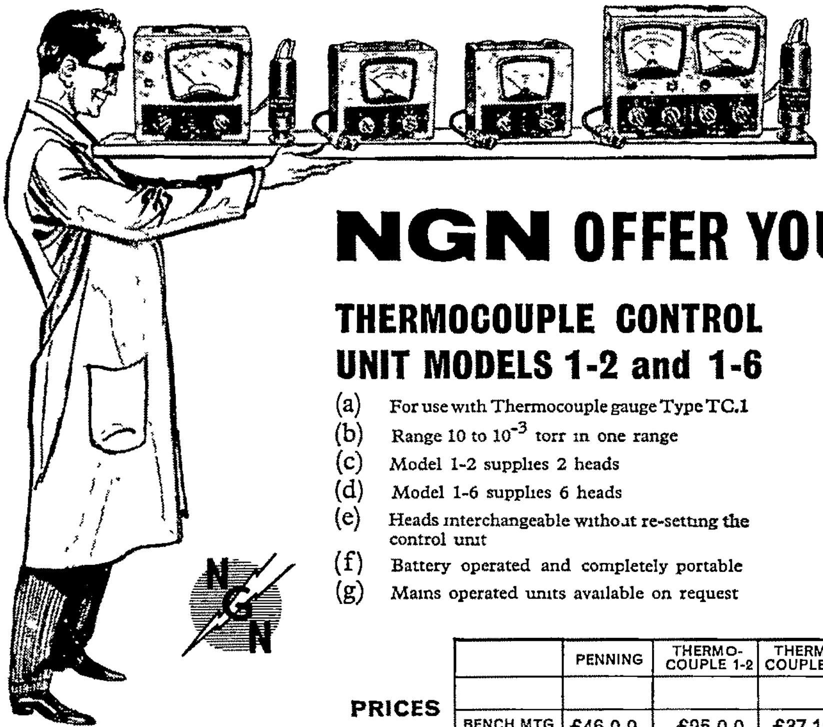

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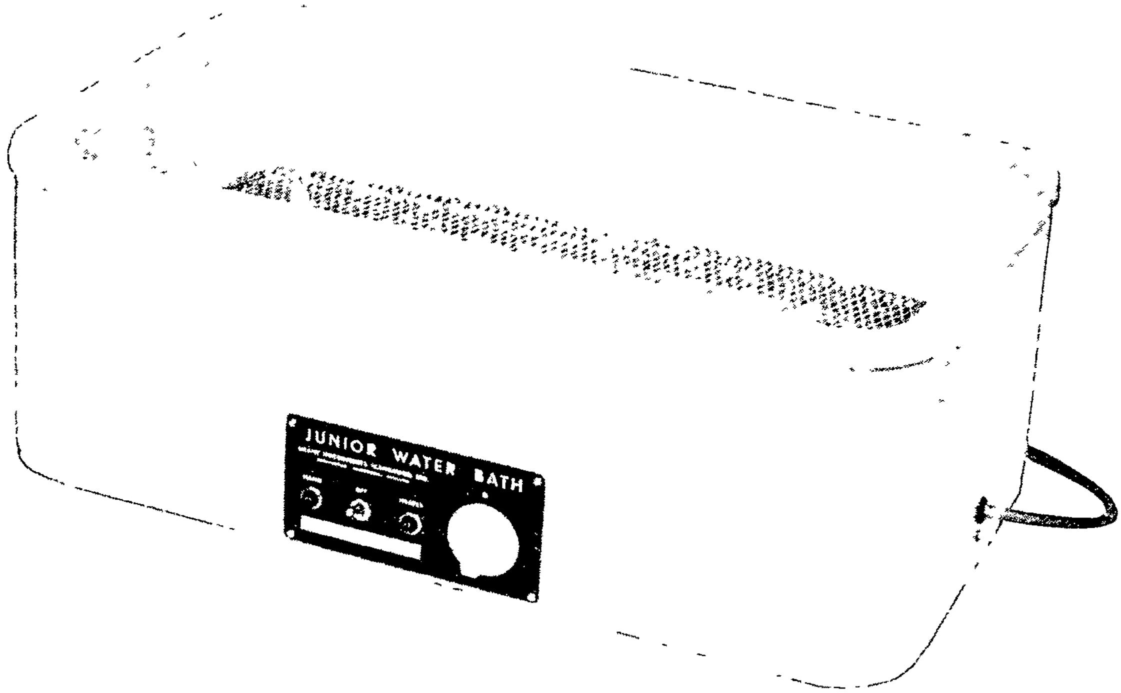

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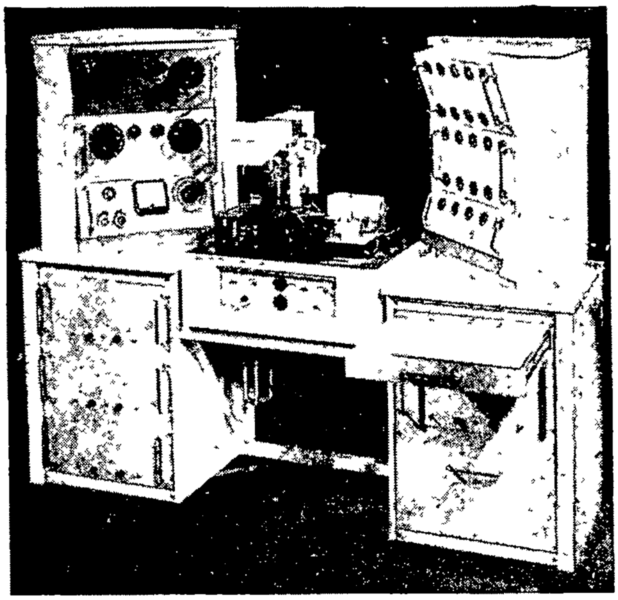

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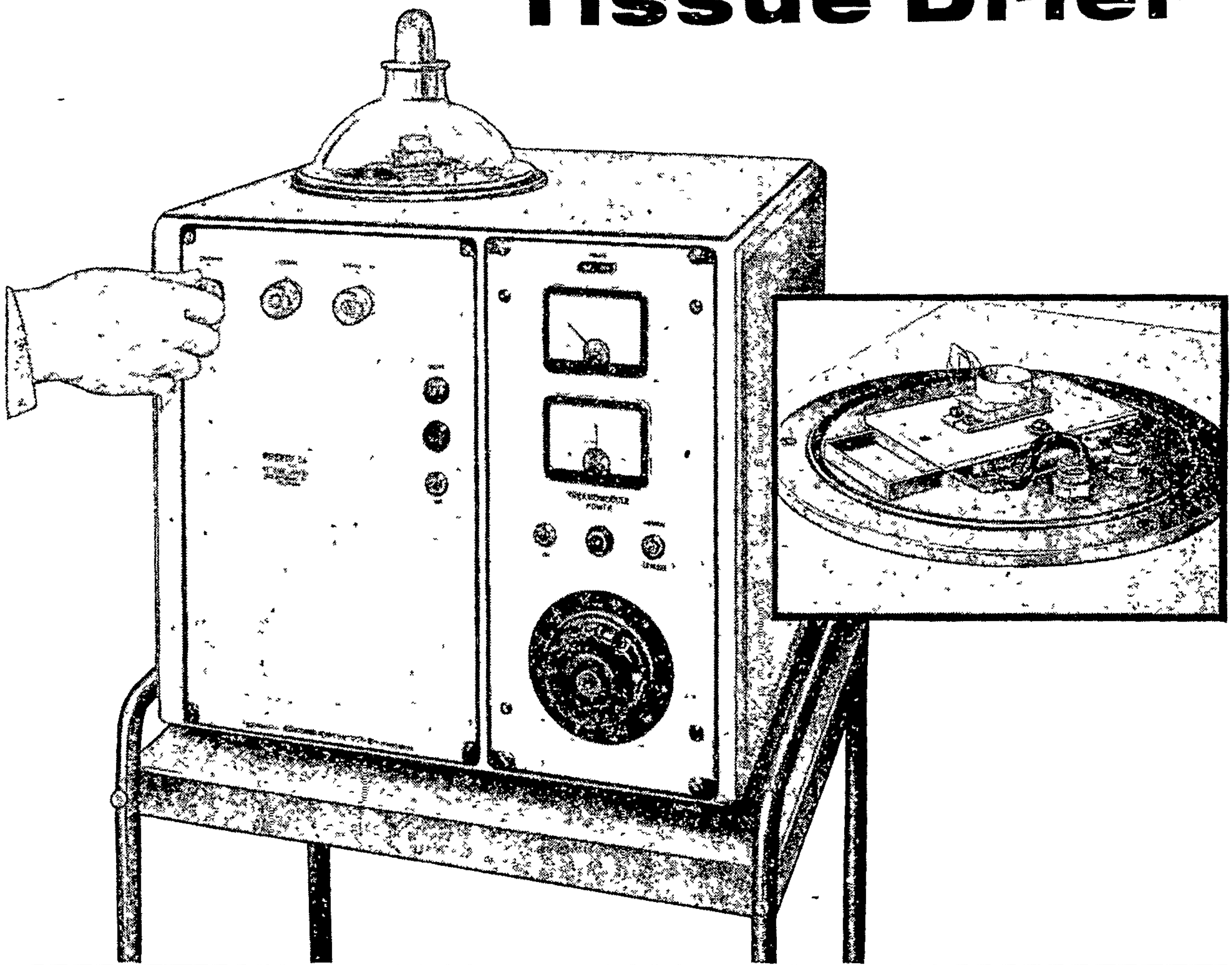

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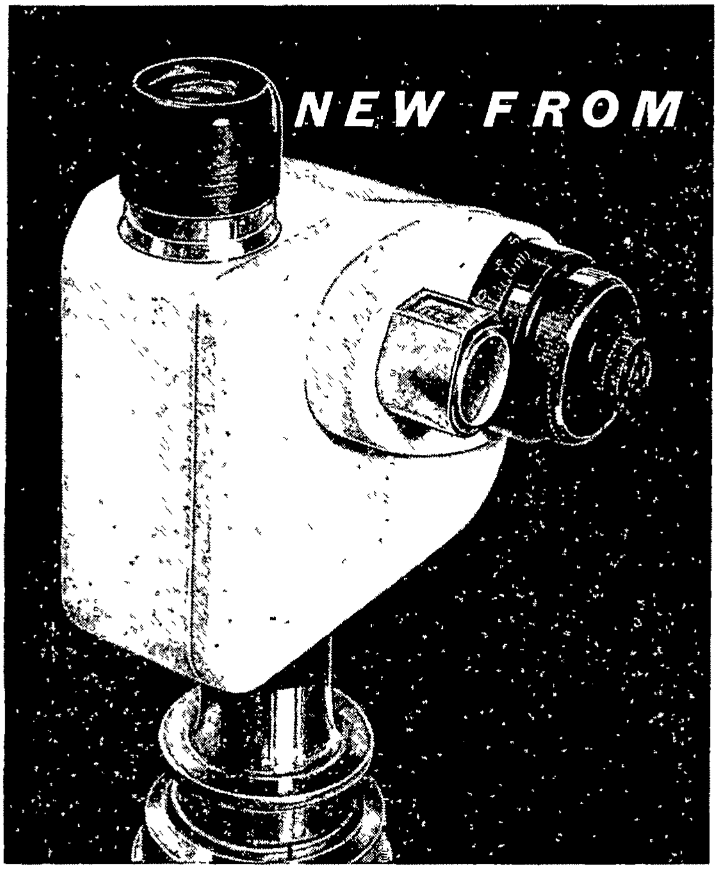

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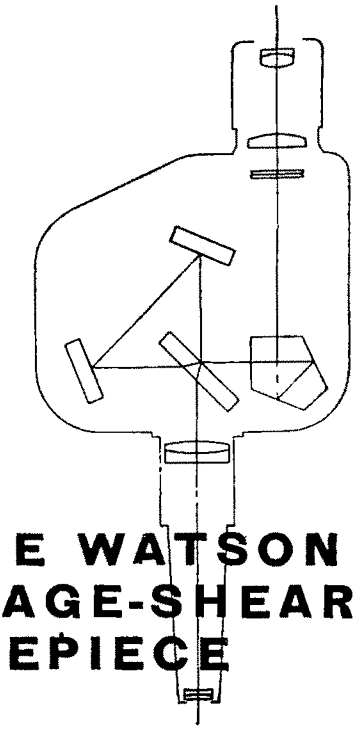

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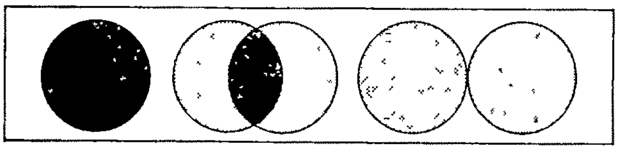

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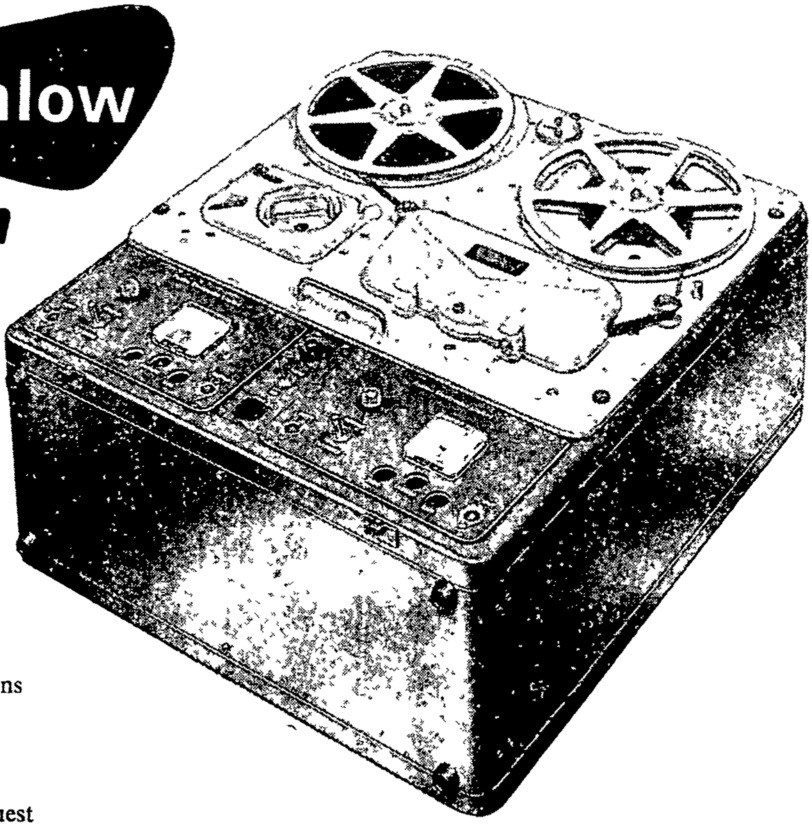

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LIBRARY SERVICES AND THE SOURCE FOR INSPIRATION

IN the *Returns from Universities and University Colleges in Receipt of Treasury Grants, Academic Year 1961–62*, the University Grants Committee continues its recent practice of including a general survey of the year. Prominence is given in this survey to the appointment last June, with the Ministry of Education, of a Library Committee, the terms of reference and the membership of which are set forth. Both terms of reference and the membership have been the subject of some criticism, and the importance of the university library has been stressed in some recent discussion of university education. It has, indeed, been urged as one reason for concentrating the university expansion on existing large universities that only such universities have any prospect of providing the essential library resources on an adequate scale.

It might be commented in this connexion that past and present parsimony has put almost all Britain's existing university libraries far behind the university libraries of the United States in their resources, nor do the returns included in the present report from the University Grants Committee indicate any improvement or prospect of improvement. Library expenditure in Great Britain as a whole remains at no more than 3·8 per cent of university expenditure as compared with 3·8 per cent in 1951–52 and 4·0 per cent in 1959–60, and the total expenditure is now only £2·8 million compared with £988,000 in 1951–52 and £2·35 million in 1959–60. The total expenditure on books is little more than £600,000 a year and on periodicals a little more than £300,000; the combined expenditure of all the universities for these purposes is only £640,000 and £330,000, respectively. Only at the University of Oxford does expenditure on books approach £100,000 a year, and at the University of Cambridge £50,000 a year, and only at eight other universities does it approach or exceed £20,000, apart from London. Only some half-dozen universities spend £15,000 on periodicals, and the combined expenditure of £330,000 compares with £118,000 in 1951–52.

These figures are pertinent in that the new Library Committee is instructed "to consider the most effective and economical arrangements for meeting the needs of the Universities and the Colleges of Advanced Technology and central institutions for books and periodicals, taking into account expanding staff and student populations, the possible needs of other users, the growth of research, the rising costs of books and periodicals, and the increasing capital cost of library accommodation". There is manifestly much past neglect and parsimony to be made good at costs which will be inevitably the higher for delay. It is to be hoped the lesson has been learned.

The Committee was also "to assess how far greater use might with advantage be made of shared facilities, both between the institutions themselves and between the outside library systems and other institutions, and of modern methods of reproduction". If this part of its terms of reference implies recognition that university libraries are an essential part of a national library system, that may explain to some extent the membership of the Committee. It will scarcely explain some of the omissions, and it is manifest to anyone who has the slightest acquaintance with library organization in Britain as a whole why the Committee, as at present constituted, fails to command confidence, respect and enthusiasm, not only among the staff of the university libraries but among the Standing Conference of National and University Libraries, the National and University Libraries Committee of the Library Association or the Association of Special Libraries and Information Bureaux. Such confidence and respect are essential, however, if the Committee is to prove effective, and there is little in its present membership to offset doubts as to its possessing either the professional expertise and insight or the imagination and drive which its work demands.

The most appropriate background against which the work of this new Committee is to be assessed is, however, provided by the forthright address which Dr. J. N. L. Myres, the Bodley's Librarian, delivered as president at the annual conference of the Library Association on July 7. Dr. Myres quoted a comment from Mr. R. Vosper, of the University of California, that Illinois, with 3·5 million volumes, was the largest State university library in the United States and the only one that had undertaken to compete with Harvard in the massive and persistently expanding support that is essential to library excellence. He asserted that unless Britain's university libraries, both new and old, were given this massive and persistently expanding support and had the priority over other university needs, which Mr. Vosper also claimed was so at Illinois, there was no possibility in the present acutely competitive condition of the international book trade that they would achieve or retain the value or the status necessary to make their universities worthy of the name. It is not simply a question of money, though the figures already quoted indicate sufficiently how poorly placed are the university libraries of Britain to compete with the effect which this massive American buying of older books and manuscripts has had and still is having on the dwindling sources of supply in Europe or on the general level of prices. Dr. Myres was thinking far more of the need for consistent and sustained generosity in providing library funds and of the overwhelming and urgent need for a far higher level of financial support for learned libraries generally in Britain.

Dr. Myres began his consideration of the position of the university or learned library by referring to the challenge presented to statesmanship in the policy to be adopted in providing libraries for the new and recently created universities—a challenge which he urged confronted not only the newly appointed librarians and library authorities, but no less their vice-chancellors, the University Grants Committee and the Treasury. Dr. Myres thought that there was grave risk that, in spite of apparently generous initial grants, the assumption might be made that the main requirement was to provide for the current needs of undergraduate study with a limited range of standard text-books, periodicals and works of reference, and that, for anything outside that range, recourse should be had to the borrowing facilities already available either regionally or nationally from older and better stocked collections. Admittedly these new university libraries will require all the assistance they can obtain in their early years from outside sources, and may take many years to create for themselves even the basic minimum stock which will justify their existence as learned libraries

Even if Dr Myres is a little pessimistic as to the time required for that purpose, he is fully justified in his assertion that the notion that the needs of these libraries beyond a certain point ought not to be satisfied permanently, or for a moment longer than is absolutely essential, by reliance on borrowing from existing libraries, even from national loan collections such as the National Lending Library for Science and Technology or an equivalent institution in the humanities that may be created, is a dangerous delusion Dr Myres does not decry or minimize the value of such facilities He insists, nevertheless, that such facilities should never be regarded as an adequate substitute for the possession on the spot of books that are needed on that spot Moreover, no university library worthy of the name will ever be formed on the principle that anything it requires but does not possess need not be bought if it can be borrowed from elsewhere

That principle needs assertion to-day and not simply in relation to the expansion of existing or the creation of new university libraries but in regard to the development of an efficient and economically sound national library system It is of the first importance that the new Library Committee should bring home to the University Grants Committee that the only sound policy for a university library, on grounds of economy no less than efficiency, is one that aims at providing within its own walls its own copies of everything which the members of the university need for the promotion of their own studies or the advancement of learning. Reasons of space or scarcity may impose the use of modern copying methods in place of originals, but that does not invalidate the principle, and clear thinking on this point is a first essential

But the principle reaches further and it is two-edged University libraries are important participants in the National Central Library's present system, and most university librarians can tell of requests for the loan of books and other publications the cost of which is sometimes no more than that of the postage and packing involved Government departments are notorious offenders in this respect, and unless a sense of proportion obtains and due vigilance is exercised, efficient loan systems could founder through unreasonable demands There is already some concern over the policy adopted by the National Lending Library for Science and Technology, and this uneasiness was not removed by the talk which Miss Webb gave to Aslib last May on the new National Library of Science and Invention, of which she is to be in charge

The new Library is to restrict its holding of periodicals to those less than 50 years old, the old volumes being transferred to the British Museum Library or to the National Lending Library for Science and Technology With the discontinuance of a loan service from the remains of the Science Museum Library in London, access to the older periodicals, particularly open access, will be considerably more limited or impeded This may be a serious matter for those working on the history of science, and it has its bearing on the extent of the stocks which a university library should hold and on the validity of a policy based on the alleged fact that a periodical article is only consulted once on average Averages are misleading and even worthless where the scholar is concerned even if the basis of compilation is not open to challenge The only sound basis for determining policy as regards stocks of older periodicals and books, whether for loan or access, is in relation to a national scheme which takes full account of the resources of all national institutions, including those of the learned societies and universities, as well as of the public libraries One cannot, for example, at the same time assert that insufficient use is made of existing knowledge and claim that the present records of use are necessarily a sound guide to future requirements

Dr Myres, in the concluding part of his address, was particularly concerned about the acquisition of research material in the face of American demand and resources It was the situation there that led him to conclude reluctantly that there was no will, in the quarters that control Britain's policy on the finance of higher education to provide the support which alone can build new university libraries worthy of the name, or enable the older ones to maintain their proper status in the world of learning, but there is little evidence to suggest that his fears generally are unjustified The unwillingness of the University Grants Committee to earmark grants for library purposes for fear of interfering with academic freedom is scarcely justifiable to-day, and if the appointment of the new Library Committee means anything it should point a way around that difficulty Certainly, while Dr Myres was urging the Library Association itself to use some of its own influence to impress those responsible for public policy in this matter, his words should not escape the attention of either the new Committee or the Minister for Science himself

Nor is it simply a matter of books and of buildings and equipment Of vital importance to the quality of the service given by a university library is the ability of its staff from the librarian downward The grounds for disquiet are no less substantial here While in the statutes for the new University of Strathclyde (see *Nature*, **199**, 1137, 1963) the university librarian is given the status of professor and a place on the Senate, only a handful of universities as yet accord such recognition to their librarian The scales advocated by the University Grants Committee are honoured in the breach rather than in the observance Even if the university librarians generally were placed in the professorial grade with the appropriate salaries, many of them would still find it almost impossible to attract and retain staff of the appropriate ability and enthusiasm in the existing salary situation—a position which, it should be noted, is equally characteristic of much of the public library service

A satisfactory library service for the universities and for the nation generally depends in some measure on a more generous and enlightened attitude to the librarians and their staff As part of such a policy, a more rational attitude may well be required to the organization of library work so that specially trained or skilled staff is not wasted excessively on duties which require no special professional knowledge or aptitude Here, the responsibilities of professional bodies are involved and they have essential contributions to make, and for that reason alone the Minister of Education can scarcely allow difficulties over the reorganization of local government to delay indefinitely the action which was recommended more than four years ago in the Roberts Report on the *Structure of the Public Library Service in England and Wales* (see *Nature*, **183**, 1289, 1437, 1959) Two reports have since been submitted to him, one on *Inter-Library Co-operation* *in England and Wales*[*] and one on *Standards of Public Library Service in England and Wales*[†], each by a working party set up to examine the technical implications of the Roberts Report

The report concerned with standards scarcely requires discussion here, though it should be noted that this working party stresses the need for an objective approach to the public library and its duties and once again emphasizes the importance of the public library as a great and developing national asset This conception is fundamental to any adequate survey of the functions which public libraries should be expected to perform and to any reasonable national library system, of which such libraries form an essential part Further, the working party recommends specifically that the public library should work in close association with the libraries of local technical colleges and of other educational institutions and with specialized libraries in other organizations It should be obvious, too, that the standards recommended by the working party on the grounds of library efficiency should contribute also to the effectiveness of the inter-library co-operation in England and Wales with which the second working party was concerned

The report of this working party recommends first that the existing eight regions of the library service should be reduced to four or five, each self-sufficient in certain types of material, and the Minister of Education, Sir Edward Boyle, indicates in a foreword dated December 1962 that he is asking the proposed advisory councils for England and Wales to consider the most appropriate relation between the English and the Welsh regions The report also recommends the replacement of the National Committee on Regional Library Co-operation by a joint committee appointed by these proposed Advisory Councils and reporting through them on all aspects of library co-operation It is also recommended that the regional councils and the National Central Library should consider the extent to which the holdings of university and other special libraries should be incorporated in regional catalogues as well as at the National Central Library

It is in respect of the National Central Library that the report touches more particularly on those aspects of a national system that are of most interest to the university librarian or to the scientist, and it is here also that some dissent is recorded by members of the working party The working party visualizes public library authorities as being made responsible in the foreseeable future for 30 per cent of the expenditure of the National Central Library It recommends consideration of the possibility of transferring responsibility for the Government grant to the Library from the Treasury to the Minister of Education as well as that the estimates of the Library should be prepared tri-annually The last proposal seems sound, but the idea that responsibility for the Library's financial resources might be placed alongside that for the National Lending Library for Science and Technology or the new National Library of Science and Invention does not appear to have been considered

There is, however, a logical argument for that course pointing, moreover, to the Minister for Science, and in line with this is Mr F G B Hutching's note of dissent regarding the cost of the National Central Library, the whole of which, in his view, should be met by the Treasury He rejects the suggestion of the earlier Roberts Report that half the income of the National Central Library should come from sources other than the Treasury, pointing out that the Library is primarily a means by which books throughout the country are made available outside their locality The closest analogy is not with the National Library of Scotland, as the Roberts Report suggested, but with the National Lending Library for Science and Technology, which is entirely supported by Government funds Mr D T Ricknell also insists that financial responsibility for developing the work of the Library should lie with the Treasury and adds that the whole service provided is national in character and its further development should be regarded as a national responsibility

These notes of dissent seem to be reinforced by the working party's finding that no case has yet been established for a new National Lending Library for the Humanities to undertake work which would be mainly an extension of what the National Central Library is already doing It recommends instead that the resources of the National Central Library for the purchase and storage of books should be considerably increased to enable it to remove many of the difficulties of research and supply in the field of the humanities The position would clearly be improved by a more generous policy in regard to university libraries generally, both in respect of the service which they are able to render to the members of their own community and the co-operation they are able to offer the National Central Library itself

Meanwhile, these reports substantiate essentially Dr Myres's plea for some more generous and realistic policy towards the university libraries both as such and as part of an adequate national system They indicate the weakness of attempts to deal piecemeal with the system and the extent to which the responsibilities of the Minister of Education in this field are interlocked with those of the Minister for Science That point emerges very clearly also from the background paper on science, economic growth and Government policy presented to the Ministerial Meeting on Science in Paris, as indeed in the report on *Science and the Policies of Government*, which was prepared by the Advisory Group on Science Policy of the Organization for Economic Co-operation and Development for the same meeting (see *Nature*, 200, 199, 1963) The importance of the work of the Library Committee appointed by the University Grants Committee and the Ministry of Education could not be better demonstrated, and in this context the Minister for Science may well be expected to take an even greater interest than the Minister of Education

BIOLOGY OF THE SEAS OF THE U.S.S.R.

Biology of the Seas of the U S S R
By Prof L Zenkevitch Translated by S Botcharskaya Pp 955 (London George Allen and Unwin, Ltd , 1963) 160s net

THE oceanic coasts of the U S S R are washed by Atlantic, Arctic and Pacific waters This vast country also surrounds, wholly or in part, the greatest and the most interesting and productive of all brackish water and inland seas the Baltic, Black Sea, Sea of Azov, Caspian and Aral Seas No other country has direct interest in seas so widely distributed or so diverse in salinity Apart from the Baltic, the Barents Sea, the Sea of Japan and the Bering Sea, in all of which other countries have made and continue to make major contributions, investigation of this large section of the seas of the world is entirely in the hands of Russian scientists working at 44 institutes and from more than 20 research vessels

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[*] Ministry of Education *Inter-Library Co-operation in England and Wales* Report of the Working Party appointed by the Minister of Education in March 1961 Pp v+34 (London H M S O , 1962) 3s net

[†] Ministry of Education *Standards of Public Library Service in England and Wales* Report of the Working Party appointed by the Minister of Education in March 1961 Pp vii+124 (London H M S O , 1962) 7s net.

In this magnificent book, described in his preface as "a considerably amended and supplemented version of the second edition of my book *The Fauna and Biological Productivity of the Sea*, published in 1947", Prof L Zenkevitch reveals how great are the contributions which he and his fellow workers have made to the science of oceanography These enable him to paint a comprehensive picture of the conditions of life and the factors controlling productivity in the 13 seas to which he gives separate consideration The Russian approach to biological oceanography has always been quantitative As an inevitable consequence of the vast areas with which they are concerned, they have had to pay initial attention to the wood as a whole, leaving detailed consideration of the individual trees until later It is the biomass of plankton, of invertebrate benthos and of fishes and the controlling effect of regenerated or inflowing nutrients which form the dominant and always fascinating themes of this book

The subject-matter is divided into northern, southern and far-eastern seas In each a preliminary account covering general characters is followed by chapters dealing with the individual seas History of exploration is followed by descriptions of geology, including submarine topography, hydrography, the nature and origin of the fauna and flora and productivity in terms of plankton and benthos, leading finally to consideration of commercial fisheries

In the northern seas we begin with the Barents Sea with its rich fauna of mixed Atlantic and Arctic origin and great productivity associated with the vertical mixing of its waters along the polar front We then progress eastward by way of the White, Kara, Laptev and Chukotsk Seas By stages the influence of the Atlantic is reduced both qualitatively and quantitatively—in the White Sea the average benthic biomass drops to one-fifth The less-saline waters of the Kara Sea contain a unique high Arctic relict brackish water fauna Finally, incursion from the Pacific comes to influence the fauna of the Chukotsk Sea An admirable account of the Baltic concludes these descriptions of northern seas

The southern seas are the enclosed seas and also those least known outside the U S S R No marine or fresh-water biologist, indeed no biologist interested in the effect of environment on life or on the distribution of living things, can fail to find matters of compelling interest in these chapters on the Black Sea Sea of Azov, Caspian and Aral Seas In his introductory chapter, Prof Zenkevitch traces the history of these inland seas and the origins of their fauna and flora To a relict community of marine Tertiary origin, much influenced later by exposure to fresh waters, have been added immigrants from fresh waters, from the Arctic and, more significantly in the Black Sea and Sea of Azov, recent invasions from the Mediterranean Although impoverished these faunas may be immense With a mere fraction of the faunal variety of the Mediterranean, the Sea of Azov has a far greater productivity

Even in the Black Sea, despite lack of oxygen in its depths, a postulated vertical circulation implies far greater productivity than has been suspected In the Caspian the immense inflow of nutrients especially from the Volga has been more fully utilized in terms of eventual production of fish by the deliberate introduction of *Nereis diversicolor* (not *N succinea* as originally thought) and other benthic invertebrates Mussels, barnacles, diatoms, fish and prawns, even a medusa and a crab from America, have all of recent years found their way into these receptive waters

The final section, dealing with the Sea of Japan, Sea of Okhotsk and the Bering Sea, has been added in this edition Here we are in the richest as well as in the deepest ocean Zones of benthic life are considered from the supralittoral to the profound depths of the Kuril-Kamchatka Trench The greater speed of growth of the Pacific, as compared with the Atlantic, cod is related to the much richer benthos and also to the shorter migrations between feeding and spawning grounds The densest populations of herring in the world occur in the Sea of Japan where, after the milt has been shed, the waters are smoothed as though oil has been poured on them Perhaps the richest of all feeding grounds for demersal fish and crabs lie on the western Khamchatka shelf The existence of an amphi-boreal fauna of recent origin can "be considered", Prof Zenkevitch writes, "as an immense experiment in acclimatization by Nature herself—a conquest of vast new habitats, often more spacious than the original ones" It is an experiment which is being emulated by Soviet biologists responsible for movement of Pacific salmon and crabs to north Atlantic waters

No short review can do anything approaching justice to this book, which opens windows on to such wide prospects of scientific knowledge It is written by a complete master of the subject and has been extremely well translated by Sophia Botcharskaya, there is only one obscure passage near the bottom of p 101 It contains 427 most illuminating figures with 329 tables, and concludes with some 50 pages of references and an excellent index I would regard this as the most important book on marine biology since the publication in 1912 of Murray and Hjort's *Depths of the Ocean* C M YONGE

MORE DEVELOPMENTS IN PLANT ANALYSIS

Moderne Methoden der Pflanzenanalyse

Begründet von K Paech und M V Tracey Fortgeführt von H F Linskens und M V Tracey 6 Band unter mitarbeit von B D Sanwal Bearbeitet von verschiedenen Fachgelehrten Pp xxiv + 512 (Berlin Springer-Verlag, 1963) 98 D M

THIS volume is the second of the supplementary volumes in the series *Modern Methods of Plant Analysis* As evidence of its quality, it is sufficient to say that it maintains the high standard set by Volume 5 The present number includes an extensive section on the general methods of enzyme chemistry, in which the new editorial combination of H F Linskens and M V Tracey was assisted by B D Sanwal In all, there are 24 separate articles, 14 in German and 10 in English

Some of the articles, although useful, require little comment since they are essentially extensions to the range of compounds covered in the earlier volumes Thus, W Heinen deals with silicon compounds, H Stern with the determination of sulphydryl groups, U Beiss with phosphatides and glycolipids, F Bohlmann and W Sucrow with naturally occurring acetylene compounds, and M Hesse and H Schmid with natural chromones Other articles deal with products characteristic of individual species or plant groups Thus, R Braun describes the production, isolation and quantitative determination of orchinol, an antibiotic formed by the tubers of a number of orchid species following incubation with the fungus *Rhizoctonia repens*. An article by J R Hudson deals with the humulones, lupulones and other constituents of hops S Shibata gives a comprehensive account of lichen substances, illustrated by 6 photomicrographs of crystals of lichen acids R Braun contributes an account of the toxins produced in plants by pathogenic micro-organisms, and J Tobiška deals with phytoagglutinins F Zilliken and R Lambert describe the isolation and analysis of bacterial cell walls

In the section dealing with enzymology, there are articles by W. Heinen on the detection of enzymatic activity and on the general characterization of an enzyme, by W. Heinen and H F Linskens on the Thunberg technique, by B D Sanwal on general methods of preparation and on enzymatic assays of amino-acids and keto-acids, by H G Boman and W Bjork on general aspects of enzymatic purification and characterization, by H G Boman on the purification of enzymes, by F Bendall on the inhibition and activation of enzymes, by G Pfleiderer on the enzymatic determination of metabolites, and by E Hofmann on the analysis of enzymes in soil Individual groups of enzymes are dealt with in the next volume, No 7 in the series

As in the previous volume, certain articles will be of special interest to many plant physiologists In his account of kinetin and kinetin-like compounds, C O Miller discusses the merits of the various bio-assay systems available An article by R Knapp on gibberellins includes accounts of their chemical properties, of their occurrence in plants and of the chemical, physico- chemical, and biological methods available for their detection

There is little need to stress the great value of this volume, and indeed of the entire series, to those concerned with almost any aspect of plant analysis All the favourable comments made in the earlier review of Volume 5, about the printing and general production of the book, apply equally to the present volume

A Allsopp

POLYCHAETE PHYSIOLOGY

Annelids

By R. Phillips Dales Pp 200 (London Hutchinson and Co (Publishers), Ltd, 1963) 15*s*

This book does not live up to the expectations of its title which could, with minor deletions, have been, more appropriately, "Aspects of the Physiology of Polychaetes"

Following a comprehensive introduction there are nine chapters, the first treating the general organization of the body with a proper emphasis on segmentation, and on the origin and fate of the cœlom and mesodermal tissues Thereafter there follow seven chapters, which, though sufficient in their coverage of polychaete physiology and reproduction and development, pay but lip-service to the existence of any other group of Annelida or other branches of zoology

If Chapter 3 on the evolution of polychaetes is aimed at the student population it can have but scant appeal If students can make their way through the maze of family group names they will deplore the absence of substantiated fact What is the point of telling them that the goniadids, the Sphaerodoridae and Pisionidae are derivatives of Nephthydidae and Glyceridae without, at least, extensive morphological comparisons, with the aid of diagrams, in view of the total lack of supporting embryological and palæontological evidence? This type of ill-considered assertion is repeated throughout this chapter Indeed, if a chapter on the evolution of Polychaeta is necessary—which I doubt—it is incorrect to say, "there are no fossils worth mentioning" Very large populations of polychaete jaws are known from the Ordovician and the great variety of the polychaete fauna of the Cambrian Burgess shales, and the information on the past history of the group these provide should have been mentioned

Chapter 9 deals with the origin and classification of the oligochaetes and leeches Presumably this is to make up for having given them comparatively little attention previously Students should note that the body of a leech is made up of 34 segments, 7 of which contribute to the ventral anal sucker, not 33 and 6 as on p 181. Reference is made in this chapter to the excellent and comprehensive text-book by K H Mann, *Leeches* (Pergamon Press, 1962), as a source for the identity of British leeches, and readers should know that this work in fact provides a key for the identification of genera of marine leeches throughout the world and species of fresh-water leeches from central Europe, Britain and North America

A surprising omission is that no chapters have been included on distribution, either ecological or zoogeographical Much recent and comprehensive work on marine pollution, encrustation, and zonation together with extensive soil and forest-fauna surveys have thus been overlooked, which would have been welcomed by student and research worker alike.

Yet, perhaps, the major omission is that the important chromosome studies by Omodeo and Muldal on lumbricid earthworms have been reduced to a nebulous sentence of some nineteen words (p 179) and their respective works not even included in the bibliography Finally, the one other place where reference to chromosomes appears (p 182) refers briefly to the leech work by Wendrowsky, which is also omitted from the bibliography

N. Tebble

AN OUTLINE OF HISTOCHEMISTRY

Histochemistry

Theory, Practice and Bibliography By Tibor Barka and Paul J Anderson Pp x + 660 (New York and London Hoeber Medical Division, Harper and Row, Publishers, Inc, 1963) 18 50 dollars

The authors have set out to provide a general outline of histochemistry, a concise practical and theoretical guide to histochemical methods, and a detailed bibliography of methods and their applications The bibliography is fairly extensive, consisting of almost 5,000 references, and in fact forms more than one-third of the whole book With the rapid growth of the subject this cannot fail to be of great value to workers in the field of histochemistry

In the early part of the book the general techniques for preparation of tissue are surveyed, and cryostat, freeze-drying, and freeze-substitution methods are described In the case of the cryostat it is stated that "serious morphological alterations are always encountered" (p 7) In spite of this, in the section devoted to demonstration of succinic dehydrogenase, an enzyme confined to mitochondria, it is stated that "cryostat sections of fresh tissue are necessary for the histochemical reaction" Moreover, it is known that cryostat sections can be regularly prepared in which neither mitochondria nor lysosomes show evidence of morphological damage (Bitensky, L, *Brit J Cancer*, 14, 696 (1960), *Ciba Found Symp on Lysosomes*, 362, Churchill, London, 1963) The authors realize that in histochemistry a critical attitude is necessary, but no indication of the particular standards is given In discussing the methods for hydrolytic enzymes, it is surprising that only passing reference is made to the work of De Duve, that it only relates to acid phosphatase, and fails to mention the importance of the lysosome with reference to all acid hydrolases On p 205 a list of factors which may interfere with the demonstration of a particular enzyme is given Yet, on p 238 in the section dealing with acid phosphatase, techniques are recommended that result in at least 50 or 60 per cent inactivation of the enzyme If strict criteria are to be observed, then such methods should surely be regarded as useless Again, on p 238 it is stated that "their demonstration (i e, acid phosphatases) in cryostat sections is unrealistic, even if hypertonic or 'osmotically protected' incubating solutions are employed" Other workers have found that localization of acid phosphatase can be constantly demonstrated within the lysosomes in cryostat sections of normal tissues which have been suitably treated.

In a work of this kind, which is presumably intended to encourage workers in histochemistry, it is disappointing to find that the authors regard the subject in such a narrow fashion. It would appear that their initial approach is purely morphological, and that is certainly no disadvantage. When it is stated that "deriving its theoretical foundations from chemistry, histochemistry remains a morphological tool" (p. 342) one feels that the extensive possibilities of the subject remain unexplored by the authors. This is decidedly surprising for much attention has been directed to the demonstration of enzymes which are essentially dynamic in nature. Again on p. 342 they say that "finite measurement is not the immediate goal of microscopic histochemistry". The large number of biochemical comparisons made in recent years between normal and abnormal tissues have served to emphasize that while qualitative differences do exist they are greatly outnumbered by quantitative variations. It is hard to see how these differences can be identified if quantitative histochemistry is not developed. Biochemistry has done much to advance our knowledge of the cell, but a precise localization of chemical components can be achieved only by histochemical methods. Whether the method of study be the demonstration of carbohydrates, lipids, proteins, or enzymes, or the rate of uptake of labelled metabolites, the aim is the same, namely, to piece together our information on the metabolism of the cell. At the present time it is too much to hope that one discipline will provide the answer to any one problem, but it is quite certain that histochemistry in its future and fuller development will play a vital part in the understanding of cellular physiology and pathology. It would indeed be sad if it merely demonstrated a number of new structures, the function of which could not be determined.

GEORGE J. CUNNINGHAM

VIRUSES

Advances in Virus Research

Vol. 9. Edited by Kenneth M. Smith and Max A. Lauffer. Pp. viii + 312. (New York and London: Academic Press Inc., Publishers, 1962.) 92*s*.

ONE-THIRD of this number of *Advances in Virus Research* is occupied by an article by St. Groth called "The Neutralization of Viruses". The article is in fact more limited than this for it deals almost exclusively with the influenza viruses. This makes it rather specialized, but it is written in a lively and colourful manner so that the reader is carried along in the stream in spite of possible disagreement with many of the *obiter dicta*. The influenza viruses are an admirable vehicle for carrying the general argument for they have an unusual number of measurable activities—infectivity in several hosts, toxicity, an enzymic activity, and hæmagglutination—all of which are inhibited by suitable antibodies. St. Groth discusses the peculiarities and limitations of each type of measurement, the effects on them of virus and antibody heterogeneity, and, when possible, the mechanism of the action. Wisely, each section is followed by a summary so that the reader unfamiliar with work of this type can keep track of what he has been told.

The scope of the article by Porterfield on the arthropod-borne viruses, that is the viruses that can multiply in both mammalian and arthropod hosts as distinct from those that are transmitted mechanically by arthropod hosts, is somewhat similar. The article is factual rather than interpretative; it shows the complexity of the antigenic structure of the viruses and the variation in the hosts' responses to them. Non-specialists would have understood the article more fully if the editors had explained the terminology, especially the system of units used. It ends with the comforting conclusion that there is no good evidence for the appearance of new viruses in this group though the antigenic components may regroup in suitable conditions.

The causative agents in a group of clinically rather ill-defined infections of the respiratory tract, and of some organs in the abdomen and elsewhere, in man and animals are grouped together as the adenoviruses. Electronmicrograms of several members of the group show icosahedrons with rounded corners. Brandon and McClean discuss both the structure of the virus particles and the reactions of the infected host to them. The story is fascinatingly complex with a cytopathic protein made by the infected host, at least three antigens that seem to be fragments of the intact virus and, in long-established infections, intranuclear crystals that seem to be virus aggregates. As the authors point out, this multiplicity of effects makes the group peculiarly suitable for an investigation of the general nature of virus infection.

The article by Smith is more general in scope. He gives a great deal of information on what might be called the natural history of the arthropod viruses and shows that many of the old ideas about them are ill-founded. They are by no means as host-specific as used to be thought, several can multiply in both insects and plants and cause pathological changes in both hosts, and latency is so widespread as to render all claims for the chemical induction of new virus infections suspect. The last phenomenon is particularly interesting because an uncongenial environment, or even a change of diet, can make the latent virus patent. There seem to be no general differences in morphology or chemical composition between arthropod viruses and those of plants or mammals, but the arthropod host seems able to tolerate an unusually large amount of virus before it finally succumbs—the only analogies with this extensive replacement of host tissue by virus is found in some of the infective mammalian warts, and in nitrogen-deficient plants.

Markham contributes a cheerful article on the information that can be gained about viruses by ultracentrifugation. He stresses that the instrument is easy to use, the calculations easy to make, and that a great deal can be found out with even one run on unpurified material. There is much shrewd and entertaining comment here. Those who were engaged in the early work on tobacco mosaic virus assumed that linear aggregation would have little effect on sedimentation constant; they will therefore be surprised to learn that the fact that this is so is unexpected.

Ten years ago, few virus workers could tell a regular from a rhombic dodecahedron; but we are all Platonists nowadays and the icosahedron is our juju. The significance of the regular solids (or the helix) is, however, widely misunderstood. These structures are likely to be common because they offer unusual opportunities for the formation of a stable shell. Speaking anthropomorphically, for the sake of brevity, the two main problems confronting a virus are: to find a system in a host that can be deluded into synthesizing virus, and to be stable enough to avoid the host's scavenging systems. We may therefore expect certain structures to appear often, as caffeine appears in at least 14 families of plants and the benzene ring (probably) in all organisms, though it serves many purposes and is made in many different ways. That is a reaction to the final article, by Andrewes, on the classification of viruses. We classify from a point of view and with an end in view. At one point Andrewes agrees and says "taxonomy is an artificial business"; he seems nevertheless to be searching for a classification that will command the general agreement that Linnean classification gets. But satisfactory Linnean classification summarizes the course of evolution, and there is no reason to assume that viruses, except within closely related groups, have undergone a similar process.

N. W. PIRIE

TECHNOLOGY AND ECONOMICS AND THEIR RELATION TO PUBLIC POLICY*

By The Right Hon LORD ROBBINS, C.B., F.B A

Chairman of the Committee on Higher Education

In common with the Rector of the Imperial College of Science and Technology and nine other colleagues, I have already expressed my views on the subject of higher education and its future† I therefore offer here a few reflexions on technology and economics in relation to public policy I shall deal first with technology *per se*, then with its relations with economics, then their joint status in regard to public policy, and finally briefly with the use of skills and knowledge they involve in the conduct of public business

I do not need to spend time on the immense direct increase in our power to satisfy our various wants which is due to technical progress One has only to travel in some backward area where the major operations of agriculture and construction are still done by hand or beast to realize how much we owe to the machine in this respect It is more interesting, I suggest, to contemplate the subtler influence on social structure and social relations which are due to technical change We have all heard of the social changes which have followed the discovery of methods of production bringing together large masses of workpeople in towns and in big factory installations The influence on contemporary politics of the introduction of television is a matter of common observation But I wonder how many people have reflected on the immense change in the social position of women which is due to the internal combustion engine—an invention which has equalized the power of the sexes to control the lighter types of vehicle and thus freed the physically weaker from dependence in this respect on husbands or coachmen

Such influences are so continuous and so widespread that some have gone so far as to claim for them an almost exclusive status as ultimate historical explanation The history of tools is the history of mankind, it is said. But although this may be a very healthy corrective to the histories which tend to explain all change in terms of the influence of kings or priests or politicians, it certainly goes too far, in regard to both detail and broad outline Our tastes are not all conditioned by technical availabilities, it is a significant circumstance that the age of the electric razor should have witnessed a proliferation of beards The arts are not exclusively determined by present-day modes of production, in spite of certain museum labels of the Stalin era, the paintings of Cezanne are not "a characteristic product of the period of heavy industry" The broad structure of human society is certainly not only a by-product of mechanical convenience, the division of the Western World into independent sovereign States and all the ups and downs which are the consequences thereof are tragic examples of the fact that influences other than technology play their part in human destiny It is true that the growth of States was aided by the invention of gunpowder, and it may be that the invention of the hydrogen bomb will eventually induce their consolidation, but if this happens—and the outcome is by no means certain—a great deal will have occurred in between which, in any sane view, cannot be interpreted in terms of technological influences

But whether or not technology has determined the course of history in the past, it is sometimes said that it ought to determine it in the future I do not think the technologists have often made this claim they are usually much better balanced personalities than those who romanticize them But the idea of a world planned and run by technologists has swum before the eyes of many social visionaries, from St. Simon onwards One of the most forceful expressions of this occurs in Thorstein Veblen's *The Engineers and the Price System*, where, speaking of the modern industrial system, he asserts "It is a mechanically organized structure of technical processes designed, installed and conducted by production engineers Without them and their constant attention the industrial equipment, the mechanical appliances of industry, will foot up to just so much junk The material welfare of the community is unreservedly bound up with the due working of this industrial system and therefore with its unreserved control by the engineers, *who alone are competent to manage it*" [my italics]

I am quite sure that this is a mistake As I shall be arguing later, I am very much in favour of the increased employment of technologists in high places But it will not be their knowledge as technologists which will determine the ultimate decisions For the ultimate decisions involve problems which technology as such is quite unable to solve

Consider for a moment a very simple example—the problem of housing construction Let us assume for purposes of discussion that the supply of houses is on a public basis so that there are no complications arising from the relation between the State and private enterprise, and let us assume further that the amount of house-room to be provided has already been decided There remains, therefore, the question what techniques to adopt, and at first sight this might appear to be entirely technological But this is not so The techniques of house construction run through an almost infinite range of possibilities, from those which provide mud huts to those which provide buildings of almost limitless durability, from the provision of bare utility to the last sophistications of opulence Now, clearly, if no other considerations were involved, we should opt for maximum durability and maximum amenity, and, æsthetics apart, the information we needed would be technological But actually we are at once confronted with the hard fact that the different kinds of labour and materials involved are also involved in hundreds of alternative forms of production, in factory construction, in shipbuilding, in the production of tractors and weapons, to say nothing of road building and agriculture, and that, in conditions of reasonably full employment, there are not enough to satisfy all these demands to the point of satiety Hence, if the general production plan is to be rational, account must be taken of such other uses Just to give complete priority to the production of housing units of the maximum durability and convenience would not be sensible The choice of techniques in any one branch of production must therefore depend on the general availability of resources and their allocation between different alternatives Now there is no purely technological calculus which can solve the allocation problem There is no technological principle which by itself can tell us how much steel or how much labour to allot to this branch of industry and that

It is here that economics has a contribution to make —although, as I shall be arguing later on, it is only a contribution, not a final solution For it is one of the specific tasks of the economists to study the distribution of resources which are in limited supply; and it is his business, given appropriate information regarding the possible range of techniques on one hand, and the conditions of demand for possible products on the other, to devise criteria of appropriate allocation. Given information of this sort, he ought to be able to show what proportions of existing resources should be allocated to house building and consequently which of the various techniques available it would be most suitable to adopt I would not wish to be thought to be saying that he can do this, for a host of difficulties is concealed in the assumption that the relevant information is available But such at least is the kind of problem and the kind of solution with which his prescriptions are concerned

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* Substance of the address delivered by Lord Robbins as Special Visitor to the Imperial College of Science and Technology, University of London, at the annual Commemoration Day ceremony in the Royal Albert Hall on October 24 (see also p 632 of this issue)

† Committee on Higher Education Higher Education Report of the Committee appointed by the Prime Minister under the Chairmanship of Lord Robbins Pp xvi+335 (Cmnd 2154) (London H M S O, 1963) 15s net

Now the last thing I should want to do would be to suggest that all that scientists and technologists do is to supply parameters for our equations On a long view of history, who can question that technologists have done far more for humanity than anything which economists have been able to do up to date? If the technologists like this way of putting things better, they can simply say that the other factors which the economic calculus has to take into account are simply so many necessary constraints on their perfection I certainly would not quarrel with that at all, it would be just another way of bringing out the point that I am trying to make that the two kinds of consideration, though related, are not identical and that technical optima are not necessarily economic optima. For the technical optimum in any line of production is achieved when, given the existing state of knowledge, the construction is as efficient as can be conceived, regardless of cost But the economic optimum is essentially related to cost—what you go without when you do one thing rather than another, it involves a balance of alternatives not technically related Hence, technical and economic optima can only coincide in a world in which there are no costs—that is to say, in a world in which the economic problem has ceased to exist This, to put it mildly, is some way ahead

But economics too has its limits As we have seen, if it is told what people want and in what quantities at different prices, it can combine this information with the information it gets from the various technologists, so as to depict the most economical use of the available resources But it does not tell us why people want things, or whether they ought to do so Think for a moment of the problem of steel allocation Some of the steel will be wanted for goods which eventually find their way to the market, and if we are content with the results in that sector of the economy of allowing people to spend their money according to their fancy, then market demands can be taken as ultimate data But some steel will doubtless be wanted for the production of weapons and warlike stores and some for the construction of schools and hospitals, and in such cases there is no market in the final sense, and the decision what to produce and how much is essentially one which is a matter of political judgment

In the case of defence, this is obvious But it is no less true with education and health Attempts may be made to measure the commercial return on teaching and hospital treatment and, in their way, they have their uses But, if taken too seriously, they tend to ignore social consequences which are not at all susceptible of such measurement, yet which in the long run are even more important Even in the market sector, the decision to leave things to the play of individual and group demand is also in essence political It involves both an implicit acceptance of the existing distribution of power to spend and an acceptance of the view that in this sphere the individuals concerned are the best judges of what is good for them I do not say that this is wrong On the whole, where private goods are concerned, I personally prefer markets to regulation and where the distribution of wealth is held to be unsuitable, I prefer taxes and subsidies to rationing All that I am trying to drive home is that, even here, economics is open-ended and by itself furnishes no final guide to policy Equally with technology, although on a different plane, it is instrumental rather than ultimate

If this is true, it follows that there are no easy solutions. Neither technology nor economics can provide the answers to the ultimate questions Where are we going ? What do we want to do ? In so far as these can be answered in general terms, we must still depend on moral and political philosophy and on the vision of inspired thinkers In so far as day-to-day business is concerned, we are still at the mercy of what Adam Smith described as that "insidious and crafty animal, vulgarly called a statesman or politician, whose counsels are directed by the momentary fluctuations of affairs" They may be a somewhat disillusioning conclusion, yet it is difficult to see how it can be avoided In the last analysis decisions regarding human destiny rest on volitions and valuations which do not lie within the compass of either of our respective techniques

But at this point it is important that I should avoid misapprehension on the part of readers The fact that neither technology nor economics in themselves can afford ultimate guidance concerning social choice and social action does not in the least mean that in coming to decisions of this sort the information they do provide can be sensibly neglected The political philosopher who speculates on what shapes and contents of the desirable society is likely to waste a good deal of time unless he takes account of what shapes and contents of societies are *possible*—as we can see from the intellectual wreckage of many philosophical Utopias and the pure futility of much of the exhortation which we have to endure from such quarters Moreover, the insidious and crafty animal seeking to control "the momentary fluctuations of affairs" is also likely to land himself in all sorts of difficulties if he does not pay at least some heed to the technological and economic limitations of alternative stratagems

Indeed, in a complex society such as ours, resting on advanced technology and involving the most intricate and far-reaching economic relationships, the statesman who would seek to make fundamental decisions without some knowledge and advice of this sort is about as likely to succeed as a would-be artist who would take it on himself to carry out some vast decoration without a knowledge of the properties of pigments or the elements of perspective and drawing. He may succeed as some artists so deficient have succeeded But the success is inevitably in the nature of a fluke and carries with it no guarantee of continuity Or, if I may vary my simile, he is like a would-be pilot of a modern aircraft who attempts to fly without knowledge of the capacity of his craft or appropriate navigational aids It is true that neither aeronautical engineering nor navigational instruments will solve the ultimate questions where he wants to go But, given his ultimate goal, he will be lucky indeed if he gets there without instrumental knowledge

It is, therefore, a matter of considerable concern to all of us to see that the conduct of those who rule over us is sufficiently informed by technological and economic knowledge As I see things, there is a two-fold problem here There is the problem of ensuring that there is available in the machinery of Government a sufficiency of expert knowledge of this sort, and there is the further problem of seeing that the conduct of Government as such is sufficiently informed by an awareness of the importance of such knowledge and its relevance to ultimate decisions so that it is called into operation in the appropriate context

At the present day the first of these problems does not seem any longer to present insurmountable difficulties Doubtless, there are all sorts of questions of detail which still await solution, and I should be the last to say that our provision in this respect is adequate—it is still deficient in both fields But the need for technological and economic advice as such seems to me to be fairly generally acknowledged, and we must not complain too much of a certain time-lag in meeting it, though we should all do our best in our respective ways to help to abbreviate it We must be careful, too, in making public criticisms, that we get our facts right. It is a sad comment on the quality of public discussion of these matters that, at a time when various publicists are denouncing the poverty in this respect of the public service in general and the Treasury in particular, more than half the top administrators in the Treasury have degrees or similar qualifications as economists

It is the second problem which worries me more the problem how to bring it about that the general conduct of public affairs and the state of mind of those who rule over us is sufficiently informed of the relevance of technology and economics and the powerful assistance they can give I think this problem is very important If the quality of actual discussion in this respect is compared to what it might be, it is all too painfully obvious what a long way there is to go But I do not see any simple answer As I have tried to show earlier, I do not think we shall get anywhere by pitching our claims too high—by contending that either technology or economics holds the key to human destiny For that would be false, and to rely on that kind of argument would simply provide yet another pretext to the Philistines for not taking us at our true worth In the long run I should hope much from the presence in *non-specialized* jobs in the public service and business of more persons with a technological or economic educational background; the moral value of that sort of training with its objectivity and its humility *vis-à-vis* the ultimate problems of the universe is still often insufficiently appreciated I think also that much can be done by patient participation by technologists and economists in the public discussion of questions to which their expert knowledge is germane The business of politics is much too serious nowadays to be left to the politicians I hope too that, as time goes on, teaching in schools and universities will come to convey even to those who do not aspire to be experts in these fields some idea of their general relevance and the part they can play in human progress

But this brings me to the threshold of a subject which at the outset I declared it my intention to avoid I will conclude, therefore, by expressing my conviction that, in the creation of this broader understanding and this more enlightened outlook, the Imperial College of Science and Technology has a notable part to play, and my firm confidence that it will not fail to rise to the opportunity

INTERGROUP SELECTION IN THE EVOLUTION OF SOCIAL SYSTEMS

By Prof V. C. WYNNE-EDWARDS
Department of Natural History, University of Aberdeen

IN a recent book[1] I advanced a general proposition which may be summarized in the following way (1) Animals, especially in the higher phyla, are variously adapted to control their own population densities (2) The mechanisms involved work homeostatically, adjusting the population density in relation to fluctuating levels of resources; where the limiting resource is food, as it most frequently is, the homeostatic system prevents the population from increasing to densities that would cause over-exploitation and the depletion of future yields. (3) The mechanisms depend in part on the substitution of conventional prizes, namely, the possession of territories, homes, living space and similar real property, or of social status as the proximate objects of competition among the members of the group concerned, in place of the actual food itself (4) Any group of individuals engaged together in such conventional competition automatically constitutes a society, all social behaviour having sprung originally from this source

In developing the theme it soon became apparent that the greatest benefits of sociality arise from its capacity to override the advantage of the individual members in the interests of the survival of the group as a whole The kind of adaptations which make this possible, as explained more fully here, belong to and characterize social groups as entities, rather than their members individually This in turn seems to entail that natural selection has occurred between social groups as evolutionary units in their own right, favouring the more efficient variants among social systems wherever they have appeared, and furthering their progressive development and adaptation

The general concept of intergroup selection is not new It has been widely accepted in the field of evolutionary genetics, largely as a result of the classical analysis of Sewall Wright[2-4]. He has expressed the view that "selection between the genetic systems of local populations of a species . . has been perhaps the greatest creative factor of all in making possible selection of genetic systems as wholes in place of mere selection according to the net effects of alleles"[4] Intergroup selection has been invoked also to explain the special case of colonial evolution in the social insects[5-7]

In the context of the social group a difficulty appears, with selection acting simultaneously at the two levels of the group and the individual It is that the homeostatic control of population density frequently demands sacrifices of the individual, and while population control is essential to the long-term survival of the group, the sacrifices impair fertility and survivorship in the individual. One may legitimately ask how two kinds of selection can act simultaneously when on fundamental issues they are working at cross purposes At first sight there seems to be no easy way of reconciling this clash of interests, and to some people consequently the whole idea of intergroup selection is unacceptable

Before attempting to resolve the problem it is necessary to fill in some of the background and give it clearer definition. The survival of a local group or population naturally depends among other things on the continuing annual yield of its food resources. Typically, where the tissues of animals or plants are consumed as food, persistent excessive pressure of exploitation can rather quickly overtax the resource and reduce its productivity, with the result that yields are diminished in subsequent years This effect can be seen in the overfishing of commercial fisheries which is occurring now in different parts of the world, and in the overgrazing of pastoral land, which in dry climates can eventually turn good grassland into desert Damage to the crop precedes the onset and spread of starvation in the exploiting population, and may be further aggravated by it The general net effect of over-population is thus to diminish the carrying-capacity of the habitat

In natural environments undisturbed by man this kind of degradation is rare and exceptional the normal evolutionary trend is in the other direction, towards building up and sustaining the productivity of the habitat at the highest attainable level Predatory animals do not in these conditions chronically depress their stocks of prey, nor do herbivores impair the regeneration of their food-plants Many animals, especially among the larger vertebrates (including man), have themselves virtually no predators or parasites automatically capable of disposing of a population surplus if it arises

It is the absence or undependability of external destructive agencies that makes it valuable, if not in some cases mandatory, that animals should be adapted to regulate their own numbers By so doing, the population density can be balanced around the optimum level, at which the highest sustainable use is made of food resources

Only an extraordinary circumstance could have concealed this elementary conclusion and prevented our taking it immediately for granted Some eight or more thousand years ago, as neolithic man began to achieve new and greatly enhanced levels of production from the land through the agricultural revolution, the homeostatic conventions of his hunting ancestors, developed there as in other primates to keep population density in balance with carrying-capacity, were slowly and imperceptibly allowed to decay We can tell this from the centrally important place always occupied by fertility-limiting and functionally similar conventions in the numerous stone-age cultures which persisted into modern times[8] Since these conventions disappeared nothing has been acquired in their place growing skills in resource development have, except momentarily, always outstripped the demands of a progressively increasing population, there has consequently been no effective natural selection against a freely expanding economy. So far as the regulation of numbers is concerned, the human race provides a spectacular exception to the general rule

A secondary factor, tending to obscure the almost universal powers possessed by animals for controlling their numbers, is our everyday familiarity with insect and other pests which appear to undergo uncontrolled and sometimes violent fluctuations in abundance In fact, human land-use practices seldom leave natural processes alone for any length of time vegetation is gathered, the ground is tilled, treated or irrigated, single-species crops are planted and rotated, predators and competitors are destroyed, and the animals' regulating mechanisms are thereby, understandably, often defeated In the comparably drastic environmental fluctuations of the polar and desert regions, similar population fluctuations occur without human intervention

The methods by which natural animal populations curb their own increase and promote the efficient exploitation of food resources include the control of recruitment and, when necessary, the expulsion and elimination of unwanted surpluses The individual member has to be governed by the homeostatic system even when, as commonly happens, this means his exclusion from food in the midst of apparent plenty, or detention from reproduction when others are breeding The recruitment rate must be determined by the contemporary relation between population density and resources, under average conditions, therefore, only part of the potential fecundity of the group needs to be realized in a given year or generation

This is a conclusion amply supported by the results of experiments on fecundity versus density in laboratory populations, in a wide variety of animals including Crustacea, insects, fish and mammals, and also by field data from natural populations[1]. But it conflicts with the assumption, still rather widely made, that under natural selection there can be no alternative to promoting the fecundity of the individual, provided this results in his leaving a larger contribution of surviving progeny to posterity[9] This assumption is the chief obstacle to accepting the principle of intergroup selection

One of the most important premises of intergroup selection is that animal populations are typically self-perpetuating, tending to be strongly localized and persistent on the same ground This is illustrated by the widespread use of traditional breeding sites by birds, fishes and animals of many other kinds, and by the subsequent return of the great majority of experimentally marked young to breed in their native neighbourhood It is true of non-migratory species, for example the more primitive communities of man, and all the long-distance two-way migrants that have so far been experimentally tagged, whether they are birds, bats, seals or salmon, have developed parallel and equally remarkable navigating powers that enable them to return precisely to the same point, and consequently preserve the integrity of their particular local stock Isolation is normally not quite complete, however Provision is made for an element of pioneering, and infiltration into other areas; but the gene-flow that results is not commonly fast enough to prevent the population from accumulating heritable characteristics of its own. Partly genetic and partly traditional, these differentiate it from other similar groups

Local groups are the smallest racial units capable of continuous existence for long enough to undergo evolutionary differentiation In the course of generations some die out, others survive, and have the opportunity to spread into new or vacated ground as it becomes available, themselves subdividing as they grow In so far as the successful ones take over the habitat left vacant by the unsuccessful, the groups are in a relation of passive competition Their survival or extinction is partly a matter of chance, arising from various forms of *force majeure*, including secular changes in the environment, for the rest it is determined, in general terms, by heritable qualities of fitness

Gene-frequencies within the group may alter as time passes, through gene-flow, drift (the Sewall Wright effect), and selection at the individual level Through the latter, adaptations to local conditions may accumulate Population fitness, however, depends on something over and above the heritable basis that determines the success as individuals of a continuing stream of independent members It becomes particularly clear in relation to population homeostasis that social groups have highly important adaptive characteristics in their own right

When the balance of a self-regulating population is disturbed, for example by heavy accidental mortality, or by a change in food-resource yields, a restorative reaction is set in motion If the density has dropped below the optimum, the recruitment rate may be increased in a variety of ways, most simply by drawing on the reserve of potential fecundity referred to earlier, and so raising the reproductive output Immigrants appearing from surrounding areas can be allowed to remain as recruits also If the density has risen too high, aggression between individuals may build up to the point of expelling the surplus as emigrants, the reproductive rate may drop, and mortality due to social stress (and in some species cannibalism) may rise These are typical examples of density-dependent homeostatic responses

Seven years of investigation of the population ecology of the red grouse (*Lagopus scoticus*) near Aberdeen, by the Nature Conservancy Unit of Grouse and Moorland Ecology, have revealed many of these processes at work[10] Their operation in this case depends to a great extent on the fact that individual members of a grouse population living together on a moor, even of the same sex, are not equal in social status Some of the cock birds are sufficiently dominant to establish themselves as territory owners, parcelling out the ground among them and holding sway over it, with a varying intensity of possessiveness, almost the whole year round During February–June their mates enjoy the same established status

In the early dawn of August and September mornings, after a short and almost complete recess, the shape of a new territorial pattern begins to be hammered out In most years this quickly identifies a large surplus of males, old and young, which are not successful in securing any part of the ground and consequently assume a socially inferior status They are grouped with the hens at this stage as unestablished birds, and day by day their security is so disturbed by the dawn aggressive stress that almost at once some begin to get forced out, never to return By about 8 a m each day the passion subsides in all but the most refractory territorial cocks, after which the moor reverts to communal ground on which the whole population can feed freely for the rest of the day. As autumn wears on and turns to winter, the daily period of aggression becomes fiercer and lasts longer; birds with no property rights have to feed at least part of the time on territorial ground defended by owners that may at any moment chase them off. More and more are driven out altogether; and since they can rarely find a safe nook to occupy elsewhere in the neighbourhood, they become outcasts, and are easily picked up by hawks and foxes, or succumb to malnutrition. Females are included among those expelled, but about February the remaining ones begin to establish marital attachments, and at the same time, quite suddenly, territories are vigorously defended all day. Of the unestablished birds still present in late winter, some achieve promotion by filling the gaps caused by casualties in the establishment. Some may persist occasionally until spring, but unless a cock holds a territory exceeding a minimum threshold capacity, or a hen becomes accepted by a territorially qualified mate, breeding is inhibited.

Territories are not all of uniform size, and on average the largest are held by the most dominating cocks. More important still, the average territory size changes from year to year, thus varying the basic population density, apparently in direct response to changes in productivity of the staple food-plant, heather (*Calluna vulgaris*). As yet this productivity has been estimated only by subjective methods, but significant mutual correlations have been established between annual average values for body-weight of adults, adult survival, clutch-size, hatching success, survival of young, and, finally, breeding density the following year. As would be expected with changing densities, the size of the autumn surplus, measured by the proportions of unestablished to established birds, also varies from year to year.

There are increasing grounds for concluding that this is quite a typical organization, so far as birds are concerned, and that social stratification into established and unestablished members, particularly in the breeding season, is common to many other species, and other classes of animals. In different circumstances the social hierarchy may take the form of a more or less linear series or peck-order. Hierarchies commonly play a leading part in regulating animal populations, not only can they be made to cut off any required proportion of the population from breeding, but also they have exactly the same effect in respect of food when it is in short supply. According to circumstances, the surplus tail of the hierarchy may either be disposed of or retained as a non-participating reserve if resources permit.

It is not necessary here to explore in detail the elaborate patterns of behaviour by which the social hierarchy takes effect. The processes are infinitely varied and complex, though the results are simple and functionally always the same. The hierarchy is essentially an overflow mechanism, continuously variable in terms of population pressure on one hand, and habitat capacity on the other. In operation it is purely conventional, prescribing a code of behaviour. When a more dominant individual exerts sufficient aggressive pressure, usually expressed as threat although frequently in some more subtle and sophisticated form, his subordinates yield, characteristically without physical resistance or even demur. It may cost them their sole chance of reproduction to do so, if not their lives. The survival of the group depends on their compliance.

This has been taken as an example to illustrate one type of adaptation possessed by the group, transcending the individuality of its members. It subordinates the advantage of particular members to the advantage of the group, its survival value to the latter is clearly very great. The hierarchy as a system of behaviour has innumerable variants in different species and different phyla, analogous to those of a somatic unit like the nervous or vascular system. Like them, it must have been subject to adaptive evolutionary change through natural selection, yet it is essentially an 'organ' of a social group, and has no existence if the members are segregated.

A simple analogy may possibly help to bring out the significance of this point. A football team is made up of players individually selected for such qualities as skill, quickness and stamina, material to their success as members of the team. The survival of the team to win the championship, however, is determined by entirely distinct criteria, namely, the tactics and ability it displays in competition with other teams, under a particular code of conventions laid down for the game. There is no difficulty in distinguishing two levels of selection here, although the analogy is otherwise very imperfect.

The hierarchy is not the only characteristic of this kind. There are genetic mechanisms, such as those that govern the optimum balance between recombination and linkage, in which the benefit is equally clearly with the group rather than the individual. Without leaving the sphere of population regulation, however, we can find a wide range of vital parameters, the optima of which must similarly be determined by intergroup selection. Among those discussed at length in the book already cited[1] are (1) the potential life-span of individuals and, coupled with it, the generation turnover rate, (2) the relative proportions of life spent in juvenile or non-sexual condition (including diapause) and in reproduction, (3) monotely (breeding only for a single season) versus polytely, (4) the basal fecundity-level, including, in any one season, the question of one brood versus more than one.

These and similar parameters, differing from one species or class to another are interconnected. Their combined effects are being summed over the whole population at any one time, and over many generations in any given area. It is the scale of the operation in time and space that precludes an immediate experimental test of group selection. An inference that may justifiably be drawn, however, is that maladjustment sufficient to interfere persistently with the homeostatic mechanism must either cause a progressive decline in the population or, alternatively, a chronic over-exploitation and depletion of food resources: in the end either will depopulate the locality.

There still remains the central question as to how an immediate advantage to the individual can be suppressed or overridden when it conflicts with the interests of the group. What would be the effect of selection, for example, on individuals the abnormal and socially undesirable fertility of which enabled them and their hereditary successors to contribute an ever-increasing share to future generations?

Initially, groups containing individuals like this that reproduced too fast, so that the overall recruitment rate persistently tended to exceed the death-rate, must have repeatedly exterminated themselves in the manner just indicated, by overtaxing and progressively destroying their food resources. The earliest adaptations capable of protecting the group against such recurrent disasters must necessarily have been very ancient; they may even have been acquired only once in the whole of animal phylogeny, and in this respect be comparable with such basic morphological elements as the mesoderm, or, perhaps, the cœlom. Once acquired, the protective adaptations could be endlessly varied and elaborated. It is inherently difficult to reconstruct the origin of systems of this kind, but genetic mechanisms exist which could give individual breeding success a low heritability, or, in other words, make it resistant to selection. This could be relatively simply achieved, for example, if the greatest success normally attached to heterozygotes for the alleles concerned, creating the stable situation characteristic of genetic homeostasis[11].

A more complex system can be discerned, as it has developed in many of the higher vertebrates where the breeding success of individuals is very closely connected with social status. This connexion must necessarily divert an enormous additional force of selection into promoting social dominance, and penalizing the less fortunate subordinates in the population that are prevented from breeding or feeding, or get squeezed out of the habitat. Yet it is self-evident that the conventional codes under which social competition is conducted are in practice not jeopardized from this cause selection pressure, however great, does not succeed in promoting a general recourse to deadly combat or treachery between rivals, nor does it, in the course of generations, extinguish the patient compliance of subordinates with their lot

The reason appears to be that social status depends on a summation of diverse traits, including virtually all the hereditary and environmental factors that predicate health, vigour and survivorship in the individual. While this is favourable to the maintenance of a high-grade breeding stock, and can result in the enhancement through selection of the weapons and conventional adornments by which social dominance is secured, dominance itself is again characterized by a low heritability, as experiments have shown In many birds and mammals, moreover, individual status, quite apart from its genetic basis, advances progressively with the individual's age Not only are the factors that determine social and breeding success numerous and involved, therefore, but the ingredients can vary from one successful individual to the next A substantial part of the gene pool of the population is likely to be involved and selection for social dominance or fertility at the individual level correspondingly dissipated and ineffective, except in eliminating the sub-standard fringe

Such methods as these which protect group adaptations, including both population parameters and social structures, from short-term changes, seem capable of preventing the rise of any hereditary tendency towards anti-social self-interest among the members of a social group Compliance with the social code can be made obligatory and automatic, and it probably is so in almost all animals that possess social homeostatic systems at all In at least some of the mammals, on the contrary, the individual has been released from this rigid compulsion, probably because a certain amount of intelligent individual enterprise has proved advantageous to the group In man, as we know, compliance with the social code is by no means automatic, and is reinforced by conscience and the law, both of them relatively flexible adaptations.

There appears therefore to be no great difficulty in resolving the initial problem as to how intergroup selection can override the concurrent process of selection for individual advantage. Relatively simple genetic mechanisms can be evolved whereby the door is shut to one form of selection and open to the other, securing without conflict the maximum advantage from each, and since neighbouring populations differ, not only in genetic systems but in population parameters (for example, mean fecundity[12]) and in social practices (for example, local differences in migratory behaviour in birds, or in tribal conventions among primitive men), there is no lack of variation on which intergroup selection can work.

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GEOMAGNETIC PARAMETERS AND PSYCHIATRIC HOSPITAL ADMISSIONS

By PROF HOWARD FRIEDMAN and PROF ROBERT O. BECKER

Syracuse Veterans Administration Hospital, New York, and State University of New York Upstate Medical Center

AND

PROF. CHARLES H. BACHMAN

Syracuse University, New York

ALTHOUGH the relationship of natural environmental physical forces to human behaviour has always been a matter of general interest and speculation, there has been a paucity of systematic investigations in this connexion among modern scientific workers Buettner[1], in a critique of the literature bearing on correlations between bioclimatological parameters and human health and behaviour, pointed to the general confusion in the field as well as to the very limited unequivocal findings consisting of the establishment of two 'potentially harmful' weather types The literature in the field of geomagnetic parameters and human behaviour is more restricted in quantity and in significance of findings

Possibly the most ambitious pertinent investigation is that of Dull and Dull[2] In an analysis of approximately 40,000 cases over a period of 60 months they demonstrated graphically a clearly observable relationship between the 67 magnetic storms occurring during this time, and the incidence of nervous and mental diseases and suicides Their results, however, were not subjected to statistical treatment Berg[3], reviewing the literature pertaining to the relationships between environmental geophysical parameters and the human population, concluded that firm evidence for or against such relationships is still lacking

The relative indifference of the behavioural scientists to this problem may possibly be due to the lack of that type of theoretical framework which could permit the interrelationship of the two parameters to be derived as a logical consequence There has been, however, a growing appreciation of, and body of empirical findings related to, one circumscribed aspect of the overall area, the biological effects of magnetic fields[4–6], particularly with reference to infra-human organisms A parallel related concern has been the investigations into the electrical organizations of the living organism Thus, in a review of recent work in the relationship between direct current electricity and living organisms[7], it was suggested that the direct-current electrical system of the organism could be conceived as a control system which could be influenced by external force fields These considerations led to a pilot investigation[8] which examined the relationship between gross manifestations of extreme human psychological disturbance and natural magnetic field intensity The psychiatric admissions to two Syracuse, New York, hospitals were correlated with K-index[9] sums as measured at the Fredericksburg Magnetic Observatory, Virginia, for approximately a four-year period The statistically significant correlation ratios obtained for non-linear relationships, η, of 0 26 and 0 27 suggested that at least some relationship exists between the incidence of psychiatric disturbance and some geophysical parameter associated with the magnetic field

The investigation reported here is an extension of the pilot work utilizing a larger sample of psychiatric hospital admissions and, as advised by Bartels[10], a linear measure of the magnetic field intensity, ak, the equivalent 3-h range, rather than the quasi-logarithmic scale of K-indices In addition, ap, the planetary counterpart to ak was used

Daily admissions to seven central New York State psychiatric hospitals and to the Psychiatric Service of a Veterans Administration General Medical and Surgical Hospital during July 1, 1957–October 31, 1961, were obtained Transfers from other psychiatric institutions were excluded Total admissions included in the data are 28,642

For the same period of time K-indices, as determined at the Fredericksburg Magnetic Observatory, were obtained from the Coast and Geodetic Survey, U S Department of Commerce The K-index, as described by Bartels[10], reflects the amplitude, a, of the most disturbed component of the magnetic field, measured every 3 h The values of a are customarily condensed, for convenience of reporting, into a quasi-logarithmic scale for K, ranging from 0 to 9 In this work each 3-h K-index was reconverted into the equivalent 3-h range, ak, by means of a table of Bartels[10], thus providing a linear measure The eight daily values of ak were then summed to arrive at a daily ak sum

The planetary 3-h K-indices, Kp, as furnished by the U S Department of Commerce, Bureau of Standards, Central Radio Propagation Laboratory, Boulder, Colorado, were also reconverted into 3-h equivalent planetary amplitudes, ap, and then summed to obtain a daily ap sum

It was decided to examine 7-, 14-, 21-, 28- and 35-day time-periods to correlate with the corresponding periods of the geomagnetic parameters for the following reasons First, the time between onset of psychiatric disturbance necessitating hospitalization and actual admission varies with a host of uncontrollable factors, not the least of which are differing hospital admission procedures It is also impossible to specify *a priori* the most meaningful duration and extent of geomagnetic event Secondly, inspection of the hospital admission data readily revealed one artefact among the many possible contributors to the number of uncontrolled variables in the crude measure of this parameter Every Sunday was marked by a striking drop in admissions, undoubtedly reflecting differing admission policies and procedures The selection of the aforementioned time-periods thus provided a constant number of Sundays when all the possible 7-, 14-, 21-, 28- and 35-day intervals were examined

To obtain the 7-, 14-, 21-, 28- and 35-day values, the number of admissions for every possible consecutive 7-, 14-, 21-, 28- and 35-day period was summed and correlated with the geomagnetic parameter sums for the corresponding periods Thus 7-day periods yielded 7 correlations obtained from using days 1 through 7, 8 through 14, 15 through 21, etc , for the first correlation, then days 2–8, 9–15, 16–22, etc , for the second correlation, and continuing on in similar fashion to the seventh correlation which used days 7–13, 14–20, 21–27, etc This provided 3 correlations with 226 pairs of comparisons (N), and 4 with 225 pairs The other time-periods were treated similarly, thus giving 3 correlations with N's of 113 and 11 correlations with N's of 112 for the 14-day periods, 10 correlations with N's of 75 and 11 with N's of 74 for the 21-day periods 17 correlations with N's of 56 and 11 with N's of 55 for 28-day periods, and 10 correlations with N's of 45 and 25 with N's of 44 for the 35-day periods

In order to compare admissions to the greater intensities of geomagnetic disturbance, a listing of the principal magnetic storms from July 1, 1957, until October 31, 1961, identified by the Fredericksburg Magnetic Observatory, Virginia, was obtained from reports contained in the *Journal of Geophysical Research* and *International Geophysical Year Bulletins* The number of days of storm in the 7-, 14-, 21-, 28- and 35-day periods were then correlated with the hospital admissions for the corresponding time-periods

The product moment correlation coefficients, r, derived from the comparison of total geomagnetic activity parameters, as reflected in ak and ap, with hospital admissions are negligible in magnitude regardless of time-period used. Table 1 presents the correlation coefficients obtained from the comparison of the 7-, 14-, 21-, 28- and 35-day periods of hospital admissions with the number of days of magnetic storm for the corresponding periods of time The correlations between the number of days of magnetic storm and admissions for 7-day periods can be considered as negligible The 14 correlation coefficients obtained by using the 14-day periods range from +0 077 to +0 221, the latter figure being statistically significant ($P < 0\ 05$) The correlations of the 21-day periods range from +0 116 to +0 305 with 5 statistically significant ($P < 0\ 05$) coefficients The 28-day correlations range from +0 167 to +0 332, and 13 are statistically significant ($P < 0\ 05$) The 35-day correlation coefficients range from a low of +0 222 to an unexpected high of +0 345, and 11 are statistically significant ($P < 0\ 05$)

Table 1 CORRELATIONS BETWEEN DAYS OF MAGNETIC STORM AND PSYCHIATRIC HOSPITAL ADMISSIONS

Admission periods	No of r's	Range	Median r
7-day	$3(N = 226) + 4(N = 225)$	−0 010–+0 122	+0 043
14-day	$3(N = 113) + 11(N = 112)$	+0 077–+0 221	+0 117
21-day	$10(N = 75) + 11(N = 74)$	+0 116–+0 305	+0 182
28-day	$17(N = 56) + 11(N = 55)$	+0 167–+0 332	+0 261
35-day	$10(N = 45) + 25(N = 44)$	+0 222–+0 345	+0 279

The use of unscreened heterogeneous hospital admissions as a measure of alteration in human behaviour is admittedly crude The host of uncontrolled variables makes this at best a gross approximation to psychological disturbance in the human population at large If one becomes intrigued with ætiological considerations there are then the further problems of the unknown time-period between occurrence of geomagnetic event and its significant effect on the organism, and the specification of the duration and extent of meaningful geomagnetic events Since the purpose of this work was simply to investigate whether any relationship could be found to exist between geomagnetic parameters and human behaviour, it was hoped that some of the effects of the uncontrolled variables in the behavioural parameter could be crudely minimized by randomization within a large sample, and that the problem of unknown time for effect of geomagnetic event could be attenuated in part by the use of 7-, 14-, 21-, 28-, and 35-day time-periods The results indicate that statistically significant low to marked linear relationships exist between geomagnetic parameters and a gross measure of human disturbance These relationships are evident when the measure of the geomagnetic parameter is restricted to those periods of higher disturbance which can be categorized as magnetic storms The total range of of geomagnetic activity, as reflected in planetary (ap) and more local (ak) natural magnetic field intensity parameters, does not reveal any significant relationship with psychiatric hospital admissions

The use of the five time-periods provides no definitive information about the optimal time between geomagnetic event and onset of psychological disturbance or time to achieve hospital admission However, inasmuch as the magnitude of the maximum correlation tends to increase with the size of the time-period used it suggests in part, that the optimal time may be closer to thirty-five days than seven days In part, it probably also reflects the attenuation of transient artefacts inherent in the hospital admission data

Although correlational investigations shed but little light on causal relationships, they nevertheless do provide a fertile field which can yield more significant testable hypotheses In view of the diversity of the two variables under investigation and the grossness of the measures, the present investigation offers correlations of a surprising magnitude In general, the tentative conclusion of the pilot study can be reaffirmed a significant relationship has been shown between psychiatric disturbance as reflected in hospital admissions and natural magnetic field intensity In any interpretation of this relationship it should be cautioned that it is beyond the scope of this investigation to determine whether the more meaningful geophysical parameter is magnetic field intensity *per se*, or some other geophysical parameter intimately associated with it Speculatively, the results are in keeping with the conception of the behaviour of an organism being significantly influenced, through the direct-current control system, by external force fields Attention is thus invited to a hitherto neglected dimension in the complexity of psychopathology specifically, and perhaps generally in all human behaviour

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SATELLITE GRAVITY MEASUREMENTS AND CONVECTION IN THE MANTLE

By Prof S K. RUNCORN

Physics Department, University of Newcastle upon Tyne, and Jet Propulsion Laboratory, California Institute of Technology

THE discovery of non-axial and odd axial harmonic terms in the Earth's gravitational field from surface gravity observations[1] and, more recently, from the orbits of satellites[2], has been widely interpreted as showing that the Earth's mantle possesses a finite strength and that in consequence the occurrence of convection currents in the mantle is excluded The argument is that if the mantle acts as a fluid over periods of millions of years and convection currents are occurring in it, the continents should lie over the descending currents Munk and MacDonald[3] compared the coefficients of the spherical harmonics which represent the distribution of the oceans and continents with those of the gravity observations and found that no correlation exists, so that a strong argument against convection seemed to have been found (Jeffreys[1] had shown, on any reasonable isostatic hypothesis, that the gravitational attraction of the continental masses would be annulled by the deficiencies of mass supporting them below to about a milligal)

Because of their wave-lengths, the sources of the low harmonic gravity anomalies are reasonably located in the deeper parts of the mantle[4] These gravity anomalies

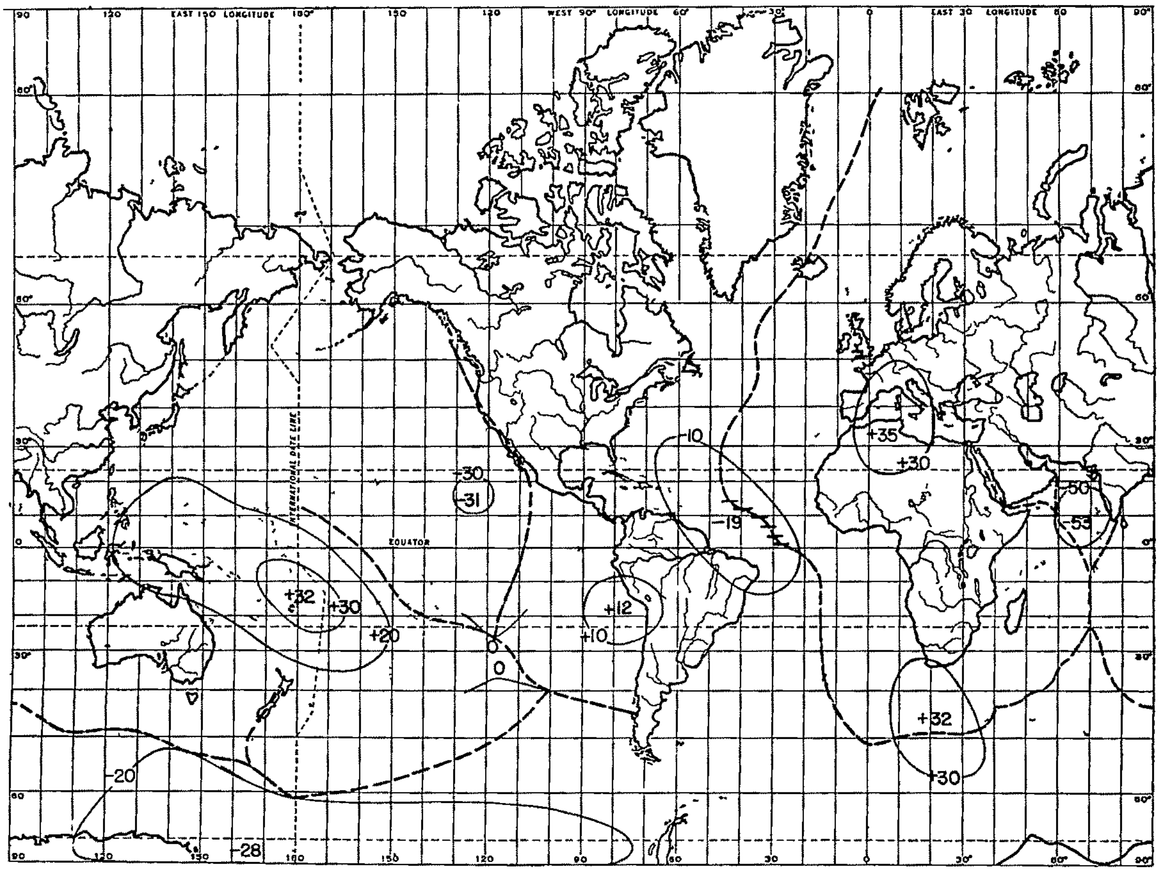

Fig 1 Dotted lines are the ocean rises[9], thin lines are contours of geoid surface[10] (in metres) (numbers given in centres of contour show positions and values of gravity highs and lows)

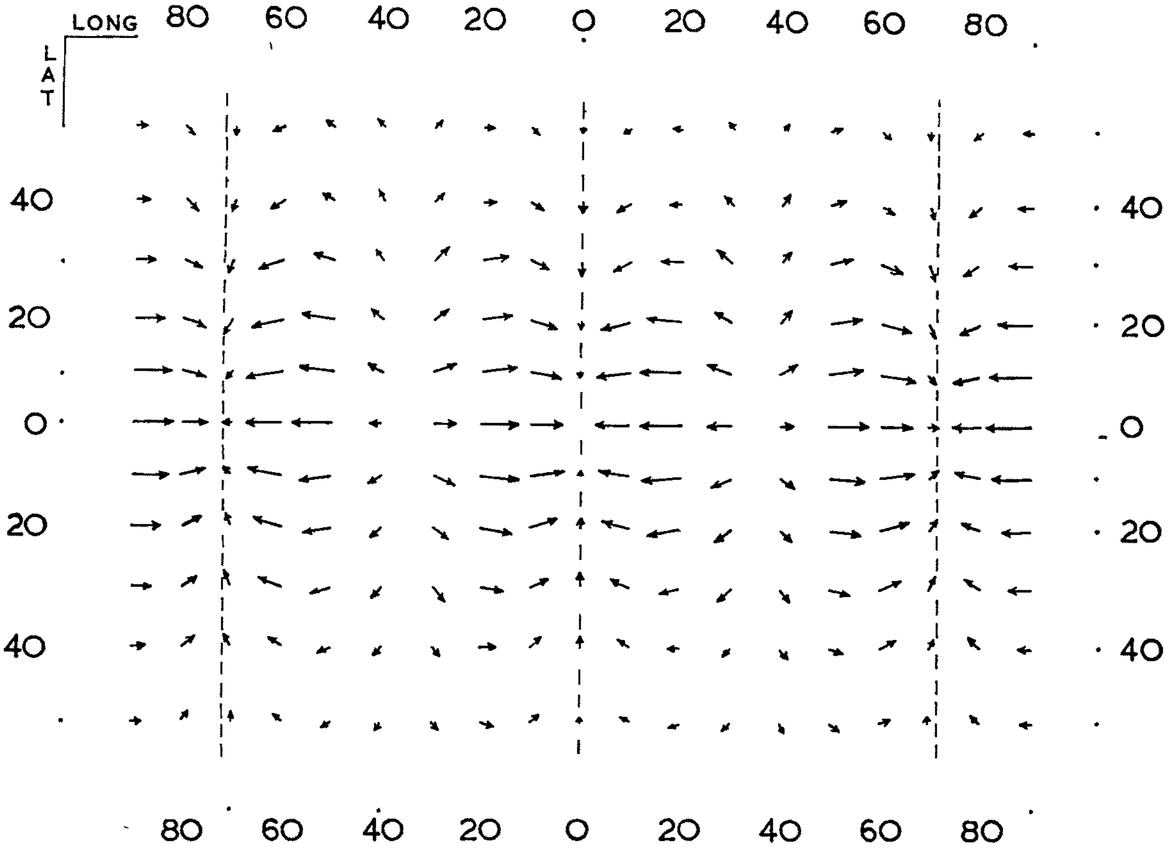

Fig 2. Horizontal flow distribution for a fifth degree sectorial harmonic convection current. Horizontal velocity components are proportional to lengths of arrows

must arise from deep-seated inequalities of density in the mantle, which, if flow were to be ruled out because of the finite strength of the mantle, result from events in the remote past of the Earth. For example, it has been recently suggested[5] that they are the scars left in the Earth by the breaking away of the Moon. This line of reasoning depends on a belief that the interior of the Earth can retain strains over 10^9 years. Until I recently suggested an alternative explanation[6], there seemed to be incontrovertible support for this view from the case of the Moon, the differences in the moments of inertia of which are thirty times those to be expected if the Moon were in hydrostatic equilibrium. This non-hydrostatic bulge towards the Earth of about 2 km is interpreted by Jeffreys[1] as a fossil tidal bulge, left from the period (10^9 years ago) when the Moon was much closer to the Earth. The internal stress difference required to support this bulge is 20 bars. It does not seem to have been recognized by the proponents of these views that their explanation of these departures from hydrostatic equilibrium in the interiors of the Moon and Earth (that the mantles have finite strength over 10^9 years) invokes remarkable mechanical properties. To cause the bulge on the Moon to subside completely, a strain of 10^{-3} would be required. If this has not occurred in 3×10^9 years, it means that the creep rate must have been less than 10^{-20} sec^{-1}. Thus what is demanded of the Earth and Moon are creep rates of less than 10^{-20} sec^{-1} under stresses of about 5 per cent of the breaking stress of rocks at ordinary temperatures. It is remarkable that our geophysical colleagues have not made widely known to engineers their technological breakthrough in discovering a material which, at elevated temperatures, has a creep rate only one millionth millionth of those encountered in the laboratory.

The explanation of the low degree harmonics of the Earth's gravitational field and the bulge on the Moon may more plausibly be sought in thermal convection[7,8]. An order of magnitude argument establishes the reasonableness of this solution for the Earth. I[7] showed that if a value for the viscosity of the mantle is taken of 10^{21} poise, and the velocities of the convection currents are inferred from the rates of displacement of the continents the temperature differences between the rising and falling streams on the equipotential surfaces are of the order of 1/3° C. Taking a volume coefficient of expansion for the mantle of 3×10^{-5} per °C, the deficiency of mass per unit surface in the uprising volume compared with the downgoing one is about 5×10^3 g/cm^2. This gives a gravity anomaly of the order of 10^{-2} cm/sec^2, or 30 m in geoid height, which are the values given by Jeffreys and those who have reduced the satellite gravity observations (see Fig 1).

But, as already explained here, Munk and MacDonald's negative correlation of the topography of the Earth with the satellite gravity anomalies has been a stumbling block. Further, although convection has been used to explain the ocean ridges, the convection currents being supposed to rise under the ridges, it has not been clear what relationship the satellite gravity observations have to these essentially linear features. It is the purpose of this communication to point this out.

Convection currents in a sphere or spherical shell may best be described using spherical harmonic functions $S_n(\theta,\varphi)$ when θ is co-latitude and φ is the longitude. The northward and eastward horizontal velocity components are proportional to $\frac{Sd_n}{d\theta}$ and $\frac{1}{\sin\theta}\,\frac{dS_n}{d\varphi}$ respectively. As an example, Fig. 2 shows the horizontal velocity pattern for the fifth degree sectorial harmonic, which is thought to be prominent in the present flow in the mantle[7,11]. It will be seen that there are five lines of longitude from which flow diverges: these would be lines along which tension would develop causing the formation of the central rifts in the rises, which have been found in the Atlantic and

Carlsberg ridges. But, because of the fact that the divergence of the velocity vector is zero, the components of flow along a ridge line cannot be zero all along the ridge. This is clear from Fig. 2, which shows that along the ridge in the southern hemisphere there is a southward component of flow parallel to the ridge and in the northern hemisphere a northward component. Only on the equator at intervals of 36° of longitude does the horizontal flow completely vanish. These are above the main rising and falling columns of fluid.

It is thus necessary to determine from the ocean bottom topography where these points are before examining if they have any relation to the negative and positive gravity anomalies. The ocean rise is therefore a line from which flow diverges but having, in general, a component of flow along it. The positions of the rising columns are therefore to be located at the intersection of two oceanic rises. For at this point the foregoing condition for an ocean rise, that is, that the velocity perpendicular to it is zero, results in zero horizontal velocity. Fig. 1 shows the distribution of the ocean rises after Menard[9]. There are five clear cases where the ridges bifurcate or sharply change direction: (1) the intersection of the Pacific Antarctic rise with the rise running northwards into New Zealand, (2) the branching of the mid-Indian Ocean rise, (3) the join of the north and south Atlantic rises, associated with the east–west fractures near the equator, (4) the join of the East Pacific rise with the Pacific Antarctic rise, and (5) the northern extremity of the East Pacific rise in the Gulf of California.

Izsak[10] has recently published a map showing the 10-m contours of the satellite geoid heights. These are ovals surrounding four points of maximum and five of minimum gravity. Fig. 1 shows the nine positions of these maxima and minima, their magnitudes and the contours surrounding them. It will be seen that a good correlation exists between the five points of minimum gravity and the five points of uprising currents determined from the topography. As this satellite gravity map is based on only those harmonic terms up to and including the fourth degree and as the fifth is of equal importance with the third and fourth in the spherical harmonic expansion of the Earth's topography, the correlation is as good as it is reasonable to expect. Doubtless the inclusion of higher harmonic terms will move the points of maximum and minimum gravity to some extent.

On this view we would naturally associate the positive anomalies with the descending currents and therefore with zones of compression. The two zones of positive anomaly in south Europe and the Andes agree with this expectation very well. The elongated positive anomaly between the mid-Pacific and the East Indies[12] covers a region of strong compression discovered by Vening Meinesz and the Tonga trench. The positive anomaly south of South Africa is of considerable interest, for little is known of the topography of the ocean floor in this region and points the need for investigation there.

It will at once be objected that the Tertiary mountain chains other than the Alps and Andes are not associated with positive anomalies. However, we must recall that on the theory of continental drift some continents have moved considerable distances in the past million years. Palæomagnetic evidence suggests that both India and Australia have moved considerable distances northward in this time. Both are now north of negative gravity anomalies and will still be experiencing a further northward force which may explain the Himalayas. Similarly, North America lies between two regions of uprising currents which must be exerting compressive forces which may have a fundamental connexion with the Rocky Mountain orogenies.

I thank Dr. H. W. Menard and Dr. R. W. Girdler for many discussions on the significance of the ocean ridges and Dr. J. H. Parry and Mrs. B. Gray for help with Fig. 2. I was stimulated to think about the problem presented by the gravity data discussion during a U.S. National Science Foundation conference on artificial satellites at the Virginia Polytechnic Institute, Blacksburg, Virginia, and with Dr. R. Kershner and his colleagues at the Applied Physics Laboratory, Silver Springs, Maryland.

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OBITUARY

Dr. W. J. Dowson

WALTER JOHN DOWSON, who was widely known for his research and teaching in plant pathology, died recently in Cambridge at the age of seventy-six. His career had been unusually full and varied. Originally a Scholar of Christ's College, Cambridge, he obtained first-class honours in the Natural Sciences Tripos, and followed this by two years' research in Germany. Then in 1913 he went as Government mycologist to Kenya, where he is remembered for his work on control of the coffee leaf rust fungus, *Hemileia vastatrix*. In 1920 he returned to Great Britain as mycologist to the Royal Horticultural Society at Wisley, where he published work on a variety of diseases caused by fungi. In 1928 he went abroad again, this time as Government plant pathologist in Tasmania, where his human qualities and professional ability combined to make him a great success with the growers.

The year 1932 marked a significant divide in Dowson's professional life, for the twenty years remaining until his official retirement, he was to be both a university teacher and a more specialized research worker in a field that he was to make peculiarly his own. As a student at the Cambridge Botany School, he had early attracted the attention of Prof. F. T. Brooks. When, around 1932, a generous benefaction from the Rockefeller Foundation enabled a University Sub-Department of Mycology to be established within the Botany School, Brooks secured the appointment of Dowson as a University lecturer and as his colleague in the new Sub-Department. From that time until Brooks's retirement from the chair of botany in 1948, the two of them were responsible for all the teaching and research in mycology, bacteriology and plant pathology carried out in the Botany School, and both will long be remembered by their former research students, now all over the world.

After consultation with Brooks on his return to Cambridge, Dowson decided to take up the investigation of plant diseases caused by bacteria—at that time a decidedly neglected branch of plant pathology. This was a far-sighted decision, for the next twenty years and more, Dowson and his research students added greatly to our knowledge of these diseases. He was not content to confine himself to bacterial diseases occurring in Britain but extended his investigations to tropical plant pathogens, and carried on a voluminous correspondence with plant pathologists all over the world Third-year students at the Cambridge Botany School benefited by a facility not available elsewhere in Britain—a comprehensive course of lectures and practical work devoted to bacterial pathogens of crop plants Dowson's book, *Manual of Bacterial Plant Diseases*, first published in 1949, was the direct outcome of this course, as the most concise and readable text on this group of pathogens, it enjoyed a wide success In 1957 a second edition was published, this time by the Cambridge University Press, under the title *Plant Diseases due to Bacteria*

When Brooks retired from the chair of botany in 1948, Dowson became the second director of the Sub-Department of Mycology, and continued as such until his own retirement in 1952 He did not retire from research, however, but continued active in laboratory work and in publication almost to the time of his death eleven years later He will be remembered not only for an impressive contribution to his own subject, but also as a man who was inspired by lasting enthusiasm for his work purely for its own sake His scientific merit was recognized by the University of London through its award of the D Sc degree; later, he also obtained the Sc D., of Cambridge He did not, perhaps, receive his full share of the material benefits and public recognition to which his scientific achievements had undoubtedly entitled him For this, there could have been two reasons First Dowson only began his best work after reaching the age of forty-five, and his international reputation came considerably later. Secondly, he was an extremely modest and even somewhat diffident man , people who did not know him or his work well were somewhat surprised to be told that his system of classification for plant pathogenic bacteria had gained such ready acceptance What Dowson perhaps lost in his reluctance to thrust himself forward, he undoubtedly gained in human relationships A most kindly man, with an ever-ready and generous interest in the affairs of other people, he never lost the common touch which had made him as popular with the apple-growers of Tasmania as with a generation of students at Cambridge

S D GARRETT

NEWS and VIEWS

The 1963 Nobel Prize for Physics and Chemistry

ONE-HALF of the 1963 Nobel Prize for Physics has been awarded jointly to Prof Maria Goeppert-Mayer, professor of physics in the University of California, and Prof J Hans D Jensen, professor of theoretical physics in the University of Heidelberg, the other half has been awarded to Prof Eugen P Wigner, professor of mathematical physics in Princeton University The Chemistry Prize has been awarded jointly to Prof Karl Ziegler, of the Institute for Carbon Research, Mullheim-Ruhr, and Prof Giulio Natta, of the Institute of Technology, Milan

The Royal Society of London: Medal Awards

THE following awards of medals have been made by the President and the Council of the Royal Society *The Copley Medal*, to Sir Paul Fildes, formerly director of chemical bacteriology at the Medical Research Council, for his pioneer contributions to bacteriology, *The Davy Medal*, to Dr E J Bowen, Aldrichian praelector in chemistry at the University of Oxford, for his distinguished work on the elucidation of photochemical reactions, and for his study of fluorescence and phosphorescence in relation to the molecular processes concerned, *The Hughes Medal*, to Prof F C Williams, professor of electrical engineering in the University of Manchester, for his distinguished work on early computers, *The Leverhulme Medal* (which was founded in 1960 by the Trustees of the Leverhulme Trust Fund to mark the occasion of the tercentenary of the Royal Society), to Dr A J P Martin, director of the Abbotsbury Laboratories, Ltd , for his distinguished and fundamental discoveries in chromatography and its application

Rutherford Memorial

UNDER the terms of the scheme to commemorate the late Lord Rutherford of Nelson, the following appointments have been made Sir George Thomson, formerly Master of Corpus Christi College, Cambridge, as Rutherford Memorial Lecturer, to lecture in August 1964 in New Zealand Mr H S Tan, formerly of Imperial College of Science and Technology and later at the Post Office Research Station, Dollis Hill, to hold a Rutherford Scholarship for three years from September 1963, to carry out research in the department of physics, McGill University, in solid-state physics

Research Professorship

THE title of a Royal Society Research Professor has been given to Dame Honor Fell, Messel Research Fellow from 1931 until 1943 and Foulerton Research Fellow since 1943 Dame Honor Fell, who is director of the Strangeways Research Laboratory, Cambridge, is well known for her work in the field of cell biology

Visiting Professorship

DR A M. LWOFF, of the Institut Pasteur, Paris, has been appointed to be the Royal Society Visiting Professor for the academic year 1964–65 He is expected to take up appointment in April 1965 and to work in the division of virus research at the National Institute for Medical Research, Mill Hill, London, N W 7 Dr Lwoff, who is a Foreign Member of the Royal Society, is distinguished for his work on the morphology and nutritional requirements of Protozoa, and for his researches on lysogenic bacteria and bacteriophage Recently he has become interested in the growth of animal viruses and in normal defences against virus replication

Guests

ACADEMICIAN E N PAVLOVSKII of the Academy of Sciences of the U S S R and his daughter Dr. Irena Bykhovskaya-Pavlovskaya arrived in London on October 31 for a stay of three and a half weeks as the guests of the Royal Society, collaborating with the British Council The visits have been arranged under the agreement between the Royal Society and the Academy, which provides for the annual exchange visits of five senior scientists Pavlovskii became an Academician in 1939, and in 1944 he became an Academician of the Academy of Medical Sciences of the U S S R He is director of the Institute of Zoology and Parasitology of the Academy, lieutenant-general of medical services, president of the Geographical Society of the U S S R and an Honorary Fellow of the Royal Society of Tropical Medicine and Hygiene While in Britain, Academician Pavlovskii and his daughter will visit universities and other scientific institutions in London, Edinburgh, Liverpool and Cambridge They will both deliver a series of lectures

Imperial College of Science and Technology, University of London

THE day after the publication of the Report of the Committee on Higher Education, Lord Robbins, the chairman, was the Special Visitor at the annual Commemoration Day ceremony of Imperial College held recently in the Royal Albert Hall (see p 621 of this issue). That the College itself is poised on the threshold of a new epoch and ready to accept another challenge is evident from the report by Sir Patrick Linstead, the Rector The initial target of 3,000 full-time students has been reached. The figure includes 1,175 postgraduates and 120 women students New targets will soon be set and double the number of women will be welcome The academic staff now numbers more than five hundred and has been strongly reinforced at all levels, particularly at the top, where the recruitment of several outstanding men contrasts with gloomy utterances on the emigration of scientists

A radical change in the structure of the College is that 20 per cent of the students are in residence compared with 8 per cent last session More than six hundred students live within a quarter of a mile of the Queen's Tower of the College At the beginning of the term, H R H The Princess Margaret launched the great addition to the residential and social facilities in Prince's Gardens. A most welcome anonymous benefaction of a quarter of a million pounds will make it possible to build the next hall of residence in the same area, where a long-term plan is gradually taking shape The main task of demolition is over Two major building projects were finished last year—the Civil Engineering building and the South Side building in Prince's Gardens Four large academic building projects are now in hand

Dunn Nutritional Laboratory, Cambridge:

Dr. L. J. Harris

DR LESLIE J HARRIS, who became the first director of this laboratory on its inception by the Medical Research Council in 1926, retired on August 29 A native of Liverpool, and a graduate of the University of Manchester, he went to Cambridge as a young research student under the late Sir Frederick Gowland Hopkins There he soon won distinction, and in 1925 the Meldola Medal of the Institute of Chemistry, for his pioneer work in applying the theory of titration curves to amino-acids, polypeptides and proteins His ingenuity enabled him to distinguish clearly between zwitterion and non-zwitterion molecules After his appointment at the Dunn Nutritional Laboratory his interests were concentrated on vitamins He originated the assessment of human nutritional status, by saturation tests, and also the bradycardia test, with rats, for thiamin deficiency He developed chemical methods commonly used for the assay of ascorbic and nicotinic acids, discovered 'monkey pellagra', and made illuminating investigations with rats on the mechanism and psychology of appetite During the Second World War he supervised joint work between the Medical Research Council and the Food Investigation Board on methods for the preservation of foods, usually by dehydration, without the excessive destruction of their vitamins He played a large part in the foundation of the Nutrition Society (Great Britain), of which he was the first honorary secretary (1941–47) and later president (1953–56) He was also a joint founder and secretary-general (1946–60) of the International Union of Nutritional Sciences, a body which has held six highly successful triennial congresses, most recently in Washington (1960) and Edinburgh (1963) His semi-popular book, *Vitamins in Theory and Practice*, has been widely read and translated into several languages To mark the completion of his long period as director, Dr Harris was presented, by present and past members of his staff, with his portrait, painted in oils by Mrs B J Miles

Dr. E. Kodicek

DR EGON KODICEK has been appointed to succeed Dr Harris Dr. Kodicek has been working at the Dunn Nutritional Laboratory since 1939. He graduated M D. in Prague in 1932 and later became assistant physician at the Department of Internal Medicine at Charles University. In 1938 he was appointed physician-in-charge of the Endocrinological Out-patient Unit In the same year he was awarded the Diploma of Specialist for Diseases and Metabolic Disorders Dr. Kodicek came to Britain in 1939 as a Scholar of the Society for the Protection of Science and Learning He obtained the Ph D degree of the University of Cambridge in 1942 for his thesis *Biochemical Studies on Nicotinic Acid* The same year he was appointed a member of the Scientific Staff of the Medical Research Council. During the War he took part in investigations on the effects of dehydration on the vitamin B content of foodstuffs and was a member of various Government sub-committees Since the War, Dr Kodicek has carried out experimental work on a wide range of topics which have included bacterial metabolism and the effect of ascorbic acid deficiency on wound healing He has also made investigations into the nature of bound nicotinic acid and its relation to the pathogenesis of pellagra More recently, Dr Kodicek has made an exhaustive study of the distribution and metabolism of vitamin D in the animal body, this work was greatly facilitated by his successful synthesis of ^{14}C-labelled vitamin D Dr Kodicek has been a visiting lecturer to a number of universities in the United States and was elected a Committee Member of the Biochemical Society in 1961

Inorganic Chemistry in the University of Sheffield

Prof. R. Mason

PROF RONALD MASON, who was recently appointed to the newly established chair of inorganic chemistry in the University of Sheffield, took up his duties there on October 1 Dr Mason began his scientific career by taking a first degree at University College, Cardiff, and thereafter studied under Dame Kathleen Lonsdale at University College, London, obtaining his doctorate in 1953 in crystallography For the next seven years he was Research Fellow of the British Empire Cancer Campaign in the Department of Chemistry at University College and undertook research work into the structure of biologically active molecules, X-ray studies of thermal vibrations and the applications of quantum chemistry to problems in molecular biology From 1960 he was lecturer in inorganic chemistry in the Imperial College of Science and Technology, London His more recent work has been concerned with structural investigations on a variety of transition metal complexes, notably those where there is both σ- and π-bonding, and on paramagnetic anisotropies of single crystals of tetrahedral cobalt(II) species, potassium ferricyanide and di-π-*cyclo*pentadienyl nickel and their relation to the electron distribution in these complexes He has also had a continuing interest in energy and charge migration phenomena in molecular solids and biological systems

Prof Mason is the author of some fifty research papers and co-editor of the series *Advances in Crystallography*, *Advances in Radiation Biology* (to appear shortly) and of *Physical Processes in Radiation Biology*, and he has been visiting research professor in the Department of Biophysics at Michigan State University and visitor to the Brookhaven National Laboratory, Upton, Long Island The understanding of modern inorganic chemistry is especially dependent on structural analysis, and Prof Mason's expert knowledge in this area, coupled with his theoretical background and broad interests, should enable his new University to develop a thriving school of contemporary inorganic research in the widest sense

The Rockefeller Foundation

A statement, *Plans for the Future*, issued by the Trustees of the Rockefeller Foundation, indicates that, as was foreshadowed in the recent annual report of the Foundation (see *Nature*, 200, 97; 1963), during the foreseeable future the Foundation's efforts will be concentrated in five areas of pressing need. Three of these—the strengthening of that part of the present programme which is directed to human nutrition, and involving increasing the quality and quantity of food, as well as the search for new knowledge leading to more efficient use of land and water resources; initial research on the population problem, including programmes on the dynamics and the stabilization of populations; and strengthening selected centres of learning in the emergent nations—were specifically mentioned in that annual report. To these are now added programmes designed to give increasing numbers of citizens better educational opportunities to develop their full potential and to take their rightful place in society. Moreover, programmes are included to aid cultural development in the humanities and the creative and performing arts, using selected colleges and universities as the principal media. In its efforts toward these five goals—provision of an adequate food supply, stabilization of population, the training of promising individuals and development of institutions in areas where lack of leadership retards progress, advancement of equality of opportunity for all, and cultural development—the Foundation will, as it has traditionally done, seek to sharpen definitions of the problems and to support promising pilot projects which may open the way for greatly expanded organized effort.

The Nuffield Foundation Science Teaching Project

Criticism of school science courses is nothing new. Changes in content and a more experimental approach in the teaching have been advocated in many quarters for decades. The present wave of dissatisfaction has swept into Europe from across the Atlantic and with it has come the idea of a combined operation to set science teaching on a new path. The Nuffield Foundation Science Teaching Project is one such operation, and the first *Progress Report* gives some idea of its scope (Pp. iii+40. London: The Nuffield Foundation, 1963). It aims, initially, to produce a range of teaching resources—texts, teachers' guides, visual aids, apparatus and laboratory notes—for each of four courses. These are biology, chemistry and physics for 11–16-year-olds and science for 9–13-year-olds. Stress is laid on the encouragement of critical enquiry. Some indication is given of the approach planned and one is pleased to note that, while subject-matter is to be selected for its relevance to "the understanding of science as it is to-day" and with the educated layman in mind, there is a recognition of the danger of building up "a picture of science by authority". The chemistry section underlines the need for clear distinction between experimental fact and theoretical explanation, a distinction too easily blurred in the chase for modern knowledge, and a similar feeling is expressed in the biology section by reference to the practical evidence on which generalizations are based. Preparation has been made for trying out materials in schools, and a list of these, the teachers and others involved in the project, is included. The appearance of the actual teaching materials will be awaited with interest.

Economic Growth and Manpower

The report of the 1963 spring conference of the British Association for Commerce and Industrial Education presents the preliminary findings of a group of economists who, for the past two years, have been constructing a computable model of the British economy at the Department of Applied Economics of the University of Cambridge (Pp. 55. London: British Association for Commerce and Industrial Education, 1963. 10*s*.) The objective of the group is to investigate the problems of increasing the rate of British economic growth. As the work progresses every effort is being made to discuss its various aspects with those engaged in practical activities so that the results are both realistic and consistent. As one of its contributions to National Productivity Year, the Association provided a platform for discussion of the results which are now beginning to emerge from the group's calculations. Prof. R. Stone, P. D. Leake professor of finance and accountancy in the University of Cambridge, stated that "If we are to reach the goal proposed by the National Economic Development Council . . . we need to see clearly, quantitatively and with as much notice as possible, what is expected of each of us . . . A small group cannot produce a finished picture in one go from published information: it needs the help of other groups and individuals engaged in practical life, who alone can contribute the necessary range of knowledge and experience. But all this knowledge must be organized within a coherent framework . . . Until a framework exists, the task of organization cannot be started". The three economists who followed at the conference covered: (*a*) Industrial outputs in 1970—how the model is used to estimate the levels of output which each industry will have to attain by 1970 if the programme of growth is to be realized. (*b*) Industrial requirements for manpower in 1970—estimates under (*a*) interpreted into manpower requirements for thirty-one industries (the estimate for 1970 is 21,482 million as opposed to 20,469 million in 1960). (*c*) The composition of manpower requirements—the spectrum of skills; labour skills in 1951, in 1961 and in 1970. Each paper is accompanied by appropriate diagrams.

Fauna Conservation

The latest issue of *Oryx* (7, Nos. 2 and 3; August 1963) contains reports on several species of mammals that are thought to be in need of special protection because of their diminished numbers. A survey in southern Nepal by E. P. Gee shows that the population of Indian rhinoceros in the adjacent valleys of the Rivers Rapti and Rue is now increasing. The estimated number of 300 rhino in Nepal in 1959 dwindled to 160 in 1961 owing to increased poaching during a time of political unrest. By 1962 after the re-establishment of more settled conditions the number had risen to 185. The author recommends additions to the area of the national park, vigorous suppression of poaching, and the encouragement of tourism, as measures to ensure the conservation of the species. A paper by Dr. and Mrs. Colin Bertram states that the manatee is now seen less frequently in the waters of British Guiana than formerly, but that its status is not easy to assess because the animals are shy and difficult to observe; the increasing use of power boats may be driving them from some reaches of the rivers. Research is needed into the biology of the manatee, particularly its breeding, about which almost nothing is known. Manatees are efficient clearers of canals and channels choked with aquatic vegetation, but their introduction into other parts of the world to clear more open waters cannot be practicable until much more is known about them. Barbara Harrisson gives an interesting account of the attempt to return to the wild orphaned orang-utans seized from illegal captivity. The experiment has achieved some success owing to the zeal and devotion of Mrs. Harrisson, but the real way to conserve rare animals in the wild is to leave them alone and ensure that nobody interferes with them.

Radioactive Contamination of Food in the United Kingdom

The annual report for 1962–63 of the Agricultural Research Council Radiobiological Laboratory, Wantage, has recently been published (*ARCRL* 10 Pp x+108 London Agricultural Research Council Obtainable from H M S O 7*s* 6*d* net) It is the tenth in the series of reports on the Laboratory's work on radioactivity in the diet The Radiobiological Laboratory has continued to measure the levels of iodine-131, strontium-89, strontium-90, cæsium-137 and barium-140 in foodstuffs in the United Kingdom The major effort was devoted to milk, samples being regularly examined from depots which handle more than 40 per cent of Britain's total production The average ratio of strontium-90/calcium in milk was 12 picocuries/g in 1962, about 13 pc /g for the twelve months to the end of March 1963, and about 15 pc /g for the twelve months to the end of June Those levels reflect the increased rate of fall-out in 1962, and at the end of the year the twelve-month average was about 20 per cent greater than the previous highest value recorded in 1959 following the 1958 nuclear tests The average ^{90}Sr/Ca ratio in the total mixed diet in 1962 was 10 pc /g, which was about 60 and 10 per cent higher than in 1961 and 1959, respectively, but was still less than 8 per cent of the 'cautionary' working-level of 130 pc /g recommended by the Medical Research Council However, not all the components of the diet reflected the increased rate of fall-out in 1962 In particular, the ^{90}Sr/Ca ratio for flour was below that for the previous year due to the delay between the harvest of grain and the consumption of the flour The values rose, however, in the 1962 crop, which did not enter the diet until 1963 The levels of iodine-131 in milk for the first six months of 1963 continued to be below the limit of detection (11 pc /l) The average for the twelve months to the end of June was below 15 per cent of the Medical Research Council's recommended 'cautionary' level of 130 pc /l

One-sided Tolerance Limits and Variables Sampling Plans

A MONOGRAPH recently published by the Sandia Corporation contains a wide selection of tables based on the non-central *t* distribution (*SCR*–607 *Factors for One-sided Tolerance Limits and for Variables Sampling Plans* By D B Owen Pp 412 Albuquerque, New Mexico Sandia Corporation. Available from Office of Technical Services, Department of Commerce, Washington 25, D C , 1963 5 dollars) Previous work in this field has thus been extended and, in some cases, corrected. Sampling from a normal distribution is considered throughout, the principal tables are designed for use with acceptance procedures (single-sample variables sampling plans) described in the author's abstract as follows "The probability is γ that at least a proportion P of a normal population is below $\bar{x} + ks$, where $\bar{x}$ has a normal distribution with mean μ and variance σ^2/n and fs^2/σ^2 has an [independent] chi-square distribution with f degrees of freedom" Typically γ and P are fixed according to manufacturer and consumer interests Values of k are tabulated for suitable values of γ, P and the sample sizes n and f Other applications include the power of Student's *t*-test and "expected coverage tolerance limits" The tables are balanced by a valuable commentary In this, Dr Owen explains very clearly how to use the tables, he discusses various approximations arising in their construction, and he gives the mathematical derivations on which they rest Dr Owen claims that the variables sampling plans discussed are to be preferred in particular, when the protection of the consumer is of primary interest and the costs of items are high He does not argue this point, but the reader who feels at all doubtful is free to seek reassurance by sampling the bibliography of some eighty items

The Night Sky in December

NEW moon occurs on Dec 16d 02h 07m U T and full moon on Dec 30d 11h 04m The following conjunctions with the Moon occur Dec 18d 14h, Venus 0 5° S , Dec 20d 14h, Saturn 2° N., Dec 24d 08h, Jupiter 4° N In addition to these conjunctions with the Moon, Mercury is in conjunction with Mars on Dec. 7d 03h, Mercury being 1 4° S , and Mercury with Mars on Dec 30d 05h, Mercury being 2° N There is a total eclipse of the Moon on December 30, invisible at Greenwich Mercury is an evening star, but is not well placed for observation Venus is an evening star, setting at 17h 15m, 17h 40m and 18h 30m on Dec 1, 15 and 31, respectively Its stellar magnitude is −3 4, its distance decreases during the month from 141 to 128 million miles, and the visible portion of the apparent disk decreases from 0 914 to 0 862 Mars is too close to the Sun for observation Jupiter sets at 2h 20m, 1h 20m and 0h 25m on December 1, 15 and 31, respectively It is in Pisces, its stellar magnitude is −2 2 and its distance from the Earth on December 15 is 425 million miles Jupiter is stationary on December 6, after which it resumes its eastward motion among the stars Saturn sets about 4h after the Sun, but conditions for observation are now becoming unfavourable Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich Dec 2d 1h 20 6m, ζ Tau (*D*), Dec 2d 2h 19 0m, ζ Tau (*R*), Dec 29d 2h 18 6m, 106 Tau (*D*) *D* and *R* refer to disappearance and reappearance, respectively. The Geminid meteors are active during December 9–14, with maximum on December 13, the radiant is near R A 7h 28m, Dec +32°, and conditions for observation are favourable The Ursid meteors are active during December 20–22, with maximum on December 22, the radiant is near R A 14h 28m, Dec +76°, and conditions for observation are favourable The winter solstice occurs on Dec 22d 14h

Announcements

PROF C F A PANTIN, professor of zoology in the University of Cambridge, president of the Marine Biological Association and a member of the Nature Conservancy, has been appointed chairman of the newly constituted Trustees of the British Museum (Natural History) Prof Pantin, who was awarded the Royal Medal of the Royal Society in 1950, and was President of the Linnean Society from 1958 until 1960, is distinguished for his work in marine biology and experimental zoology, and well known for his interest in providing the broadest possible basis for future specialists

AT a general monthly meeting of the members of the Royal Institution held on November 4, the Right Hon Lord Brabazon of Tara was appointed honorary professor extraordinary of the Royal Institution Lord Brabazon has been a Member of the Institution for twenty years and was president for fifteen years from 1948 until 1963 He was succeeded as president by Lord Fleck

A MEETING of the Plant Phenolics Group on "Polysaccharides" will be held at the School of Pharmacy, London, on January 7 Further information can be obtained from Mr A H Williams, Research Station, Long Ashton, Bristol

A COLLOQUIUM on "The Decomposition and Ignition of Peroxides", organized by the Combustion Institute, will be held in the University of Oxford on January 7 Further information can be obtained from Dr W G Parker, Department of Chemistry, College of Advanced Technology, Birmingham 4

THE 1964 Winter Gordon Research Conferences on "Polymers" and "Electrochemistry Electrode Reactions" will be held in Santa Barbara, California, during January 27–February 7 Further information can be obtained from the director of the conferences, Dr W George Parks, University of Rhode Island, Kingston, R I

THE ROWETT RESEARCH INSTITUTE FOR ANIMAL NUTRITION

GOLDEN JUBILEE

THIS year the Rowett Institute celebrates its origin in 1913, when a Joint Committee of the University of Aberdeen and the North of Scotland College of Agriculture was formed for the prosecution of research in animal nutrition The late Prof E P Cathcart, who in 1913 was a lecturer in the Institute of Physiology in the University of Glasgow, was first approached and accepted the offer to go to Aberdeen to start this research work Later, he decided to take a chair of physiology in the University of London and, when intimating his change of mind, recommended that Dr John B Orr be offered the post Thus, Dr Orr became the first director of what not long after grew into the scale of an institute, and in this capacity he served until his retirement in 1945, when he was succeeded by Dr D P Cuthbertson, also from the Institute of Physiology, Glasgow, but who had been seconded at the time to the Medical Research Council During its fifty years, therefore, the Institute has had only two directors

The Governing Body of the Institute is now tripartite, with five persons appointed by each of the following University of Aberdeen, North of Scotland College of Agriculture and the Secretary of State for Scotland The Principal and Vice-Chancellor of the University is its chairman

Following his retirement in 1945, Sir John Orr became the first director-general of the Food and Agriculture Organization of the United Nations The need for the creation of such an organization owed much to Orr's vision and his previous researches culminating in his book *Food, Health and Income* Orr had also been responsible for a large-scale demonstration of the nutritive value of milk for children, and the evidence from this and comparable trials was largely responsible for the introduction of the milk and finally the meals-in-schools scheme He had felt that so much useful knowledge had been acquired about animals that it was time it was applied to man himself

The Commonwealth Bureau of Animal Nutrition does not share the Jubilee that the Rowett Institute is celebrating it is only thirty-two years old, but, as in some ways an offshoot of the Institute, it shares its history and celebration, for Orr was its first director, followed in more recent years by Dr I Leitch and Dr D Harvey The present director of the Institute is consultant director to the Bureau and editor-in-chief of its publication, *Nutrition Abstracts and Reviews*

The early and subsequent history of the Institute, its Reid Library, its farm—the Duthie Experimental Stock Farm—and its residential hall—Strathcona Club—are described in a unique volume entitled *Progress in Nutrition and Allied Sciences*, which is edited by the present director and published by Oliver and Boyd at the modest price of 50*s* for its 452 pages (*Nature*, 200, 201, 1963) This provides an account of the present research work of the Institute in relation to the existing state of knowledge, and some account is also woven into it of the past research of the Institute A few distinguished ex-members of staff have contributed topics not at present being investigated at the Institute There are thirty-nine chapters Among the significant topics dealt with are those which have been in recent years the continuing concern of the Institute These are the physiology, biochemistry and microbiology of digestion, particularly in the ruminant, the nutritional significance of the 'non-protein nitrogen' fraction of plants, physiological individuality, minerals in relation to the skeleton, the teeth and functioning of the nervous system, mammalian glycosidases and their inhibition, growth and reproduction, family diets, infection and resistance The Institute's present contribution to our better understanding of the nutrition of dairy cattle, sheep and their shelter, poultry and pigs and the recent system for intensive beef production introduced in Britain by the Institute are also described The Institute continues to assist in defining where the application of man's scientific knowledge of agriculture to world needs can best be directed

The present staff of the Institute numbers two hundred and fifty persons, of whom some fifty-five belong to the scientific officer class Dr R L M Synge, Nobel prizeman, is probably its most distinguished member of staff He has written a challenging article on the state of our ignorance concerning the nutritional role of the constituents of the non-protein fraction of plants at this point in time

The Institute publishes each year an annual report which is free to universities, colleges and non-commercial research organizations

At its Founders' Day Dinner in this year of its Golden Jubilee, the Secretary of State for Scotland, the Right Hon Michael Noble, gave the principal speech and proposed a toast to "The Founders"

D P. CUTHBERTSON

VEGETATION OF THE HUMID TROPICS

A SYMPOSIUM on the vegetation of the Humid Tropics was held at Kuching, Sarawak, during July 2–10, it was sponsored jointly by the South-east Asia Science Co-operation Office of Unesco and the Government of Sarawak, and was attended by some 36 participants coming from most of the countries of South-east Asia, as well as from Europe, the United States and Australia The United States was represented by Dr F R Fosberg of the Pacific Vegetation Project, Washington, chairman of the Humid Tropics Committee of Unesco, and by Dr George Gillett of the University of Hawaii; participants from the United Kingdom were Sir George Taylor, director of the Royal Botanic Gardens, Kew, Mr P Greig-Smith, reader in botany at the University College of North Wales, Bangor, and Prof P W. Richards. Mr L. Mattsson, director of the South-east Asia Office at Djakarta, was the official representative of Unesco This symposium was the third of a series on tropical vegetation organized by the South-east Asia Office of Unesco and followed those held at Tjiawi, Java, in 1958, and at Goroka, New Guinea, in 1960 (see *Nature*, 189 24, 1961)

The present policy of the South-east Asia Science Co-operation Office is that each symposium should have one or more principal themes, as well as giving experts on tropical vegetation the opportunity of discussing their work and problems with colleagues whom of necessity they cannot meet very often At the Goroka symposium, the Proceedings of which have recently been published*, the emphasis was on the impact of man on the vegetation of the humid tropics At the recent symposium at Kuching the chief themes were recent advances in tropical ecology and the application of quantitative methods to the study of tropical vegetation Although according to the programme the latter topic was the subject of only one morning's session, it was in fact a recurrent theme, both in the regular sessions and in the less formal, but no less animated, discussions 'out of hours' The papers presented to the symposium indeed covered a wide field, from the "Entropy Concept in Ecology" (F R Fosberg) to the "Ecology of Pitcher Plants (*Nepenthes*)" (B E Smythies), and provided a cross-section of the main fields of activity in present-day tropical ecology

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* Unesco Science Co operation Office for South-east Asia and Administration of the Territory of Papua and New Guinea (Government Printer, Canberra, 1963)

Sir Alexander Waddell, Governor of Sarawak, declared the symposium open at an inaugural session in the afternoon of July 2, under the chairmanship of Mr B E Smythies, conservator of forests, Sarawak In his speech the Governor stressed the importance of ecological research to a country such as Sarawak, much of which consists of undeveloped land covered with forest and other vegetation in a relatively natural state He also quite rightly directed attention to the considerable contribution to research on tropical vegetation which is being made by the staff of the Sarawak Forest Department

The one session devoted entirely to quantitative methods was opened by Mr P Greig-Smith, who strongly emphasized the importance of statistically reliable sampling procedures and directed attention to some of the pitfalls which surround attempts to apply quantitative methods of description and analysis to plant communities as rich in species and lacking in homogeneity as the forests of the humid tropics, he also discussed the problems involved in the selection and delimitation of sample plots. The type of quantitative method to be used must be modified, Mr Greig-Smith considered, to suit both the nature of the vegetation to be studied and the ultimate object in view This opening paper led to a long and lively discussion in which many modern techniques such as 'plotless sampling', gradient analysis, etc , were considered

This paper was followed by two others dealing with related problems Dr P S Ashton gave an account of his work (shortly to be published as an *Oxford Forestry Memoir*) on the Mixed Dipterocarp forests of Brunei The forests of this small country—no larger than an English county—are extraordinarily rich in species, there are more than 150 species of large trees belonging to the family Dipterocarpaceae alone Dr Ashton has, however, successfully used a modification of Bray and Curtis's ordination technique and shown that the varying composition of the forest community reflects very sensitively the innumerable small variations in topography, soil and local climate This paper high-lighted the extreme complexity of some plant communities of the humid tropics, but also gave reason for hoping that relatively simple quantitative techniques might prove adequate in providing a firm basis for correlating the endless and seemingly random variations of forest composition with the factors of environment The last paper in this session was read by Mr A J Vincent of the Forest Research Institute, Kepong, Federation of Malaya, and was entitled "Tropical Rain Forest Ecology What Does the Forester Need to Know About It and How Should He Put the Necessary and Sufficient Knowledge on a Quantitative Basis ?" As might be guessed from its title this paper was valuable in bringing the somewhat academic approach to tropical ecology which had prevailed earlier in the discussion into relation with practical realities in the newly independent countries of south-east Asia at the present day

Quantitative problems of other kinds were raised in several other papers notably in one by Mr D I Nicholson of the Sabah (North Borneo) Forestry Department on "Studies in Virgin Forest Near Sandakan, North Borneo" Among the many interesting problems raised in the discussion on this paper was the reliability of the various methods at present used for estimating the age of rain forest trees (which do not as a rule produce annual growth rings) Mr I M Qureshi (Dehra Dun) expressed the view that quantitative ecological methods should be, so far as possible, standardized as well as statistically valid

Another subject with which several papers were concerned and which led to much informal discussion was the relation between vegetation, soil and parent rock Dr L. J Webb, of the University of Queensland, in a paper on the rain forests of north-eastern Australia, directed attention to the well-known fact that in the humid tropical regions of Australia there is a marked difference between the vegetation on basalt and on other parent materials such as sandstone, the former bearing rain forest similar in many of its features to the rain forests on the Indo-Malayan region, the latter eucalypt forests of a characteristically Australian type In spite of this obvious difference in vegetation, however, soil analysis, at least using conventional methods, does not seem to reveal any marked differences in nutrient elements such as phosphorus, potassium and nitrogen between the soils formed from basalt and those derived from other parent materials A similar problem was discussed by Prof Richards in a paper dealing with soil conditions in some lowland forest communities in Brunei and Sarawak, where there are striking differences between the 'Heath forest' on the sandy *kerangas* (podsolic) soils and the Mixed Dipterocarp forests on soils of the more normal latosol type Here again the soils are obviously different and are clearly correlated with differences of vegetation, but the soil analyses appear to indicate very little significant difference in the amount of nutrients present in the soil The very distinctive character of 'Heath forest' communities was also brought out in a paper by Mr Anwari Dilmy (Herbarium Bogoriense) dealing with the vegetation of the Sampit area in Central Kalimantan (Indonesian Borneo)

It was worthy of note that although nearly every contribution to the symposium was concerned with the ecology of plant communities rather than with autecology, several speakers stressed the need for more work, especially experimental work, on the ecology of individual species Some sessions ranged into widely different fields, for example, one of the most interesting and informative dealt with palynology, a subject of great promise for the future understanding of tropical vegetation Dr D Walker of the Research School of Pacific Studies, Australian National University, Canberra, gave an account of his work on the stratigraphy of a swamp in the New Guinea highlands, and Mr J Muller, of Brunei-Shell Petroleum Co , summarized the results of his fascinating work on the pollen in Holocene peats in Sarawak In Borneo, a complete stratigraphical sequence from the Cretaceous to the present day is available for pollen analysis This has already revealed much of interest about the history of the mangroves and peat swamp, but what has already been discovered can be no more than a foretaste of results of immense significance for the understanding of the vegetation of south-east Asia

A paper by Mr T Smitinand on the vegetation of a limestone massif in Thailand and another by Dr J A R Anderson on the limestone habitat in Sarawak provided much new information on a very interesting type of tropical habitat which has hitherto received very little attention and also raised the issue of conservation which, though not one of the official themes of the symposium, was clearly one of the underlying anxieties of most participants The vegetation of the fantastically shaped limestone hills in Sarawak, Malaya, Thailand and elsewhere in south-east Asia is of the greatest interest to the botanist because of its strange and often endemic plant species, but there is a grave danger that because of the high economic value of limestone for making cement and other purposes, especially in relatively advanced countries such as the Federation of Malaya, the habitat will be destroyed before there is any adequate scientific knowledge of its flora and fauna. Similar anxieties were expressed about several other types of tropical vegetation, especially the lowland rain forest of the Malay Peninsula and the montane forests of Mount Kinabalu in North Borneo where the proposal to establish a national park needs to be pressed forward rapidly before the steady advance of shifting cultivation destroys any more of the forest vegetation.

Not the least important part of the activities of the symposium were the field excursions, which gave the opportunity for very valuable discussions of present-day problems in tropical ecology. The week at Kuching was interrupted by a very pleasant and interesting visit to the Bako National Park and Santubong Mountain at the mouth of the Sarawak River. The excellent living accommodation and well-designed paths at Bako gave the party an opportunity of seeing something of the unusual forest and heath-like plant communities on a low sandstone plateau on the coast, where *Nepenthes* and strange-looking epiphytic species of *Dischidia* and *Hydnophytum* grow in abundance and can readily be studied. Santubong at the mouth of the Sarawak river, memorable for its associations with Alfred Russell Wallace, provided a glimpse of the extraordinary variety of species and plant communities on a steep and isolated mountain less than 3,000 ft. high. Before leaving Kuching, members of the symposium were able to see the excellent 'natural' arboretum created by the Sarawak Forest Department in the Semengoh Forest Reserve and climb the 100-ft. tower which is being used to collect data on temperature and humidity at different levels in undisturbed dipterocarp forest. On the same day a short visit was paid to the famous limestone hills at Bau.

After the conclusion of the activities of the symposium in Sarawak, most of the participants were able to take part in a tour to Brunei, where one day was spent in studying the vegetation of the peat swamps and another in the magnificent Mixed Dipterocarp forest of the Andulau Forest Reserve. After this many of the party joined in a short visit to Sabah (North Borneo) and climbed Kinabalu (13,455 ft.) with its amazingly rich montane flora.

The success of the symposium owed much to the careful preparatory work of Dr. J. A. R. Anderson and other members of the Sarawak and Sabah Forest Departments, as well as to the simple, but very convenient, accommodation provided by the Sarawak Government at the Batu Lintang Teachers' Training College. The fact that it is possible to hold such a symposium at all underlines the increasing importance of tropical ecology. The foundation of a Society for Tropical Ecology in India, the formation of a Tropical Group in the British Ecological Society and the series of symposia sponsored by Unesco since the inauguration of its Humid Tropics Committee at Kandy in 1956, show that this interest is widespread and that an appreciation is developing of what ecology can contribute to scientific knowledge in a part of the world where most of the major problems are biological.

P. W. RICHARDS

ACTIVATION ANALYSIS IN MEDICINE AND BIOCHEMISTRY

ACTIVATION analysis is a sensitive and versatile technique for the determination of trace constituents through measurement of the radioactivity induced after bombardment by neutrons or other sub-atomic particles. First suggested by Hēvesy and Levi in 1936, it became practicable on a useful scale with the development of nuclear reactors and other neutron sources after the Second World War. A rapid growth of interest in biological applications was made evident during the third International Biological Colloquium, organized by the Commissariat à l'Energie Atomique and held at Saclay, near Paris, during September 26–28. The gathering of 150 research workers, drawn from many countries and many disciplines, heard 20 papers on recent developments and potential applications of the method in medicine and biochemistry.

Interest was equally divided between the experimental techniques and their applications. Discussion of techniques was concentrated on three major problems: (*a*) sources; (*b*) chemical manipulation of samples; (*c*) assay of induced radioactivity.

Thermal neutron activation is serviceable for the estimation of about 70 elements at sub-microgram levels. Consequently, the nuclear reactor is widely used as a source; most of the research reactors brought into operation in recent years, giving fluxes of 10^{11}–10^{12} n/cm^2/sec, are contributing to analytical research projects. A reactor is, however, not always essential or even desirable. For some elements the sensitivity of activation analysis using thermal neutrons is very low; for a few elements (near the beginning and end of the Periodic Table) the method is not applicable at all. Even when adequate sensitivity is available, interfering activities may be contributed by other elements present in the sample under examination. The estimation of manganese in blood can, for example, be achieved by using the reaction ^{55}Mn(n, γ)^{56}Mn. The small flux of fast neutrons inside a typical reactor will generate the same product by the reaction ^{56}Fe(n, p)^{56}Mn, giving an error of as much as 30 per cent. In this instance, a correction can be calculated, but the interference is sometimes of a more subtle kind, not so easily dealt with.

It is occasionally possible to secure greater sensitivity and accuracy by using fast neutrons. The most convenient source is a discharge-tube generator in which a tritium target is bombarded by deuterons accelerated to energies of 150–200 keV. Several models of neutron generators using this principle are available commercially, giving 14-MeV neutrons at fluxes of 10^7–10^9 n/cm^2/sec. W. Schulze (Free University of Berlin) discussed the use of fast neutrons in activation analysis and gave a comprehensive table of nuclear cross-sections (calculated and measured) for (n, γ), (n, p), (n, 2n) and (n, α) reactions. J. Wood (Services Electronic Research Laboratory, Baldock) reported new developments in the design of discharge tubes for the (d, t) reaction; with the further advances now in prospect, this work should make the use of laboratory neutron generators more attractive in terms of initial cost and tube life.

The use of charged particles for activation was exemplified in a report by A. Fleckenstein (University of Freiburg) on the determination of oxygen in biological materials, using the stable isotope oxygen-18 in the reaction ^{18}O (p, n)^{18}F, which gives excellent sensitivity. Recognition of the potentialities of activation analysis with protons or helium-3 ions has led to renewed interest in the cyclotron; it is hoped that a machine giving 8-MeV protons can be produced commercially in the United States for 100,000 dollars.

The rest of the discussion on techniques was concerned with the relative contributions of radiochemical manipulation and of instrumental assay. Broadly speaking, the activity under investigation may be separated chemically (using modifications of normal analytical procedures) or physically (by examination of the γ-ray spectrum of the induced radioactivity). In practice it is not always possible to achieve complete separation by either method alone. In biological materials, for example, the presence of sodium-24 in irradiated samples makes it difficult to measure the activity produced by trace elements present at very low levels

A lively discussion arose over the removal of interfering activities Ideally, the sample should be protected from all possible contamination until it has been irradiated, after that the purity of reagents and containers is of less importance Chemical treatment of the sample before irradiation is superficially attractive but potentially dangerous Prior separation by paper chromatography (with subsequent irradiation of the paper bearing the particular metabolic product or other compound to be analysed) is a serviceable technique in the study of complex biochemical processes, since the composition of the original tissue or product would be altered by destruction of chemical bonds during irradiation. Unfortunately the best available chromatographic paper includes impurities such as phosphorus and chlorine which limit the sensitivity of the method

Dialysis of experimental samples (such as blood) before irradiation is another attractive possibility, but the presence of contaminating trace elements in the dialysing fluids may lead to uncertainty Results obtained by this technique in the estimation of manganese in biological fluids were challenged during the Colloquium

If specimens of blood or other tissues are ashed before irradiation, some constituents may be lost by alloying in metallic crucibles or by fusion in ceramic vessels The heparin anticoagulant added to blood samples before ashing contains relatively large amounts of manganese and zinc, making the estimation of these elements difficult Even the storage of specimens in collecting bottles before irradiation may introduce contamination. Glass and polyethylene are suspect in this connexion, but 'Teflon' is more nearly inert The choice of containers for irradiation in the reactor is also important

When once the specimen has been irradiated, chemical treatment may be applied without fear of introducing contamination, though it is still important to estimate and correct for the chemical yield Ion-exchange, chromatography and dialysis are all useful at this stage in the analysis Rapid separation of the desired activity can sometimes be achieved by isotopic exchange W W Meinke (National Bureau of Standards, Washington) described a simple technique for the estimation of magnesium in blood, using a one-drop specimen After irradiation for 10 min at a thermal neutron flux of 10^{12} n/cm^2/sec the sample is brought into solution (by destruction of organic matter) and shaken for 2 min with a precipitate of magnesium ammonium phosphate The radioactive magnesium-27 then becomes concentrated in the precipitate by isotopic exchange The entire radiochemical procedure can be completed in 5 min—a necessary requirement, since the half-life of the activity to be examined is only 10 min

The speed and relative simplicity of chemical separations were emphasized by a number of contributors, but the instrumental approach, relying on γ-ray spectrometry, had convincing advocates The limit of present-day progress was illuminated by R E Wainerdi and his colleagues (Texas Agricultural and Mechanical University) Samples of biological material for activation analysis are assayed entirely by automatic instrumental methods The information obtained from a 256-channel pulse-height analyser, recorded on punched cards or magnetic tape, is transferred to an IBM 709 electronic computer in which the concentration of the element under consideration is calculated with the help of a calibration curve, obtained in advance from known amounts of the element

In a typical series, microgram amounts of silver (in rat brain) were estimated by assay of silver-110 (half-life 24 sec) Samples were irradiated for 24 sec, allowed to decay for 20 sec and counted for 30 sec to give a γ-ray spectrum, a further spectrum was recorded after the silver-110 activity had decayed to insignificance. The total computer time needed for each sample was 2 6 min and the cost of the analysis was 1 37 dollars At this price, activation analysis can compete with the more familiar methods used in hospital or agricultural laboratories

F Girardi and M Merlini (Euratom, Ispra) described a project for the estimation of trace elements in freshwater molluscs Up to 12 elements are separated chromatographically and examined by an automatic γ-ray spectrometer which supplies data on punched tape to an IBM computer for analysis and calculation

Papers on the uses of activation analysis in biological research could be classified into two groups The first was concerned with measurement of the concentration of particular elements in tissues or in the environment For some elements satisfactory techniques have now been devised, removing the uncertainties evident in earlier determinations Reported levels of manganese in tissue, estimated by activation analysis, have fallen steadily in recent years (as sources of contamination have been identified and eliminated), but now seem to have reached stability at well under 1 p p m

V P Guinn (General Dynamics Corporation, San Diego) presented relatively simple methods for the routine estimation of several elements in blood and tissue. W F. Bethard (San Diego) and Lars-Olof Plantin (King Gustav V Research Institute, Stockholm) discussed the correlation between levels of trace-elements in blood and various diseases, including leukæmia and rheumatoid arthritis

The difficult problem of estimating protein-bound stable iodine in human blood plasma (as a necessary supplement to turnover studies with radioactive tracers, such as iodine-131) has now been solved in a few laboratories. D Comar (Service Hospitalier Frédéric Joliot, Orsay) showed how activation analysis can be applied to routine determinations and to the study of abnormalities of iodine metabolism. The wider provision of such a service would fill an obvious and important clinical need

The relation of trace element metabolism to human disease was reviewed by J M A Lenihan (Western Regional Hospital Board, Glasgow), with particular reference to arsenic as a potential carcinogen Activation analysis is the only satisfactory method of measuring natural levels of arsenic in biological samples

R Soremark (University of Umeå, Sweden) reviewed the evidence for the protective action of vanadium against dental caries and described preliminary investigations by activation analysis The concentrations found in dentine and enamel from sound human adult teeth and in several foodstuffs were less than one part in 10^{11} Drinking-water levels were between one part in 10^9 and one part in 10^{10}, but parsley contained about one part in 10^6

A Fleckenstein described experiments on the study of intracellular metabolism of phosphoryl compounds The use of phosphorus-32-labelled phosphate as a tracer in this work has important limitations The radiophosphate does not readily pass through the muscle fibre membrane; if it does pass into the cell, the concentration of intracellular phosphate may increase to the point at which further access of the labelled tracer to the inside of the cell is blocked, creating the illusion of an inhibition of intracellular phosphorylation

Oxygen is a better tracer for the study of biochemical processes inside the cell Unfortunately, its radioactive isotopes have very short half-lives and are generally not very useful in such work The stable isotope oxygen-18, which forms 0 2 per cent of the natural element, can be incorporated in water at high concentrations (up to 80 per cent enrichment), but until recently could be assayed only by mass spectrometry Fleckenstein and his colleagues have used activation analysis based on the reaction ^{18}O(p, n)^{18}F, which offers a fast and sensitive method for measuring the rate of incorporation of oxygen from water into various phosphorus-containing compounds in cardiac and skeletal muscles

H Altmann (Reactor Centre, Siebersdorf, Austria) reported investigations using nucleic acids of tobacco mosaic virus to study the possible relation between trace-element levels and virus infection

R C Koch (Nuclear Science and Engineering Corporation, Pittsburgh) described work on the distribution of iodine-129, a nuclide which occurs naturally as a result of cosmic-ray reactions and spontaneous fission, but has recently been found in measurable quantities in debris from nuclear explosions The amount of iodine-129 in a typical human thyroid gland at the present time is about 10^{-11} g, and is far below the limit of chemical detection Activation analysis, using thermal neutrons to produce iodine-130 (half-life 12 5 h), enables reasonably accurate measurements to be made, as a basis for the assessment of long-term biological hazards and for studies of the movement of iodine in the biosphere

H J M Bowen (Wantage Research Laboratory, U K Atomic Energy Authority) emphasized the wide discrepancies among different laboratories in determinations of certain trace elements in biological material Experience in geochemistry and metallurgy has shown the value of standard samples, distributed for comparison of analytical results and techniques Bowen suggested that several biological materials, including algae, cabbage leaves, locusts, muscle, bone and blood, should be grown or collected under controlled conditions About 100 kg of each material would be required for use over a period of 10–15 years The samples should be dried, ground and distributed as required to laboratories working on activation analysis, so that they might compare their results on substantially identical specimens

The main conclusion which emerged from the Colloquium is that activation analysis, having passed through the stage of being an ingenious technique, now constitutes a powerful experimental weapon for biologists, biochemists and clinical research workers These beneficiaries have perhaps not appreciated fully the potentialities of new methods, but there is little doubt that the abounding enthusiasm of the effort now being applied to activation analysis will before long have a corresponding impact on biological science—including agriculture and veterinary medicine, which were not represented at all during this meeting

Some of the participants were unprepared for the 12-h working day prescribed by the organizers The ardours of this régime were handsomely mitigated by efficiency and hospitality, which culminated in a valedictory banquet at the château of Gif-Sur-Yvette under the genial chairmanship of Prof Francis Perrin, high commissioner for Atomic Energy.

J M A Lenihan

GAS CHROMATOGRAPHY

An informal symposium of the Gas Chromatography Discussion Group of the Institute of Petroleum was held at the Washington Singer Laboratories of the University of Exeter on September 27

The first paper was given by Dr I Halasz on "Gas-Solid and Gas Adsorption Layer Chromatography" He pointed out that gas-solid chromatography has now become an important subject for research again after having been displaced for a period by gas-liquid chromatography The disadvantages of gas-solid chromatography are (1) the upper limit of linearity of the isotherms of gas absorption is normally higher than it is for the solubility of gases in liquids, and (2) the number of available solids with suitable mechanical properties is strictly limited These disadvantages have now been largely overcome with the development of more sensitive detectors and new techniques Among the latter, Dr Halasz mentioned the modification of the highly polar known adsorbers by adding other solid substances, the coating of active solids with liquids for which Cremer has proposed the name 'gas adsorption layer chromatography', the coating of supports having a very large specific surface area with pulverized solids, and the coating of the inner wall of capillaries with a thin porous layer of solid In the latter case, the whole of organic and inorganic chemistry is available for experiment As regards the coating of an active solid with a liquid, preferably a polar one, some people assert that the active areas of the solid are blocked, and others suggest that the liquid changes its properties in the field of force of the solid, but Dr Halasz was of the opinion that a new stationary phase is formed, and he showed a graph in which an increase in the amount of liquid coating of alumina caused a decrease in retention volume of all C_1–C_4 components, a result in direct contrast to that obtained by gas-liquid chromatography

Two of the main advantages of gas-solid chromatography and gas adsorption layer chromatography are that mass transfer is much faster than in gas-liquid chromatography, and also that the vapour pressures of the stationary phases may be neglected or are, at the least, very low, a factor of special importance in programmed temperature operation Mass transfer will be especially fast in the case of a coating in the form of a thin porous layer, an advantage previously pointed out by both Golay and Purnell Five types of column were briefly defined as (*a*) classical packed columns in which the inner diameter of the column is larger than the particle size of the support by at least a factor of 10, (*b*) packed capillary columns having an inner diameter less than 1 mm, the particle size of the support being larger than one-fifth the inner diameter of the column, (*c*) classical open-tubes or capillaries (Golay columns) which have the inner surface coated with liquid, (*d*) porous layer capillary columns in which the inner surface is coated with a thin porous layer of active or inactive solid, (*e*) porous layer bead columns in which glass or metal beads are coated with a thin porous layer of solid support The performance of these types of column was illustrated by slides of work carried out by Heine and Horvath at Frankfurt C_1–C_4 hydrocarbons are separated fairly well on a packed capillary column of alumina in 13 sec, but although the resolution is a little better, separation took 360 sec on a classical packed column under the same conditions It has been found that hydrocarbons up to C_8 can be analysed on these packed capillary columns using alumina without much tailing of peaks but the column temperature must be relatively high (80° C) The same analysis can be carried out at 20° C by coating the alumina with 20 per cent by weight of β, β¹-oxydipropionitrile Many other similar illustrations were given, and in addition some separations using porous layer capillary columns of graphitized carbon black and silicon carbide, and porous layer glass bead columns using porous layers of zirconium oxide + triethylene glycol (for halogenated hydrocarbons) and ferric oxide + triethylene glycol (for aromatic hydrocarbons) In conclusion, Dr Halasz emphasized that his research programme was still in its early stages, and it was hoped that, in the future, better adsorbers would become available

A paper on "Modified Aluminas—A Progress Report" by C S G Phillips and C G Scott followed and was read by Mr Scott The selectivity obtainable with aluminas modified with either sodium hydroxide or a sodium halide was briefly reviewed, and it was shown that, apart from providing packings having a range of aromatic/alkene retention ratios, they could also be used to separate alkene *cis-trans* isomers. When aluminas are modified with cuprous chloride or silver nitrate, alkenes and aromatics can be held back completely, and the use of one of these packings as a subtractor was illustrated by two chromatograms of a Fischer–Tropsch Kogasin fraction. The first chromatogram, obtained on a sodium hydroxide modified alumina, gave all the alkane plus alkene peaks, and the second chromatogram, obtained on the same column to which a few millimetres' depth of cuprous chloride modified material had been added, gave the alkane peaks only. Apart from simple subtractor application in elution chromatography, the cuprous chloride and silver nitrate modified aluminas have been used to concentrate traces of alkenes and aromatics from large liquid volumes of alkane solvent. Recovery of the concentrates is achieved using gas-solid displacement chromatography. The main points to be observed with this procedure are (*a*) the displacer vapour should be more strongly adsorbed than the component to be displaced, and (*b*) the displacement fronts should be sharp—this requirement being met if the column packing is retained in the column by a flat sinter disk. When the total mass of concentrate is much less than 1 mg, it is found preferable to trap out the displacer front containing the displaced material and to re-inject it on to a gas-liquid elution column fitted with an ionization detector. The recovery of μg amounts of 1-hexene and 1- and 2-heptenes from 100 g of *n*-heptane using this procedure with 1-octene as displacer was described (see *Nature*, 199, 66, 1963). The same technique has been used successfully to recover traces of lower alcohols and ketones concentrated on sodium bromide modified material using *n*-butanol as displacer. With esters, hydrolysis occurs and the recovered material is a mixture of ester and alcohol.

Aldehydes polymerized on the column and traces cannot be recovered. The strong interaction between the adsorbates and the modified aluminas is such that appreciable amounts of adsorbate can be held on the surface with a negligible concentration in the gas phase. Thus as much as 20 mg of heptenes can be retained at 75° C on 1 g of alumina modified with silver nitrate. A column containing 50 g of packing can, therefore, hold 1 g of heptenes for displacement, and the technique can be used for preparing pure materials at this level of sample size. The specificity of the gas-solid elution columns for *cis-trans* isomers is found to be present in the strong interactions on the silver modified material, and a 1.5 ml charge of mixed 3-heptene isomers is separated into *cis*- and *trans*-fractions by displacement with 1-octene. In each case the fractions are recovered at better than 99 per cent purity. In order to obtain sharp displacement fronts, a sectional column is used having a 13 cm × 2 cm top section, a 10 cm × 1 cm centre section, and a 13 cm × 4 mm bottom section, in each of which the packing is retained by a sinter disk. High surface area solids such as carbon and silica-gel have also been used for preparative scale separations. The same apparatus holding 20 g of carbon is used to prepare ethyl propionate of better than 99.9 per cent purity. In the large-scale separation of ester mixtures and of hydrocarbon mixtures, it is found that at the change-over between pure components there is a high concentration of impurities together with a mixture of the two components flanking the change-over, and therefore the large-scale columns can be used either for the preparation of high-purity components or for the concentration of trace impurities.

"The Analysis of Surface Coating Resins by Pyrolysis-Gas Chromatography" was the subject of a paper read by Mrs. S. M. Rybicka, who emphasized the need for workers in such fields to be thoroughly conversant with the techniques of both pyrolysis and gas chromatography. She was of the opinion that many workers in the latter do not know enough about thermal pyrolysis to carry out investigations on polymers in an efficient manner, and this can easily lead to erroneous conclusions. Polymers can be divided into three main types, depending on the amount of hydrogen they contain: (*a*) those with a considerable amount, (*b*) those with a medium amount; (*c*) those with a small amount. These will give different products on pyrolysis. The thermal fission of polymers may also take place in three ways: (1) fission in the backbone of the chain which will give rise to free radicals which then become converted to stable compounds, (2) fission between the backbone and side groups, giving rise to double bonds in the chain, (3) highly cross-linked polymers, which give mainly carbonaceous material and a small amount of volatiles. The second method gives products which are ideally suited to analysis by gas chromatography, but analysis is more difficult in the third group. Pyrolysis may give some indication of the group to which a polymer belongs, but difficulties often arise when trying to discover what part the breakdown products play in the original polymer. The 'fingerprint' technique of German workers may lead to considerable errors if the full implications of the results are not properly understood. All results should be checked by infra-red spectroscopy, which in many cases will give all the information necessary without recourse to gas chromatography at all. The gas chromatographic technique should be properly used to give complete resolution of all the products obtained, and the pyrolysis should be carried out under carefully controlled conditions. The thermal stability of polymers may be investigated by pyrolysis at various temperatures. In summary, pyrolysis-gas chromatography can be a useful method of investigation, but it is best combined with infra-red spectroscopy or some other method in order to confirm the products obtained. Care must also be taken to ensure that, so far as possible, the products of reaction are stabilized before secondary reactions occur.

Dr. F. H. Pollard presented a paper on "Gas Chromatographic Studies of Compounds of Group IV A Elements", which detailed work carried out on the chromatography of organo-silicon, -germanium, -tin and -lead compounds. Most of the illustrative examples were from silicon chemistry, but many of the points made could have been quite easily taken from the chemistry of the tin compounds investigated. In the examination of such compounds, more specific problems occur than with the organic compounds of carbon itself, so that a general review of the factors likely to be involved is of interest. The injection of the sample may follow the standard procedure, but if it is readily hydrolysed, oxidized, or spontaneously inflammable, then special precautions must be taken. 'Celite' or 'Silocel' column supports often need acid-washing treatment, and complete removal of the acid is very important, especially in the analysis of chloro compounds. The stationary phase must obviously be inert to the compounds being analysed. In general, silicone oils or hydrocarbons like squalane or 'Apiezon' can be used. Other phases useful for specific purposes are: (*a*) dinonyl phthalate; (*b*) tritolyl phosphate, (*c*) 60 : 20 : 100 diethyl phthalate : silicone oil *E*301 : 'Celite', (*d*) silicone oil with a polypropylene glycol 400 plug, (*e*) 'Celite' having $n\text{C}_{16}\text{H}_{33}$-groups bonded directly to it, (*f*) nitronaphthalenes, which are particularly useful for lower chlorosilanes. As detectors, the katharometer, flame ionization gauge and gas density balance have proved to be the most useful. With the katharometer, there is a successive increase in molar response for the tetraalkyls of silicon, germanium, tin and lead, and also much less trouble than anticipated has been experienced owing to reactivity of compounds on the hot wire. The best design of flame-ionization gauge is one in which the plates are vertical. It has a high sensitivity and calls for very small samples, especially in cases such as tetramethyl tin, trimethyl ethyl silane, and similar germanium, tin and lead compounds, where a saturation effect begins at a very low level. This effect is thought to be due to actual ionization characteristics in the flame. The gas density balance has been found to be almost universal in its application since

PHYSICS [illegible]

the corrosive gases encountered so frequently in work of this type do not come into contact with the electrical parts It is found, however, that using such heavy molecular weight compounds of low boiling point, linearity is not observed except at very low concentrations—an effect illustrative of the kind of difficulty which may be encountered with work on unusual compounds The gas density balance has also been used for phosphorous chlorides, lower members of the phosphonitrilic chloride series and for a range of phosphonitrilic fluorides The problems met in the gas chromatography of organometallic compounds are well illustrated by the work described The compounds were chosen carefully so that they were amenable to study in this way, coupled with other physical methods such as infra-red spectroscopy Not only has gas-liquid chromatography enabled the analytical separation of materials to be carried out, but there have also been points of correlation between chromatographic behaviour and structure Dr Pollard emphasized the importance of gas-liquid chromatography in work of this kind, and advocated a bench-type chromatograph for every worker as standard equipment The application of gas-liquid chromatography to the groups investigated was well illustrated by slides In the case of the trimethyl silyl ethers, which hydrolyse easily and are thermally unstable on a katharometer wire, a maximum detector temperature of 150° C was used to preclude thermal breakdown, and the nitrogen carrier gas was carefully dried Although polyethylene glycol should be an ideal stationary phase for such a separation, the tendency to hydrolyse such compounds (which is virtually complete at 100° C) makes it necessary to use tritolyl phosphate or hydrocarbon phases which result in some loss of peak symmetry The methyl chlorosilanes have a narrow boiling range, and become far more liable to hydrolysis as chlorine replaces methyl The most satisfactory stationary phase is 60 20 100 diethyl phthalate silicone oil *E*301 'Celite' Preliminary pyrolysis and catalytic studies have been carried out on the methyl chlorosilanes and the trimethyl aryl silanes, and also work on rearrangement reactions involving interchange of alkyl groups using aluminium chloride as catalyst In the latter work, the number of alkyl groups used is such that it is possible to obtain 70 different products, many of which are isomeric and have closely similar boiling points Gas-liquid chromatography is virtually the only effective analytical method for such mixtures, and in order to identify individual components, most of the 70 possible compounds have been synthesized and their retention volumes determined Gas-liquid chromatography is also used for preparative work In conclusion, Dr Pollard paid tribute to similar work being carried out independently by the Oxford Group under Mr C S G. Phillips, and was able to report the good relationship and co-operation which exist between the two Groups

The last paper, on "Polymer Pyromania", by C E. Roland Jones and G E J Reynolds, was presented by Dr Reynolds He emphasized the widespread nature and application of polymers, and that pyrolysis combined with gas chromatography provided a useful method for analysis and investigation He distinguished between pyrolysis, by which he meant complete degradation to fragments, and stoving, which is a partial pyrolysis useful in thermal degradation investigations The advantages of the technique are its speed, simplicity and the use of small samples, but the interpretation of the results obtained is not always easy Very little work has been done in the quantitative field, and stringent conditions are necessary in order to obtain reproducibility of results. Four criteria are necessary to ensure this (*a*) pyrolysis must be rapid and complete, and optimum conditions must be secured in order to obtain this, (*b*) there should be a large heating area in relation to the sample, and the temperature rise should be as rapid as possible—the usual conditions used by Dr Reynolds were 700° C in 3 sec, (*c*) the possibility of gaseous diffusion must be kept to a minimum, (*d*) condensation of the products must be avoided Sample size is also very important and various workers have used samples varying from 10 mg to 20 μg, depending on the type of sample and ease of pyrolysis. It would seem that a thin film of sample is the most desirable form for rapid pyrolysis, although other forms have been used Gas chromatography for the identification of breakdown products should be supplemented by infra-red and ultra-violet analyses, and the products should be removed from the unit as quickly as possible to avoid secondary reactions taking place Slides of the pyrolysis unit and chromatograms of the breakdown products of various polymers were shown It was noted that the 'fingerprint' system mentioned by a previous speaker could, in some cases, be a useful means of identification

C E H KNAPMAN

ADVANCES IN PROGRAMMING AND NON-NUMERICAL ANALYSIS

A FEW years ago a digital computer was regarded simply as a device which could do difficult sums very fast—given the good offices of someone versed in the black arts The magical intermediary, the programmer, translated one's problem at great length into a special form of gibberish, known as 'machine code' The final programme typically bristled with flights of hair-raising ingenuity, acquired in years of struggle with acute shortage of machine time and storage space

The situation is rapidly changing The advent, first of 'autocodes', and more recently of advanced programming languages, makes it increasingly possible for the user to describe his problem in terms which come naturally to the human mind and to leave it to the machine to translate his description into its own terms Secondly, the enormous increase in speed and storage of the new generation of machines largely liberates the user from cramping preoccupations with time and space Finally the joint impact of these two developments is not only enlarging the power and range of the discipline known as 'numerical analysis' —the art and science of doing difficult sums—but has also thrown open the new territory of non-numerical analysis Here the problems do not present themselves in primarily numerical form and demand an approach based mainly on logical rather than on numerical manipulations

One of the ways in which the University of Oxford Computing Laboratory, under the directorship of Dr L. Fox, has responded to these developments has been by organizing a summer school on the theme "Advances in Programming and Non-numerical analysis" The course, which occupied the two weeks August 26–September 6, consisted of a series of some thirty lectures contributed by a panel of ten speakers In the first lectures, the general theme of the course was outlined by Prof S Gill (Manchester College of Technology and Ferranti, Ltd) and the scope of the new non-numerical applications of computers was indicated Some of these belong to areas dominated by the immediate practical need for automatic aids, such as machine translation or medical diagnosis For others, such as the discovery of new mathematical proofs, immediate utility is negligible, but the long-term impact on human thought and life is impossible to calculate

Theorem-proving was taken by one of the speakers, D C Cooper (Bloomsbury Computers, Ltd), for detailed survey, ranging from 'heuristic' techniques as utilized by the Rand Corporation group[1] to Hao Wang's recent systematic assault on the predicate calculus[2] Other selected topics were algebraic manipulation (P M Woodward, Royal Radar Establishment, Malvern), game-playing and automatic learning processes (D. Michie, University of Edinburgh) and information retrieval, machine translation and the mechanical construction of time-tables (R M Needham, University of Cambridge)

Discussion of these applications, sampled from the rising flood of non-numerical analysis, sufficiently revealed the acute dependence of such work on development of more powerful programming languages It was natural, therefore, that the lectures on programming, delivered by C Strachey and D W Barron, formed the backbone of the course The ground was prepared by an introductory account by Dr Woodward of the nature of LISP[3], the prototype of the LISt-Processing facilities which are now seen as virtually mandatory features of a programming language if it is to lend itself to non-numerical work

The talks which followed conveyed a special atmosphere of immediacy in being based on the present effort, now in full swing in Cambridge and London, to construct a language which promises in all respects to be one of the world's most advanced, namely, CPL (Combined Programming Language)[4] It was an exciting experience to be taken behind the scenes and shown work in progress in what is in effect a new, and potentially major, branch of mathematics

The relation of computational processes to the abstract formulations of mathematical logic was expounded by P Landin[5], who has based his ideas on the λ-calculus of Church More practical problems of implementation were dealt with in lectures by J M Foster (Royal Radar Establishment, Malvern) and J K Iliffe (Ferranti, Ltd)

The digital computer is a device for handling information So long as its sphere of operation is restricted to the type of information which lends itself to direct numerical expression, the term 'electronic brain' will remain a misnomer But the increasing deployment into non-numerical applications is beginning to change the look of things Concern has even been expressed—and on Dr L Fox's initiative this became a subject of lively discussion at the summer school—at the social dislocations to be expected if not only manual labour but intellectual labour too is to be taken over by machines

If first workmen and craftsmen, then clerks, and ultimately business executives, military strategists, medical diagnosticians, school teachers, political and economic planners, lexicographers, linguists, and even perhaps mathematicians themselves are to be swept aside as outmoded instruments in successive waves of the industrial revolution, What part will remain for *Homo sapiens* ? In the controversy provoked by the question, only two points of unanimity could be discerned (1) that it was right that computer scientists should from time to time give some thought to the social impact of their brain-children, (2) that an all-out national effort is called for to expand and improve our educational system in an attempt to keep pace with the technological explosion

The lectures are being collected together with the intention of publishing them in book form The book will be edited by Dr Fox, who, together with his co-sponsors Prof Gill and Mr Strachey, planned this venture and piloted it to its successful conclusion D MICHIE

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[2] Wang, H , *The Bell System Tech J* , **40** 1 (1961)

[3] McCarthy, J *Comm A C M* , 3, 184 (1960)

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A ROCKET-BORNE LOW-FREQUENCY PROPAGATION EXPERIMENT TO DETERMINE *D*-REGION ELECTRON DENSITIES

By DR J. E. HALL

Radio Research Station, Department of Scientific and Industrial Research, Ditton Park, Slough, Bucks

AND

K. BULLOUGH

Department of Physics, University of Sheffield

THIS article presents the preliminary results of a rocket experiment designed to determine the height distribution of electrons in the *D*-region of the ionosphere Two *Skylark* rockets were successfully flown at Woomera, Australia (137° E , 31° S , magnetic dip 63°), at 1351 L M T on September 20, 1962, and at 0050 L M T on August 15, 1962 In this article the results of the day-time experiment are presented A continuous wave of frequency 202 kc/s was transmitted from the ground and, in the lower ionosphere, was subject to refraction, absorption, reflexion and changes in polarization The aim of the experiment was to send a rocket through the resulting wave pattern to measure the variations in field strength, and hence to deduce the ionospheric parameters

The transmitter delivered approximately 500 W to a horizontal half-wave dipole aerial supported 10 m above the ground but, owing to ground losses, the effective radiated power was only about 10 W The rocket was equipped with three mutually perpendicular receiving aerials which were carried, folded, inside the nose cone during the early part of the flight when aerodynamic forces were great At a height of 67 km the nose cone was discarded and the aerials deployed to form two dipoles of length 4 3 m perpendicular to the rocket's axis and a monopole which, together with the instrument section of the rocket, formed a longitudinal dipole of similar length These aerials were necessarily very short compared with the wave-length, and consequently had a large capacitive impedance which depended on the properties of the ionosphere To obtain an accurate measurement of the wave-field independent of the aerial impedance, it was necessary to use receivers with a very large input impedance

Three separate receivers were used, and, by means of telemetry, a record was made on the ground of the amplitudes of the received signals Fig 1 shows a section of the record obtained The orientation of the rocket was continuously changing during the flight and the corresponding changes in the E M F received on any one aerial obscured those caused by the amplitude variations of the wave-field for example, the deep minima in the records of Fig 1 were produced when the corresponding aerial was perpendicular to the electric field in the wave

When the preliminary analysis reported here was made, the attitude of the rocket throughout the flight was unknown, and a scalar field quantity $F = \sqrt{E_1^2 + E_2^2 + E_3^2}$, which was independent of the attitude, was formed from the E M F s E_1, E_2 and E_3 received on the three aerials This quantity F is plotted on a log scale in Fig 2 as a function of height From this curve it is noticeable that:

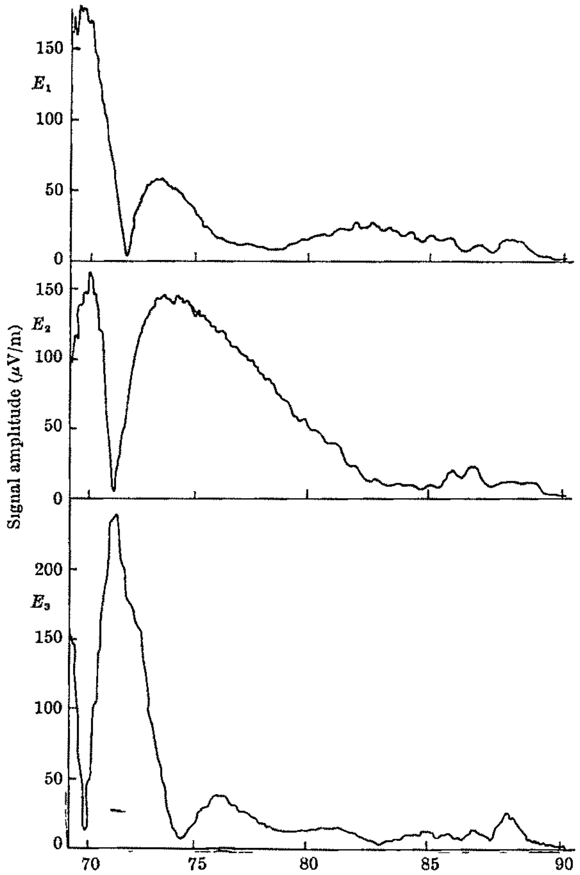

Fig 1 The height variation in the signal amplitude received on three orthogonal aerials

(*a*) ionospheric absorption causes the wave amplitude to decrease by a factor of ten between 70 and 80 km, (*b*) above 80 km the reflected wave was strong enough to produce a well-defined standing wave, (*c*) the wave-length of the standing wave increased with increasing height as the refractive index of the ionosphere decreased, (*d*) total reflexion occurred at a height of about 89 km

The results were interpreted in the following way A horizontally stratified model ionosphere was postulated in which both the electron concentration and collision frequency varied with height This was used to calculate the wave-field which would be set up in the ionosphere by a plane wave with the polarization of the transmitting aerial and incident along the line of sight from the transmitter to the rocket The calculation was made using the method described by Pitteway[1] and is based on a full-wave solution of the wave equation The model ionosphere was repeatedly adjusted until it yielded results closely resembling those deduced from the experiment

In practice it was often helpful to use the simpler ideas of magneto-ionic theory as a guide when fitting the measured field For example, as preliminary calculations had shown that the most important reflexion was near the level where $X = 1 + Y$ (the symbols have their usual meaning, see ref 2), it follows that the electron density at the reflexion height was about 4,300 cm^{-3} Furthermore, the roots of the Booker quartic could be used to calculate the spacing between the maxima and minima of the standing wave pattern and so give an approximate profile suitable for use in the initial full-wave calculation

The reflexion height and the spacing between maxima and minima depend on the real part of the refractive index which is fairly insensitive to the value of the collision frequency, but, to fit other features of the wave-field, it is necessary to know the effective collision frequency ν_{eff} as a function of height (ν_{eff} is the collision frequency appropriate to the Appleton–Hartree equation) To obtain this, the theoretical relationship $\nu_{\text{eff}} \propto \rho T$, where ρ and T are the density and temperature of the neutral air[3], has been used, and ρ and T have been taken from the 1961 *Cospar International Reference Atmosphere*[4] The constant of proportionality was obtained from the present observations as follows The depth of modulation in the standing wave pattern near the reflexion-level depends on both the collision frequency and the gradient of electron density As the latter had been determined from the spacing of the pattern, the collision frequency which produced the correct modulation depth was easily found after a few trials of the full-wave programme Molmud[3] also shows that the ratio between ν_{eff} and the simple kinetic theory value, ν, depends on whether ν is greater or less than the angular wave frequency As these become equal within the height interval of interest, ν_{eff} has been allowed to vary smoothly from one limiting value to the other between 75 and 95 km The height-distribution of

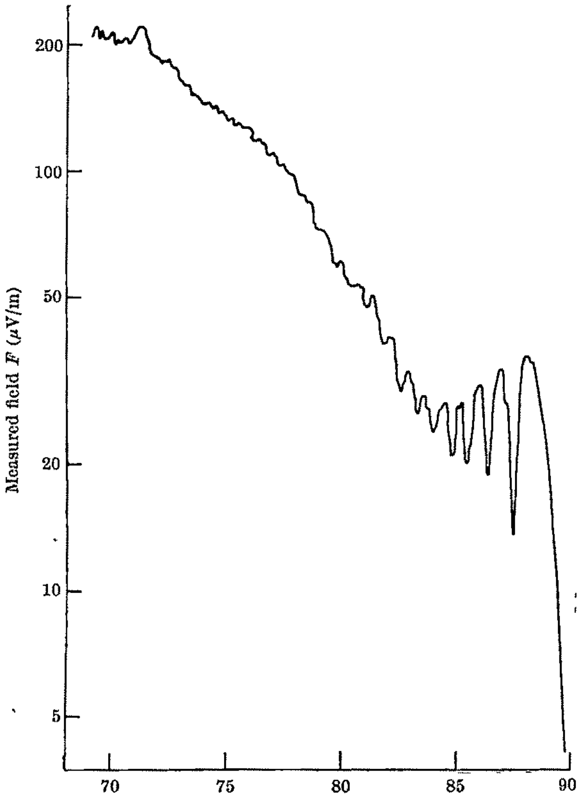

Fig 2 Observed scalar field $F = \sqrt{E_1^2 + E_2^2 + E_3^2}$ versus height

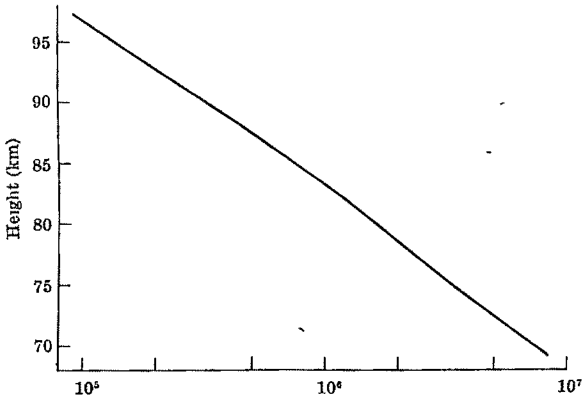

Fig 3 The height variation in the effective collision frequency, ν_{eff}, at 202 kc/s

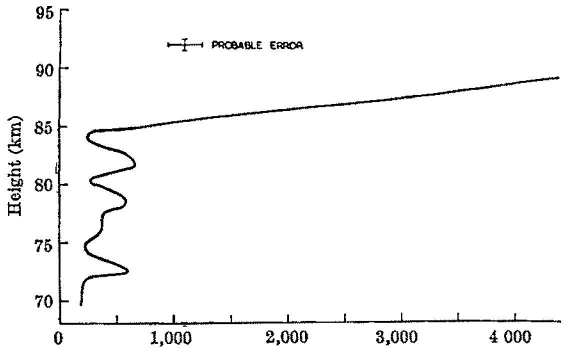

Fig. 4. Height variation of electron density deduced from the rocket experiment

the effective collision frequency at 202 kc/s which has been used in these calculations is given in Fig. 3.

The most successful electron density model is shown in Fig. 4 and the theoretical variation of the scalar field *F*, calculated using this model, is given by the continuous line in Fig. 5. This can be compared with the observed variation indicated by the dotted line. An interesting feature of the height-distribution is the contrast between the steep gradient of electron density above 84 km and the smaller variations below. We cannot attach too great a significance to the small fluctuations in electron density below 84 km since the estimated error in the density (± 150 cm^{-3}) is of similar magnitude. However, the experimental results correspond more closely to the wave-field calculated using the curve of Fig. 4 rather than to the wave-field calculated from a smooth curve which ignores the fluctuations.

A more detailed analysis of the results will be possible when information is available about the attitude of the rocket and, in particular, a knowledge of the polarization changes occurring below 80 km should lead to a more accurate estimate of the electron concentration at those heights.

A rocket experiment such as this involves too many personnel to mention individually, but we thank those, in Britain and in Australia, who have been concerned with the preparation of the equipment, the launching of the rocket and the reduction of the data.

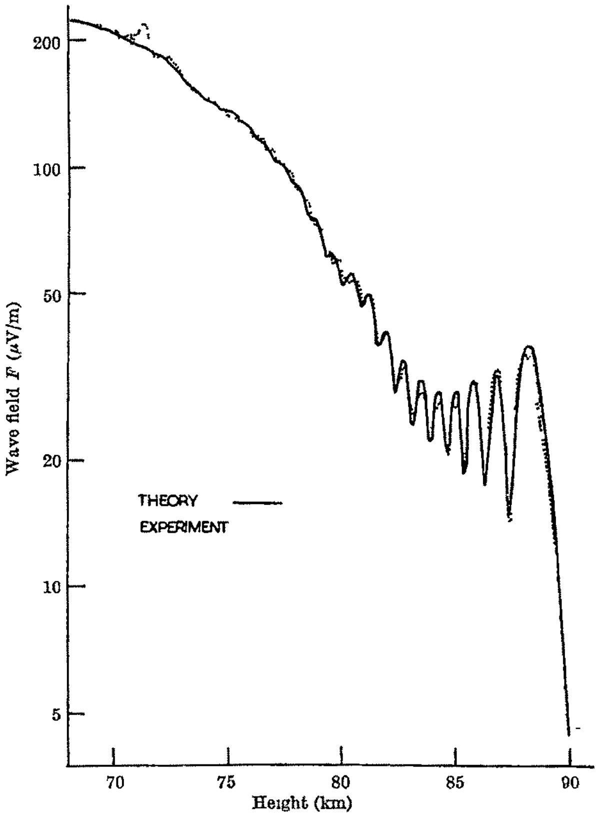

Fig. 5. Continuous line, calculated height variation of the scalar field *F* for the electron collision frequency and electron density profiles of Figs. 3 and 4; dotted line, observed height variation of the scalar field *F*

The work described here was carried out as part of the programme of the Radio Research Board, and this communication is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

[1] Pitteway, M. L. V., *Proc. Roy. Soc.* (in the press).
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OXYGEN ISOTOPIC FRACTIONATION IN THE WATER OF CRYSTALLIZATION OF GYPSUM

By DR. R. GONFIANTINI and J. C. FONTES*
Laboratorio di Geologia Nucleare del C.N.E.N., Pisa

THE isotopic composition of the water of crystallization of gypsum samples can provide information of geochemical and geological interest. For the correct understanding of such isotopic data, it is necessary to know the fractionation factor between the water of crystallization of the gypsum and the water in which the gypsum is deposited. Here, we give some results on the experimental determination of this fractionation factor. At present they are limited to $^{18}O/^{16}O$ measurements, but this research will be extended to D/H measurements.

The oxygen isotopic composition of water samples was determined by equilibrating them with carbon dioxide at 25° C, and then analysing the carbon dioxide with a mass spectrometer Atlas *M*86. The isotopic results are expressed in terms of difference in parts per mil ($\delta^{18}O$) of the $^{18}O/^{16}O$ ratio in the sample and in the 'standard mean ocean water' as defined by Craig[1]. The standard error of the isotopic measurements of water is ±0·1 $\delta^{18}O$ units for samples not too different from the working standard, as in this case.

The water of crystallization was extracted by slowly heating the gypsum sample (20–30 g) under vacuum to 400° C in a 'Pyrex' vessel, and water was trapped at liquid nitrogen temperature. Usually, with commercial pure crystalline calcium sulphate or with optically pure natural gypsum, yields of 100 per cent were obtained. With other natural samples, yields were usually lower, depending on the amount of impurities.

The reproducibility of the isotopic measurements of water of crystallization has been tested using a commercial pure crystalline calcium sulphate (product of C. Erba RP) and on optically pure natural gypsum. Four samples of the former gave respectively the following results (in $\delta^{18}O$): $-4·4$, $-4·5$, $-4·6$, $-4·6$ (average $-4·5$). Ten samples of the latter gave the following results (in $\delta^{18}O$): −3.6, −3.6, −3.8, −3.9, −3.9, −4.0, −4.1, −4.1, −4.2, −4.2 (average −3.9) respectively. On the basis of these results, we assume that the isotopic measurements of water of crystallization are affected by an error of ±0.3 $\delta^{18}O$ units.

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* Present address: Laboratoire de Géographie Physique et de Géologie Dynamique à la Sorbonne, Paris.

Isotopic exchange between oxygen of the sulphate and oxygen of the water of crystallization can be excluded during the extraction process. In order to prove this, two different tests were made.

In the first, anhydrous calcium sulphate was mixed with water of known isotopic composition in the same proportion with regard to the sulphate as in the gypsum. Afterwards, water was extracted in the method described here, as from a gypsum sample. This water preserved its original isotopic composition, not showing any appreciable exchange with the oxygen of the sulphate.

In the second test 71 g of anhydrous calcium sulphate were converted into gypsum at room temperature with 100 ml. of solution of 0.5 M sodium sulphate, following the method of Conley and Bundy[2]. The results of the isotopic analyses were (in $\delta^{18}O$): water before gypsification (100 ml.), −7.1, water after gypsification (81.2 ml.), −8.3, water of crystallization (18.8 ml.), −1.8. The isotopic balance existing between the first and the last two waters excludes any exchange with the oxygen of the calcium sulphate[3].

For the determination of the isotopic fractionation factor between water of crystallization of gypsum and water in which the deposition occurs, two sets of experiments were carried out.

(1) The aim was to obtain gypsum by evaporation of an aqueous solution of calcium sulphate keeping the isotopic composition of the water constant during deposition.

From the reservoir 1 (Fig. 1) distilled water was admitted in the container 4 through the flask 2, on the top of which the overflow 3 was fixed. The water in 4, stirred by the magnetic stirrer 5, was evaporated using the infra-red lamp 6, the level of the water being kept constant by means of the overflow. During the process, the temperature was periodically checked and it was found to be constant to about ± 1° C.

After about 100 h, the isotopic composition of the water in 4 attained a constant value (Fig. 2). When this isotopic steady state was reached, a solution of calcium sulphate (obtained by dehydration of pure gypsum) was introduced into 1 and after some hours gypsum began to crystallize in 4. During crystallization, the water in 4 showed a fairly constant isotopic composition (Fig. 2).

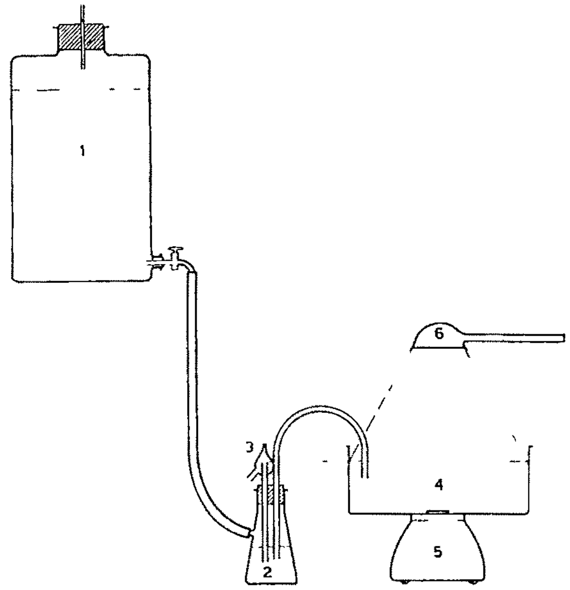

Fig. 1. Apparatus for preparing gypsum by evaporation of a calcium sulphate solution with constant isotopic composition. 1, reservoir, 2, flask with overflow 3, 4, container, 5, magnetic stirrer, 6, infra-red lamp

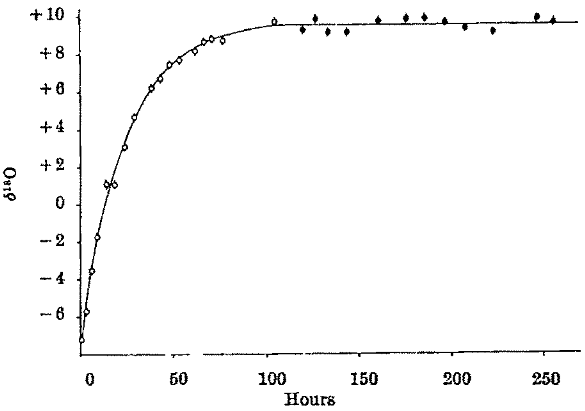

Fig. 2. Isotopic composition of water in the container 4 during the experiment illustrated in Fig. 1. Open circles, before the beginning of the crystallization of the gypsum, filled circles, during the crystallization of the gypsum

When a sufficient amount of gypsum was obtained, it was filtered and dried, and its water of crystallization was extracted and analysed for its $^{18}O/^{16}O$ ratio. Isotopic results of two experiments at 57° and 47° C are given in Table 1.

Table 1. OXYGEN FRACTIONATION FACTOR BETWEEN WATER OF CRYSTALLIZATION OF GYPSUM AND MOTHER-WATER

Temperature °C	Method	Oxygen isotopic composition ($\delta^{18}O$) Water of crystallization	 Mother-water	α
57	i	+12.7	+9.5	1.0032 ± 0.0006
47	i	+14.2	+11.3	1.0029 ± 0.0006
37	ii	−3.3	−7.2	1.0039 ± 0.0004
25	ii	−3.3	−7.4	1.0041 ± 0.0004
20	ii	−3.2	−7.2	1.0040 ± 0.0004
17	ii	−3.3	−7.2	1.0039 ± 0.0004

i, Method by evaporation in isotopic steady state, ii, method of gypsification (ref. 2).

Error for experiments following the method (i) is higher, due to the fluctuations of the isotopic composition of the evaporating water.

(2) For lower temperatures the preceding technique would require too much time because of the low evaporation rate. For this reason, we followed the method of gypsification proposed by Conley and Bundy[2].

About 30 g of anhydrous calcium sulphate were converted to gypsum by stirring with about 1,000 ml. of solution of 0.5 M sodium sulphate in a closed vessel (to prevent evaporation). As catalyst of gypsification, sodium sulphate was preferred to potassium sulphate because in the hydration sphere of Na^+ no isotopic fractionation occurs in relation to the solvent[4], so that no disturbances in isotopic composition of the solvent are expected.

The temperature was kept constant by a thermostatic bath. After 24 h the conversion was complete, the gypsum was filtered and dried, and its water of crystallization was extracted and analysed. Experiments have been made at 37°, 25°, 20° and 17° C, and the isotopic results are given in Table 1.

In both methods, the purity of the gypsum was checked by X-ray diffractometry, practically 100 per cent purity of gypsum was obtained.

The fractionation factor α is defined as

$$\alpha = \frac{(^{18}O/^{16}O)_{cw}}{(^{18}O/^{16}O)_{mw}} = \frac{1000 + \delta^{18}O_{cw}}{1000 + \delta^{18}O_{mw}}$$

(*cw*, water of crystallization, *mw*, mother-water).

The two methods of preparation of gypsum give slightly different values of α: in the first one we found 1.003, while in the second one 1.004. Taking the errors into account, we cannot determine with any degree of certainty whether the foregoing difference can be attributed to a temperature effect on the α-value in the temperature range in which the experiments have been carried out; however, this effect would be little and, in any event, would be contained in the experimental error

Our results show that the water of crystallization of gypsum is enriched in heavy isotopes in relation to the water in which gypsum was deposited This can be explained admitting that the crystallization water of gypsum is related to the water of the hydration sphere of Ca^{++}, which is presumably enriched in heavy oxygen isotope in relation to the solvent, because of the difference in vibration frequencies of the two isotopic forms of water along the bonds formed between water and cation[4]

The α-value found by us is in good agreement with that reported by Baertschi[5], who found $\alpha = 1\ 0035$, but disagrees with the data reported by Teis[6], who found that water of crystallization was depleted in heavy oxygen content in gypsum samples obtained chemically from solutions of calcium chloride and sodium sulphate This method did not give us reproducible results, and it was abandoned. Nevertheless, we should like to point out that in seven experiments of this kind, we found α-values of 1 0069–1 0029, always showing an enrichment in oxygen-18 in the water of crystallization

Assuming, on the basis of results reported in Table 1, that α is constant in the range of temperatures in which gypsum deposition occurs in Nature, it becomes possible to deduce from isotopic analyses of the water of crystallization the isotopic composition of the water in which gypsum has been deposited This can provide some important data on the environmental conditions of deposition in fact, the isotopic composition allows one to distinguish fresh waters from marine ones and to obtain information on the evaporation

Two facts have to be taken into account

(1) Gypsum could have been dehydrated and rehydrated during its geological history in such a case we are only dealing with the rehydration water which will probably be a meteoric one

(2) Isotopic exchange can occur with meteoric water percolating in the gypsum deposit Information about the kinetics of this exchange reaction is reported by Teis[6]

These difficulties can be overcome in some cases by a comparative examination of the isotopic composition of the gypsum water of crystallization and of the limestone associated with gypsum deposits, and utilizing all the available geological data From a study of this kind on the well-known gypsum of Paris, the origin of which was still under discussion, it was found that the fossil basin where deposition occurred was supplied by fresh water[7]

This work was carried out under contract with EURATOM —U L B—C N E N, No 013-61-7 *AGEC*

[1] Craig, H , *Science*, 133, 1833 (1961)

[2] Conley, R F , and Bundy, W M , *Geochim et Cosmochim Acta*, 15, 57 (1958)

[3] In this experiment the observed value of the fractionation factor was 1 0065, higher than in the other cases reported in Table 1, this could be due to the small amount of water in proportion to the calcium sulphate and to the bad stirring of the resulting mixture This could perhaps be responsible for the fact that isotopic equilibrium between gypsum and the mother-water was not completely attained

[4] Taube, H , *J Phys Chem* , 58, 523 (1954)

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FRACTURE OF LIQUIDS IN SHEAR

By J F HUTTON
"Shell" Research, Ltd , Thornton Research Centre, P O Box 1, Chester

SOLIDS fracture in both tension and shear, and it is generally accepted that liquids fracture in tension (an effect referred to as cavitation) but not in shear For some years it has been recognized that an instability occurs during the flow of non-Newtonian visco-elastic materials, for example, in the flow of molten high molecular weight polymers through tubes[1] and in cone-and-plate viscometers[2], and in the flow of soft bitumens[3] and silicone fluids[4] in cone-and-plate viscometers The purpose of this article is to present experimental results which, when interpreted in conjunction with a simple theory, demonstrate that liquids can fracture in shear The experiments were carried out on silicone fluids in two types of cone-and-plate viscometer

Fig 1 shows a typical set of results obtained with the Ferranti–Shirley cone-and-plate viscometer[5] The material is *MS* 200/30,000 silicone fluid (a polydimethylsiloxane manufactured by Midland Silicones) which has a viscosity at 30 0° C of 288 poise at low rates of shear In an experiment the rate of shear was maintained constant and the shear stress was determined as a function of time Experiments were carried out using the different values of rate of shear shown on the right of the diagram It is seen from the figure, ignoring for the moment the dotted curves, that as the rate of shear increases from the lowest value of 184 sec^{-1} the shear stress increases The stress increases less than proportionately to the shear rate, showing that the silicone is non-Newtonian At rates of shear below 900 sec^{-1} the stress is stable for at least 5 min shearing, but over the relatively narrow range of 900–1,000 sec^{-1} there is a marked transition from stable to unstable flow The instability is revealed as a fall in shear stress the higher the rate of shear the shorter the time lapse before the fall occurs and, possibly, as indicated by other experiments, the greater the fall When the shear stress nears equilibrium it oscillates considerably The vertical lines on the curves indicate the extent of the oscillation

Explanations of the fall in shear stress are usually in terms of thixotropy or of structural breakdown[2,3], with the implication that the extent of breakdown is uniform throughout the sample The results to be presented here show that uniform breakdown is unlikely The breakdown is believed to be localized on a conical fracture surface which starts at the periphery of the sample and grows radially inwards The shear stress falls because the effective shearing radius decreases as the fracture grows

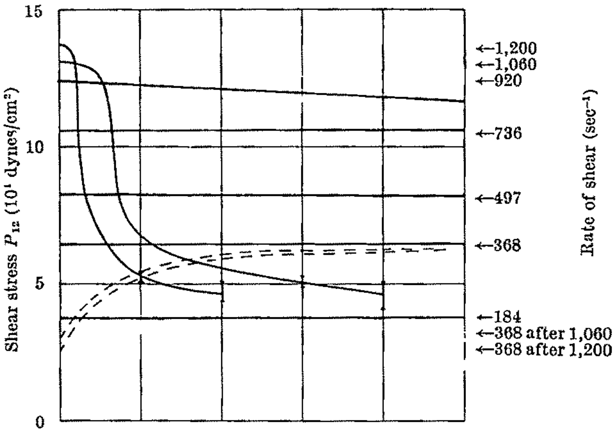

Fig 1 Shear stress as a function of time, illustrating the onset of flow instability and the recovery during stable flow after a period of unstable flow Ferranti–Shirley viscometer, 1/3° angle, 1 cm radius cone, 30 0° C, *MS* 200/30,000 silicone

Since the silicone fluid is a liquid, the fracture would be expected to heal at stresses below the transition. The dotted curves in Fig. 1 show the manner in which the stress increases after the rate of shear has been rapidly reduced from 1,060 sec^{-1} and 1,200 sec^{-1} to 368 sec^{-1}. The stress in each case recovers completely to the normal equilibrium value corresponding to the rate of shear of 368 sec^{-1}. Molten polyethylene also shows such a stress recovery[2].

At still higher rates of shear the sample aerates and is thrown out of the shearing zone. In this event recovery is not observed.

Near the point of instability the Weissenberg effect is quite pronounced, the excess liquid around the viscometer cone climbing a little way up the outside of the rotating cone. In addition, the normally curved meniscus of the excess liquid divides into two, one enclosing a collar of liquid on the cone and the other enclosing a bank of liquid on the stationary plate. This division suggests fracture. With large diameter (7·5 cm), large-angled cones (1° angle between the cone surface and the surface of the flat plate) the gap between the shearing surfaces at the periphery of the cone is large enough to permit the observation of a division of the liquid inside the main shearing zone.

The temperature rise of the liquid measured at the surface of the stationary plate is negligibly small, thus indicating that the observed fall of stress is not due to heating effects. Tests with the near-Newtonian liquid treacle, having a viscosity of about 150 poise at 30° C, also prove that viscous heating plays no part. Treacle shows no fall of stress at power dissipations of up to three times that at which a silicone of similar viscosity fractures.

Nor is the effect due to turbulence. Low viscosity Newtonian mineral oils can be tested in the cone-and-plate viscometer without turbulence at Reynolds numbers many orders higher than the Reynolds numbers at which the silicone fluids fracture.

Rather, the effect appears to be one of fracture, and the observation of a Weissenberg effect indicates that it is linked with the elasticity of the liquid. The following theory is based on these conclusions.

An elastic solid in a state of strain possesses elastic energy which is released when the solid is allowed to recover. Similarly a visco-elastic liquid in a state of flow possesses elastic energy, and when the shear is stopped, the liquid recoils because of the release of this energy. It is postulated that when the total elastic energy contained in the sheared sample of liquid exceeds a critical value the liquid will fracture, and that the fracture surface in a cone-and-plate viscometer is a cone with apex coincident with that of the viscometer cone. It is further postulated that fracture will occur only when the total elastic energy is at least sufficient to supply the energy requirement of the new surface formed. Therefore fracture will occur when

$$fGS_e^2V/2 \geqslant 2\pi R^2\gamma \qquad (1)$$

where G is the shear modulus, S_e, the recoverable elastic shear strain and γ, the surface tension of the liquid, V is the volume of the sample, determined by the cone radius, R, and angle φ, f is a fraction representing the part of the elastic energy which is converted to surface energy. For the uniform laminar shearing motion obtaining in a cone-and-plate viscometer there is a choice of expressions for G and S_e. Jobling and Roberts[6] quote Weissenberg's theoretical results as follows

$$G = P_{12}^2/(P_{11} - P_{22}) \qquad (2)$$

and

$$S_e = (P_{11} - P_{22})/P_{12} \qquad (3)$$

where $(P_{11} - P_{22})$ is the normal stress difference and P_{12} is the shear stress acting in the liquid. Both are measurable.

However, Lodge[7], on the basis of his network theory, calculates the values

$$G = 2P_{12}^2/(P_{11} - P_{22}) \qquad (4)$$

and

$$S_e = (P_{11} - P_{22})/2P_{12} \qquad (5)$$

Substitution into equation (1) leads to the result that fracture will occur when

$$(P_{11} - P_{22}) \geqslant n\gamma/R\varphi \qquad (6)$$

where n is $6/f$ or $12/f$ depending on whether the Weissenberg or the Lodge theory is used.

For a liquid in Newtonian flow the elastic energy is zero $(P_{11} - P_{22})$ is zero and fracture in shear does not occur. For a liquid that behaves visco-elastically $(P_{11} - P_{22})$ increases with rate of shear and fracture will begin to be observed at the critical value given by

$$(P_{11} - P_{22})_c = n\gamma/R\varphi \qquad (6a)$$

The shearing must be allowed to continue sufficiently long for the equilibrium theory to apply.

Preliminary results have been obtained to prove the validity of the theory. For polydimethylsiloxanes of 1 poise viscosity and greater, γ is practically independent of molecular weight and lies between 21 and 22 dynes/cm (say, 21·5 dynes/cm) (ref. 8). The equation (6a), therefore, predicts that all higher molecular weight polydimethylsiloxanes will fracture at the same critical normal stress difference. It also predicts a linear relationship between $(P_{11} - P_{22})_c$ and $1/R\varphi$. The results given in Table 1 were obtained with the Ferranti–Shirley cone-and-plate viscometer on which only the shear stress P_{12}, but not the normal stresses, can be measured. Results are given for the smallest shear stresses at which fracture was observed in a 5-min shearing period. In making the measurements care was taken to remove excess liquid round the cone. The effect of excess liquid was to increase the critical fracture stress to an extent that increased with decrease in cone radius. On the other hand, the amounts of excess liquid involved had no influence on the shear stresses. The curves in Fig. 1 were obtained with a small excess of test liquid, and they indicate a critical fracture stress of about 12 × 10^4 dynes/cm^2, whereas the correct value (Table 1) is 8·0 × 10^4 dynes/cm^2. Results are estimated to be accurate within about 10 per cent. The low values for *MS* 200/100,000 are significantly different from the values obtained for the other three fluids. According to measurements made by Benbow and Howells[9] the values of $(P_{11} - P_{22})$ corresponding to shear stresses in the range 5·0–6·0 × 10^4 dynes/cm^2 lie between 3·5 and 5·0 × 10^4 dynes/cm^2. This correspondence, to a first approximation, is not dependent on molecular weight or temperature. Similarly, the shear-stress range 7·0–8·5 × 10^4 dynes/cm^2 corresponds to a normal stress range of about the same values, say, 7·0–8·5 × 10^4 dynes/cm^2.

Hence the predictions that the critical normal stress difference is independent of molecular weight but dependent on $R\varphi$ are supported.

Normal stresses have been measured on the 2,000-poise silicone in the Weissenberg rheogoniometer[3,6]. The

Table 1. SHEAR STRESSES AT THE ONSET OF FRACTURE FOR SILICONE FLUIDS IN THE FERRANTI–SHIRLEY VISCOMETER (TEMP. 30·0° C)

Silicone fluid		Shear stress at fracture (10^4 dynes/cm^2)	
Designation	Zero shear viscosity (poises)	Medium cone } R=2·0 cm, φ=0·00603 rad	Small cone } R=1·0 cm, φ=0·00606 rad
MS 200/12,500	108·5	5·5	8·5
MS 200/30,000	288	5·5	8·0
MS 200/100,000	944	5·0	7·0
MS 200/200,000	about 2,000	6·0	8·5

rate of shear is adjustable in increments of about 25 per cent on this instrument, so the onset of fracture cannot be exactly pinpointed In the results given in Table 2 the observed limits of normal stress difference correspond to rates of shear at which fracture was and was not observed after 5-min shearing. No detailed assessment has been made of the accuracy of these results

Table 2 NORMAL STRESS DIFFERENCES AT THE ONSET OF FRACTURE OF *MS* 200/200,000 SILICONE FLUID IN THE WEISSENBERG RHEOGONIOMETER (TEMP , 20° C)

R cm	φ rad	Normal stress difference (10^4 dynes/cm^2)
1 25	0 018	2 5 3 5
3 65	0 033	0 40 0 55

To find the dependence on $R\varphi$, values of $(P_{11} - P_{22})_c$ are plotted against $1/R\varphi$ in Fig 2 This figure includes the results in Table 2 and those in Table 1 converted by means of Benbow and Howells's results, the validity of the conversion being supported by our own results on the 2,000-poise fluid

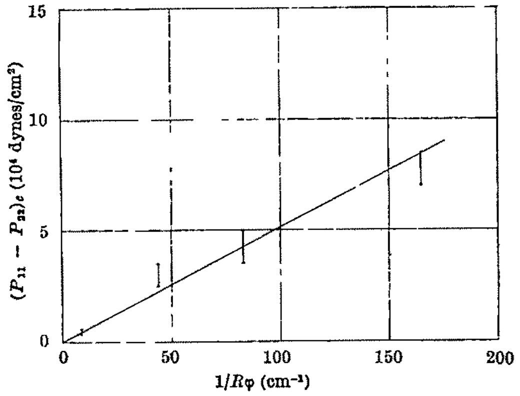

Fig 2 Variation of critical normal stress difference at fracture with the product of cone radius and angle for silicone fluids in cone-and-plate viscometers

The linear relationship predicted by equation (6*a*) is seen to hold reasonably well over a 20-fold range of values of $R\varphi$ From the slope and surface tension, n is found to be about 24 Therefore it follows that fracture will occur when the elastic energy possessed by the liquid is about four times, by Weissenberg's theory, or twice, by Lodge's theory, the free energy needed for forming the new air/liquid interface

Thus, the concept of a limit of elastic shear energy for a liquid has led to a theory that predicts the conditions for the onset of flow instability of silicone fluids in cone-and-plate viscometers The equations developed contain uncertain terms Thus, there is uncertainty about the choice of theory to calculate the elastic shear energy, the choices available leading to a difference of a factor of two There is uncertainty also about the fraction of elastic energy available for forming the fracture surfaces However, by combining these uncertainties into one adjustable dimensionless number, an equation is obtained which, after calibration to determine the number, can be used to predict quantitatively the fracture conditions

A practical use for the theory in the laboratory lies in the selection of a viscometer cone that will permit the measurement of viscosity over the widest possible range of shear rate In choosing a cone a compromise must be reached between a low value of $R\varphi$ to avoid flow instability and a high value of $R\varphi$ to achieve accuracy of measurement It is expected that the fracture phenomenon will be important in a number of other applications of fundamental and technological importance, but its occurrence will depend on the geometry of the device in which the liquid is sheared

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NATURE OF THIN OXIDE FILMS ON METALS AS REVEALED BY WORK FUNCTION MEASUREMENTS

By C. M. QUINN and DR M W. ROBERTS
Department of Chemistry, The Queen's University of Belfast

THE outstanding feature of the results so far obtained for the changes in work function ($\Delta\varphi$) resulting from the interaction of oxygen with metal surfaces is the great variation that exists for any given metal This is particularly true for oxygen on nickel, and Table 1 summarizes the values of $\Delta\varphi$ reported by different investigators at various temperatures

Table 1 SUMMARY OF $\Delta\varphi$ VALUES FOR OXYGEN ON NICKEL OBSERVED BY VARIOUS INVESTIGATORS

$\Delta\varphi$ (V)	Temperature (°C)	Technique	Ref
−1 6	−195	capacitor	1, 2
−0 55	25	photoelectric	3
−1 4	25	diode	4
~ −0 7	25	capacitor	5
−0 55	25	capacitor	6

We have investigated changes in work function during the interaction of oxygen with a number of metal films which have been prepared under high vacuum conditions (10^{-7}–10^{-8} mm) The general procedure was to admit a small dose of oxygen and to measure the associated change in work function, doses were added until the pressure was about 10^{-2} mm and $\Delta\varphi$ constant[5] In some experiments the influence of suddenly increasing the pressure from 10^{-2} mm to about 50 mm with the film at 20° C was also investigated Measurements were made by a capacitor technique and it is assumed that the work function of the gold reference electrode did not vary during an experiment

Changes in work function have been measured with three different metal film surfaces (nickel, iron and molybdenum) between −195° and 25° and up to an oxygen pressure of 50 mm In the case of nickel, only at −195° was $\Delta\varphi$ reproducible to better than 50 mV, at an oxygen pressure of 10^{-4} mm $\Delta\varphi$ was −1 45 V and became slightly more positive (−1 25 V) at 10^{-2} mm This positive contribution to $\Delta\varphi$ of about +0 2 V could be easily reversed on evacuating the cell to 10^{-6} mm Table 2 summarizes the data obtained with nickel under various conditions, clearly $\Delta\varphi$ is very sensitive to the oxygen pressure and temperature A negative value of $\Delta\varphi$ implies an increase in the value of the work function of the clean metal

In the case of iron films, $\Delta\varphi$ was reasonably insensitive to the oxygen pressure and to temperature The value of

Table 2 CHANGES IN THE WORK FUNCTION OF NICKEL FILMS UNDER DIFFERENT CONDITIONS OF OXYGEN INTERACTION

$\Delta\varphi_1$	$\Delta\varphi_2$	$\Delta\varphi_3$	$\Delta\varphi_4$
−1 45	−0 7 to −0 8	−0 9 to −1 0	−1 2

$\Delta\varphi_1$ refers to dose-wise interaction at −195°, $\Delta\varphi_2$ dose-wise interaction at 25°, $\Delta\varphi_3$ interaction at 25° after initially adsorbing oxygen at −195°, and $\Delta\varphi_4$ the addition of 50 mm of oxygen at 25°

$\Delta\varphi$ was −1 6 V at 10^{-2} mm and −195°, was virtually the same at 10^{-2} mm and 25° and did not change when the pressure was increased to 50 mm Molybdenum films showed similar insensitivity to ambient conditions and $\Delta\varphi$ was −1 8 V

With iron we know that oxygen incorporation into the lattice (oxidation) is extensive at low temperatures and pressures[7], incorporation is also thought to occur with nickel at 25° and an oxygen pressure of 10^{-2} mm (ref 8) In the case of molybdenum a stable chemisorbed layer is virtually complete at 10^{-2} mm and 25° and from which incorporation is unlikely $\Delta\varphi$ with molybdenum, therefore, refers to a chemisorbed layer only It is, therefore, necessary to rationalize why with iron $\Delta\varphi$ is insensitive to oxygen pressure but with nickel the reverse is true, yet both incorporate oxygen at 25°

The rate at which iron films oxidize is rather insensitive to changes in oxygen pressure[7] at 25° Thus, variation in oxygen pressure will make little difference to the oxide already formed on the iron surface and, therefore, will have a negligible effect on the work function of the oxide

Nickel oxide is a *p*-type semiconductor in the bulk form and the conditions at the oxide–gas interface are extremely important in oxide growth The rate of formation of such *p*-type oxides is known to be very sensitive to pressure (for example, Cu_2O where the pressure dependence of the rate is about unity[9]) and the formation of cation vacancies with associated positive holes is known to play an important part in oxide growth (equation (1))

$$2e + \tfrac{1}{2}O_2(\text{gas}) \rightarrow O^{2-}(\text{ads}) \rightarrow Ni^{2+}O^{2-} + \square\ (Ni^{2+}) \qquad (1)$$

where $\square\ (Ni^{2+})$ is a cation vacancy which will have associated with it a positive hole

Consequently, if by changing the oxygen pressure we effect extensive oxide growth and the new oxide has a different stoichiometry to that already existing on the surface we might expect a change in $\Delta\varphi$ The $\Delta\varphi$ of −1 45 V for oxygen on nickel at −195° is, therefore, considered to reflect the influence of chemisorbed oxygen on the work function of the metal The positive effect of +0 2 V is due to adsorbed molecular oxygen At 25° a semiconducting oxide is formed, and the extent to which oxidation occurs, and more particularly the density of acceptor states associated with excess oxygen, determines the observed value of $\Delta\varphi$ Clearly this is a kinetic phenomenon, and the extent to which the defects (and consequently acceptor states) are formed or annihilated will determine the change in work function observed We, therefore, find extensive variability of $\Delta\varphi$ even at 25° and 10^{-2} mm, $\Delta\varphi$ tending to be more negative if the chemisorbed layer has been first formed at −195° and then warmed to 25° than if interaction had only occurred at 25° Also $\Delta\varphi$ is very sensitive to the oxygen pressure at 25°

The results presented here, therefore, show a close relationship between the electrical properties of an oxide and possible non-stoichiometry Moreover, the apparent inconsistencies observed for $\Delta\varphi$ in the nickel–oxygen system (Table 1) can be rationalized in terms of our results and concepts

We thank the Chemical Society and the Department of Scientific and Industrial Research for grants to purchase equipment

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DETERMINING THE CO-ORDINATION NUMBER OF ALUMINIUM IONS BY X-RAY EMISSION SPECTROSCOPY

By DELBERT E. DAY
Concrete Division, Waterways Experiment Station, Jackson, Mississippi

THE wave-length of the characteristic X-radiation emitted by certain elements depends to some extent on the valence state of the element[1]. For certain elements of low atomic number, the wave-length of the characteristic X-radiation is also influenced by the co-ordination number (number of nearest neighbours) of the site occupied by the element White, McKinstry and Bates[2] reported a small difference in the wave-length of aluminium *K*α-radiation when the aluminium ions occupied four-fold and six-fold co-ordination sites This difference in wave-length was used by Brindley and McKinstry[3] and by Day and Rindone[4] to determine the co-ordination number of aluminium ions in disordered clay minerals and in sodium aluminosilicate glasses respectively This technique for determining the co-ordination number of aluminium ions is described, and the results obtained from examining several materials of widely varying composition are reported

The difference in wave-length of the aluminium *K*α-radiation produced by aluminium ions occupying different co-ordination sites results in a small but measurable angular shift in the position of the aluminium K_α peak Aluminium metal is used as reference material, and the angular position of its K_α peak is compared with the angular position of the aluminium K_α peak for the material in question The experimental quantity which is measured is the difference in these angular positions as expressed by the relationship

$$\Delta°2\theta = °2\theta\ (\text{aluminium metal}) - °2\theta\ (\text{sample}) \qquad (1)$$

This procedure is simpler and may be conducted under simpler experimental conditions than a direct measurement of wave-length

The measurements were made on a General Electric *XRD*-5 diffractometer using a chromium target at 50 kVp. and 40 m amp, a flow proportional counter, a 0 005-in soller slit, and a scanning speed of 0 2°2θ/min A helium path was used to minimize the absorption of the aluminium *K*α-radiation The chart speed was chosen so that 1°2θ was equivalent to 5 in on the chart With these conditions it was possible to measure the peak position, at half-maximum intensity, to ±0 005°2θ The analysing crystal was ethylenediamine ditartrate, with this crystal the peak position for aluminium *K*α-radiation occurs at approximately 142 6°2θ This value of 2θ is large enough so that small differences in wave-

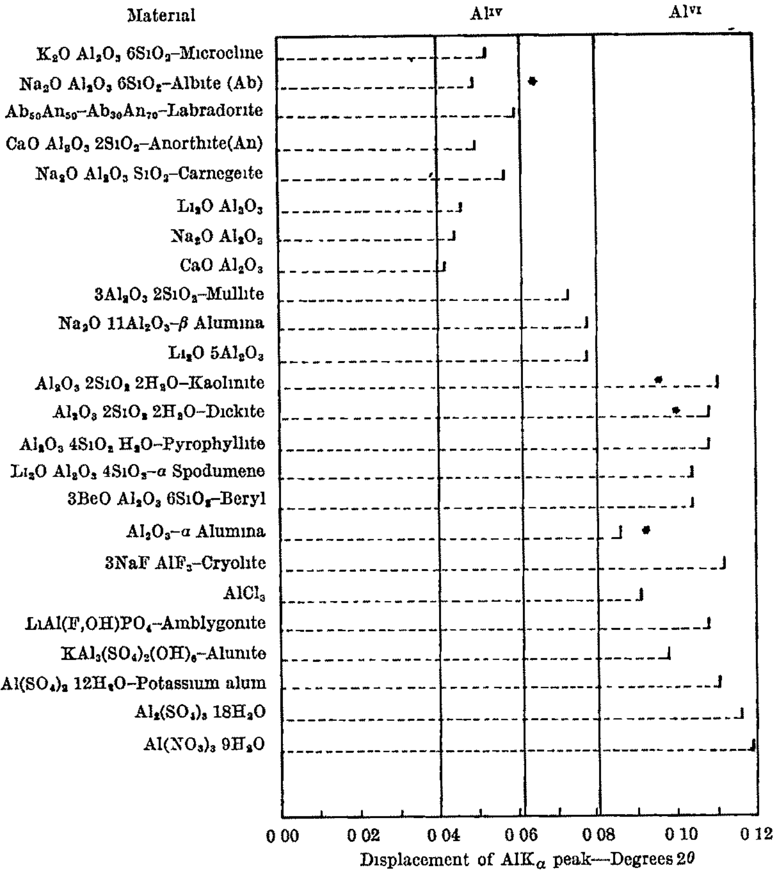

Fig. 1. Displacement of the aluminium K_α peak as calculated from the relationship $\Delta°2\theta = °2\theta$ (aluminium metal) $- °2\theta$ (sample). Al^{IV} and Al^{VI} represent the displacement for materials in which the aluminium ions occupy four-fold and six-fold co-ordination sites, respectively. Asterisks denote displacements reported by Brindley and McKinstry (ref. 3)

length can be detected by measurable shifts in the angular position of the aluminium K_α peak. All the measurements were made at room temperature with the temperature held constant to within ± 1° C. Larger temperature variations were found to change the lattice parameters of the ethylenediamine ditartrate crystal and to cause movement of the diffracted peak position.

Before each measurement, the position of the K_α peak for aluminium metal was determined at least twice. The average of these values was taken as the peak position for aluminium metal. Next, the position of the aluminium K_α peak for the sample material was determined at least twice. The angular difference in the position of the aluminium K_α peak for aluminium metal and the sample material was then determined using equation (1).

The displacements of the aluminium K_α peak, that is, the angular differences between the position of the aluminium K_α peak for aluminium metal and for the materials which have been studied, are shown in Fig. 1. The materials are arranged in the order (*a*) materials with aluminium ions occupying four-fold co-ordination sites, (*b*) materials with aluminium ions occupying more than one co-ordination site, (*c*) materials with aluminium ions in six-fold co-ordination sites. The displacements of the aluminium K_α peak for the first eight materials all occur within the range 0.040–0.061°2θ. Similarly, the displacements of the aluminium K_α peak for the materials kaolinite through $Al(NO_3)_3 9H_2O$ all occur within the range 0.080–0.120°2θ. These two regions are shown in Fig. 1 as Al^{IV} and Al^{VI}, and denote aluminium ions occupying four-fold and six-fold co-ordination sites, respectively.

The crystal structures of the materials microcline through $CaO Al_2O_3$, excluding $Li_2O Al_2O_3$, are known, and the aluminium ions in these materials occupy four-fold co-ordination sites. No information could be found for the crystal structure of $Li_2O Al_2O_3$, but the displacement of the aluminium K_α peak indicates that the aluminium ions in this material occupy four-fold co-ordination sites also.

The displacements of the aluminium K_α peak for mullite, β-alumina, and $Li_2O 5Al_2O_3$ as shown in Fig. 1 are between the regions indicated for aluminium ions occupying four-fold and those occupying six-fold co-ordination sites. These intermediate displacements indicate the aluminium ions in these three materials occupy more than one kind of co-ordination site. β-Alumina and $Li_2O Al_2O_3$ have the spinel structure with the aluminium ions distributed among four- and six-fold co-ordination sites. Similarly, the crystal structure of mullite, which resembles that of sillimanite[5], is believed to contain aluminium ions in both four- and six-fold co-ordination.

The displacements of the aluminium K_α peak for the remaining materials shown in Fig. 1 indicate that the aluminium ions in these materials occupy sites of six-fold co-ordination. The crystal structures of the materials kaolinite through potassium alum are known and confirm the existence of aluminium ions in six-fold co-ordination sites. No crystal structure information, which included the co-ordination number of the aluminium ions, could be located for $Al_2(SO_4)_3 18H_2O$ and $Al(NO_3)_3 9H_2O$. The displacement of the aluminium K_α peak for these two compounds indicates the aluminium ions should occupy six-fold co-ordination sites.

While the change in wave-length of the aluminium $K\alpha$-radiation resulting from a change in the co-ordination number of the aluminium ions is apparent in Fig. 1, the kind of anion surrounding the aluminium ion also has a small effect on the wave-length, as illustrated by $3NaF AlF_3$ and $AlCl_3$. The aluminium ions in these two compounds occupy six-fold co-ordination sites, but the displacements of the aluminium K_α peak for these compounds is 0.112 and 0.091°2θ respectively. If this trend were to continue for $AlBr_3$, it would be predicted that the aluminium ions in $AlBr_3$ should occupy four-fold co-ordination sites, since the displacement of the aluminium K_α peak would be close to the range indicated in Fig. 1 for materials containing aluminium ions in four-fold co-ordination sites. The direction of the trend indicated by $3NaF AlF_3$ and $AlCl_3$ is substantiated by the reported crystal structure for $AlBr_3$ (ref. 6) which places the aluminium ions in four-fold co-ordination sites. The influence of the kind of anion surrounding the aluminium ion has also been noted by Brindley and McKinstry[3]. They found the displacement of the aluminium K_α peak for aluminium nitride, which contains aluminium ions in four-fold co-ordination sites, differed significantly from the displacements for materials in which the aluminium ions occupied four-fold co-ordination sites, but were surrounded by oxygen ions rather than nitrogen ions.

The technique described here is simple and can be easily used for a variety of materials. Unlike X-ray diffraction, it is equally suitable for crystalline and for 'amorphous' materials having short-range order. The task of determining the presence or absence of various structural groups in amorphous materials is difficult, and in many instances structural models have been proposed on the basis of physical property measurements. This technique should be useful, therefore, in elucidating the structural groups which are thought to exist in certain amorphous materials

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DEGRADATION PRODUCTS FROM ELASTIN

Partial Structure of Two Major Degradation Products from the Cross-linkages in Elastin

THE isolation and preliminary examination of two new amino-acids derived from the cross-linkages in elastin have already been reported[1] These compounds, designated *A* and *M*, were provisionally assigned the empirical formulæ $C_{26}H_{45}N_5O_8$ and $C_{26}H_{41}N_5O_8$ respectively They have now been isolated on a larger scale directly from the acid hydrolysis products of purified elastin from bovine ligamentum nuchae, and in this communication a partial structure for both compounds is proposed

Titration experiments with *A* and *M* previously reported[1] indicated a minimum molecular weight of about 272 for both compounds, and each showed groups titrating with mid-points near *p*H 1 70, 2 40, 8 80 and 9 85 From the titration curves an iso-ionic point in the region of *p*H 5 6 would be expected, but electrophoretic mobility determinations in phosphate–citrate or sodium barbitone buffers have now shown that the isoelectric point is near *p*H 8 5 for both compounds It is thus concluded that both compounds contain a very strongly basic group, probably quaternary ammonium, which titrates above *p*H 11 5 Inspection of the titration curves shows that the alkaline isoelectric point is brought about by the presence of 0 5 equivalents of the strongly basic group in 270 g and thus the molecular weight of both substances is at least 540 In the course of the electrophoresis experiments it was observed that in buffers containing carbonate both compounds had a high net negative charge at *p*H 8 5 although the charge is zero in the absence of carbonate at this *p*H This suggests that the compounds bind carbonate ion with unusual avidity when this ion is in excess Alternatively, a carbamino compound may be formed

On treatment of compound *A* with nitrous acid in the apparatus of Van Slyke[2], 79 per cent of the total nitrogen was recovered as N_2 the value for compound *M* was 78 per cent, thus indicating that with both compounds four-fifths of the nitrogen was bound as primary amino groups When oxidized with ninhydrin by the gasometric procedure of Van Slyke *et al* [3] carbon dioxide was released equivalent to 78 per cent of the total nitrogen for compound *A* and 77 per cent for compound *M*, showing that four nitrogen atoms out of five were combined as α-amino-acid nitrogen for both compounds

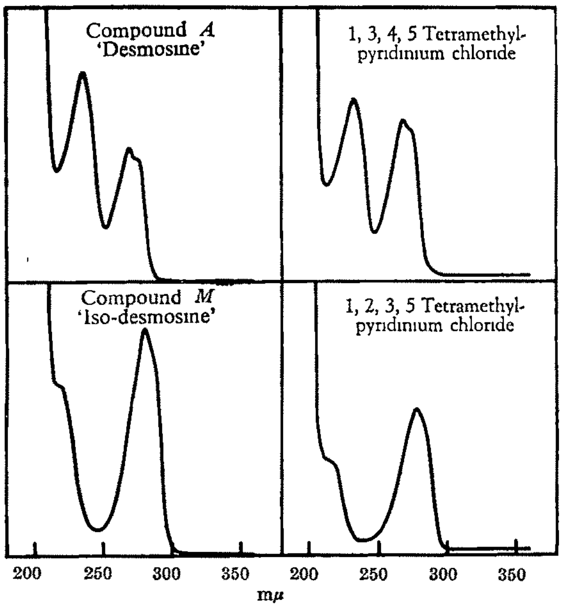
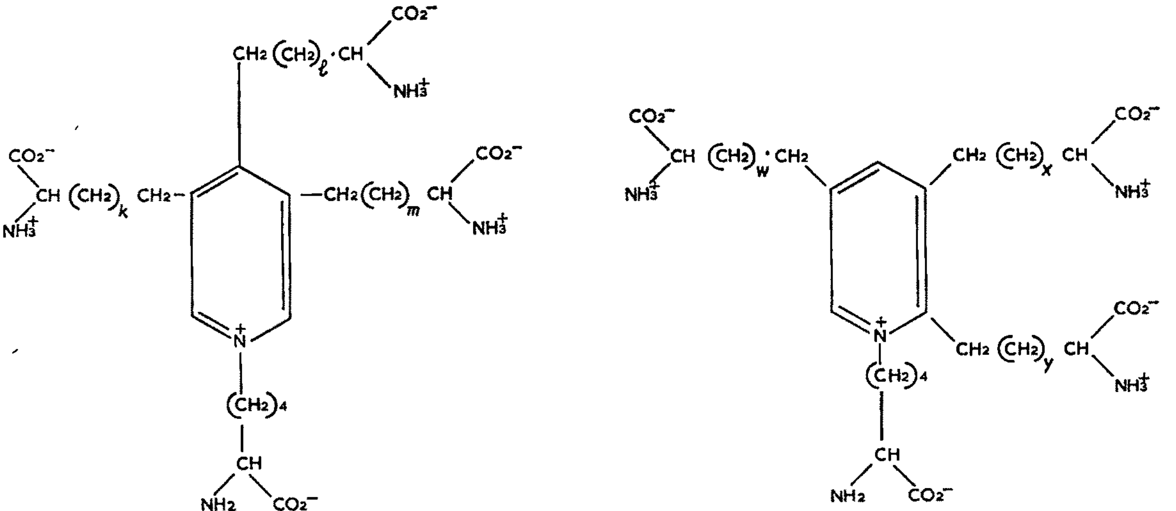


Fig 1

Determination of the molecular weight in aqueous solution using the 'Mechrolab' vapour pressure osmometer



Compound *A*, 'desmosine'

Compound *M*, 'iso-desmosine'

Fig 2

gave values of 524 for *A* and 555 for *M* These values are approximately twice the minimum mol wt determined by titration and suggest that the compounds both contain four α-amino groups, four α-carboxyl groups and one strongly basic group. Difficulty was encountered in drying specimens of the free amino-acids for elementary analysis Both compounds were very hygroscopic and removal of water appeared to be incomplete after several hours under reduced pressure at 100° Analysis of specimens dried in this way showed, for compound *A* C, 52·5, H, 7 5, N, 12 5 per cent, and for compound *M* C, 52 5, H, 7 6, N, 12 6 per cent. ($C_{24}H_{39}N_5O_8\,H_2O$ requires C, 53, H, 7 4, N, 12 8 per cent; mol wt, 543)

It was thought that the difficulty of drying might be due to the presence of the quaternary ammonium group, and to avoid this the chlorides were prepared by titrating the free amino-acids to *p*H 5 6. The chlorides could not be crystallized but the dried products were less hygroscopic and analysed as follows Compound *A* C, 49 9, H, 7 9, N, 12 5, Cl, 5 4 per cent, compound *M* C, 50 0, H, 8 1, N, 12 5, Cl, 5 6 per cent ($C_{24}H_{40}N_5O_8Cl$ requires C, 51 4, H, 7 1, N, 12 5, Cl, 6 3 per cent) From these results it was concluded that compounds *A* and *M* are isomers

On hydrogenation at room temperature and normal pressures with Adams's platinum oxide catalyst in 50 per cent aqueous ethanol or glacial acetic acid solution neither of the isomers took up hydrogen. The presence of aliphatic double bonds is therefore excluded Since both compounds show ultra-violet absorption in the range 260–280 mμ (ref 1) it was inferred that they contain an aromatic nucleus, and since the fifth nitrogen atom cannot be detected as an amino- or imino-group, but appears to form a quaternary ion, it is most probable that the substances are either N-substituted pyridines or N-substituted pyrroles The second alternative is unlikely since ultra-violet absorption spectra[1] of *A* and *M* closely resemble those of the trimethylpyridines and their *N*-methyl quaternary derivatives but differ from those of substituted pyrroles[4] These results, taken together with the marked chemical stability of the compounds under reducing conditions, suggest that the isomers are N- and C-substituted pyridines carrying four saturated aliphatic chains with α-amino-acid terminals Attempts to isolate the nucleus as a pyridine carboxylic acid by oxidation with permanganate resulted in destruction of the nucleus, presumably because of the instability introduced by N-substitution Mild oxidation with ferricyanide at alkaline *p*H also destroyed the nucleus yielding several ninhydrin-reacting degradation products One of these was isolated and identified as lysine This indicated that the structure contains two nitrogen atoms separated by a 5-carbon saturated aliphatic chain, and it was concluded that the pyridine nitrogen is quaternated by an α-amino caproic acid residue

In Fig 1 the ultra-violet spectra of compounds *A* and *M* are compared with those of the 1,3,4,5- and 1,2,3,5-tetramethylpyridinium chlorides The molecular extinctions measured in 0 05 N hydrochloric acid at the maxima were compound *A*, λ268, ε4900; 1,3,4,5-tetramethylpyridinium, λ266, ε4730, compound *M*, λ278, ε7850, 1,2,3,5-tetramethylpyridinium, λ276, ε6940

The proton magnetic resonance spectra reported in the succeeding communication confirm and extend the chemical information given here, and the data together lead to the structures proposed in Fig 2

We propose in future to refer to compound *A* as 'desmosine' and compound *M* as 'iso-desmosine'

We thank Dr A S Bailey, and Dr J. S A Brunskill of the Dyson Perrins Laboratory, Oxford, and Mr H G Willcock of Midland Tar Distillers, Ltd, for the gift of reference samples of trimethylpyridines

J THOMAS
D F ELSDEN
S M PARTRIDGE

University of Cambridge and
Agricultural Research Council,
Low Temperature Research Station,
Downing Street,
Cambridge

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Proton Magnetic Resonance Spectra of Degradation Products from Elastin

THE proton resonance spectra of desmosine and iso-desmosine (preceding communication), determined in deuterium oxide, show absorption in five spectral regions The available chemical evidence (preceding communication) and comparison with literature values (Table 1), allowed assignments as in Table 1. Peaks in the aromatic region are sharp singlets, comparison with the isomeric collidine (trimethylpyridine) methiodides indicated that desmosine and iso-desmosine are 1,3,4,5- and 1,2,3,5-tetra-substituted pyridinium salts. The remaining absorptions are broad band-envelopes, probably formed by the overlap of numerous peaks due to several signals of similar chemical shift, and complex coupling The theoretical relative areas given in Table 1 were checked by cutting out and weighing the curves and also by direct integration (on an *A60* machine) All the results were within ± 15 per cent of the theoretical The chemical and nuclear magnetic resonance evidence together lead to the structures of Fig 2 of the preceding communication '*k* + *l* + *m*' in desmosine and '*w* + *x* + *y*' in iso-desmosine are evidently 4

Table 1 PROTON RESONANCE SPECTRA AT 40 M/c*

	Aromatic		CH_2 and CH_3 attached to N^+		$-CH(ND_3^+)CO_2^-$		CH_2 and CH_3† benzyl type		CH_2 and CH_3 aliphatic	
	τ	Area	τ	Area	τ	Area	τ	Area	τ	Area
Desmosine	1 45	2	5 49	2	6 41	4	7 17	6	8 15	14
Iso-desmosine	1 43 1 70	1 1	5 43	2	6 36	4	7 15	6	8 16	14
Tetramethylpyridinium iodides										
1,2,3,5-	1 58 1 89	1 1	5 75	3	—	—	7 40 7 60	3 6	—	—
1,2,4,5-	1 57 2 32	1 1	5 90	3	—	—	7 34 7 57 7 68	3 3 3	—	—
1,3,4,5-	1 64	2	5 81	3	—	—	7 62	9	—	—
Lysine monohydrochloride	—	—	7 03	2	6 30	1	—	—	*ca* 8 3	6

* Deuterium oxide solutions, CH_3CN (τ = 7 98) as internal standard (ref 1)
† τ (CH_3) − τ (CH_2) is about 0 3 (ref 2)

G R BEDFORD
A R KATRITZKY

University Chemical Laboratory,
Cambridge

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IMMUNOCHEMICAL ANALYSIS OF RABBIT GAMMA-GLOBULIN ALLOTYPES

By Dr. A FEINSTEIN
Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge
AND
Prof P G H GELL and Dr. A S. KELUS
Department of Experimental Pathology, University of Birmingham

INTEREST in γ-globulin allotypes[1] (genetically determined variations of γ-globulin) has been increasing in recent years in view of their importance both in genetics and in γ-globulin chemistry They are definable in the rabbit in terms of six known alleles, A1, A2, A3 at one, the *a* locus, A4, A5, A6 at another, the *b* locus[2] Consequently, every rabbit has at least two and not more than four allotypic determinants in its γ-globulin, though 10–20 per cent of the γ-globulin molecules in some sera reported[3] may not carry any known allotypic markers. In this work we have investigated the distribution of the allotypic determinants, both between the whole molecules and on the fragments obtained by recently described degradation techniques

The problem as to whether the allotypic determinants in a given serum are all present on each reacting 7*S* γ-globulin molecule or are in some cases separated can be readily solved by double gel-diffusion analysis, making use of the reactions of identity, and partial or non-identity This is technically complicated by the fact that the antibodies themselves carry allotypic specificities of their own, and for clear results interactions between two adjacent antisera in the cups must be avoided We have been able, however, to demonstrate clearly a reaction of identity without any spurring between determinants at different loci in a homozygous A3,5 rabbit, indicating that all reacting molecules in this case carried both allotypic determinants In a serum examined and reported elsewhere[4], double spurring occurred in this situation In another case, we have shown a reaction of partial identity in an animal heterozygous at one locus (*a*), and homozygous at the other locus (*b*), using an antiserum to an allotypic determinant controlled by a gene at the *a* locus and one against one controlled by a gene at the *b* locus We have been unable to demonstrate unequivocally a reaction of total non-identity in a rabbit heterozygous at both loci, using an antiserum to a determinant controlled by a gene at the *a* locus and an antiserum to one controlled by a gene at the *b* locus respectively Quantitative evidence by other workers[3] has established the fact that in a heterozygote the A4 and A5 allotypes at least can occur on distinct populations of molecules

Turning now to the degradation observations, the allotypic antisera used were monospecific against each of the six known allotypic determinants A goat anti-rabbit antiserum ('Koza') was used which was mainly directed against 7*S* γ but also showed a distinct specific anti-γ-macroglobulin line; another goat anti-rabbit antiserum ('Bab') was used which did not contain this anti-macroglobulin component

Tests were made by double diffusion in 1 per cent agar in plates, and recorded as positive or negative, the concentrations of the reactants being so adjusted as to give a well-defined line

The sera used in the digestion experiments were from four rabbits homozygous at both loci (A1,4; A2,4; A3,4, A3,5), from two homozygous at the *a* locus and heterozygous at the *b* (A1,4,6, A3,4,5), from three homozygous at the *b* locus and heterozygous at the *a* (A1,2,4, A1,3,4; A2,3,4) and from three heterozygous at both loci (A1,3,4,5, A2,3 4 5 A1 2,4,5)

These were fractionated to obtain pure 7*S* γ-globulin and then fragmented (1) by papain digestion and separation of the fragments on carboxymethyl cellulose[5], (2) by mercaptoethanol in *tris* buffer to obtain cleavage into chains, which were then separated by 'Sephadex *G* 75' or '200' gel-filtration in molar propionic acid[6]. Macroglobulin fractions were separated on 'Sephadex *G* 200' gel columns[7]

The starting γ-globulin fractions showed as expected all the allotypes of the original whole sera

The macroglobulin preparations were only satisfactorily pure in two cases (A1,4,6 and A2,3,4,5) as judged by their reacting with goat antiserum 'Koza', and not with goat 'Bab' In both these samples (which happened to contain the full range of allotypes between them) there was a definite reaction with the allotypic determinants controlled by alleles at the *b* locus (A4, A5 or A6) as expected if macroglobulins share the B chain with 7*S* γ-globulins (see following) As regards the components controlled by the *a* locus, doubtful reactions were obtained with A1 and A2 determinants only, tests for A3 determinants were negative A1 and A3 determinants have been reported[8] to be present in purified macroglobulin; this problem clearly merits further investigation

Table 1 RESULTS OF GEL-DIFFUSION REACTIONS SUMMARY OF RESULTS ON TWO SERA COVERING THE WHOLE RANGE OF KNOWN ALLOTYPES. RESULTS WITH OTHER SERA WERE SIMILAR (SEE TEXT)

Rabbit allotype	Preparation	Allotypic antisera anti- −A1	−A2	−A3	−A4	−A5	−A6	Goat anti-rabbit antisera 'Koza'	'Bab'
A1,4,6	7*S* γ-globulin	+			+		+	+	+
,,	γ-macroglobulin	?			+		+	+	−
,,	Fraction I	+			+		+	+	+
,,	A chain	+			—		+	+	+
,,	B chain	−			+		+	−	−
A2,3,4,5	7*S* γ-globulin		+	+	+	+		+	+
,,	γ-macroglobulin		?	−	+	+		+	−
,,	Fraction I		+	+	+	+		+	+
,,	A chain		+	+	+	−		+	+
,,	B chain		−	−	+	+		−	−

+, Positive result, −, negative result, ?, weak or doubtful result.

The only papain fraction recorded (Table 1) was fraction I, which could be obtained in a high state of purity; and in every case (four sera covering all the allotypes) all the allotypic components of the original serum were found in this fraction Tests on fraction II gave similar results but of less significance since the fraction was not pure The preparations of fraction III were unsatisfactory

The B (light) chains from all the 12 sera examined showed the presence of the components controlled by alleles at the *b* locus (A4, A5 and A6) and the absence of those controlled at the *a* locus (A1, A2 or A3) (Table 1)

The A (heavy) chain presented some difficulty in purification. Nevertheless, in a number of cases there was no doubt that our preparations possessed the components controlled both by the *a* and by the *b* locus (Table 2)

Starch-gel electrophoresis in urea showed no detectable unreduced 7*S* γ-globulin, but agar-gel immunoelectro-

Table 2 RESULTS OF GEL-DIFFUSION REACTIONS PREPARATIONS OF A (HEAVY) CHAIN

Rabbit allotype	Allotypic antisera anti- −A1	−A2	−A3	−A4	−A5	−A6	Goat anti-rabbit antisera 'Koza'	'Bab'
A1,4	+			+			+	+
A2,4		+		+			+	+
A3,4			+	+			+	+
A3,5			+		+		+	+
A1,2,4	+	−		+			+	+
A1,3,4	+		+	+			+	+
A2,3,4		+	+	+			+	+
A3,4,5			+	+	−		+	+
A1,3,4,5	+		+	+	−		+	+
A2,3,4,5		+	+	+	−		+	+
A1,2,4,5	+	+		+	−		+	+
A1,4,6	+			−		+	+	+

phoresis of A chain fractions showed, with anti-A4 antiserum, that A4 moved with the A chain, and was not detectable in the more anodic position, where, in the absence of A chain, free B chain is found Free B chain could not, however, be expected, since immunoelectrophoresis shows recombination to be complete when a mixture of A and B chains in propionic acid is neutralized

Our results leave us in no doubt that the B (light) chain of 7*S* γ-globulin (which is shared with γ-macroglobulin) possesses the allotypic determinants A4, A5 and A6 controlled by alleles at the *b* locus

The results with A (heavy) chains are technically somewhat unsatisfactory and difficult to interpret on the reasonable genetic assumption that the A and B chains are correlated with the *a* and *b* loci respectively One would expect only the determinants controlled by alleles at the *a* locus (A1, A2 and A3) to be represented on the A (heavy) chain, as those from the *b* locus (A4, A5 and A6) certainly are on the B (light) chain We were, however, quite unable to separate anti-A4 reactivity from the A chain preparation in nearly all cases in one case out of five A5 was demonstrable, and in the preparation from A1,4,6 serum, A6 was demonstrable Attempts at further purification in 'Sephadex *G* 200' did not materially alter these results

There seem at least two possibilities either (1) that indeed the genes of the *b* locus are in control of determinants both on the B and on the A chains (for example, the determinants may be prosthetic groups added after the synthesis of the polypeptide chains), or (2) that the separation of A and B chains is not complete, and that too much B chain contaminates A chain preparations to allow of the application of qualitative methods such as those we have used Even with such methods, however, the intensity of the anomalous reactions which we have observed would imply that such contamination must be remarkably great

The reactions of the macroglobulins are consistent with expectation, in so far as the B (light) chain is supposed to be common to 7*S* γ-globulin and the γ-macroglobulin, and the allotypic determinants A4, A5 and A6 were readily demonstrable in both If it were assumed that immune globulin molecules contain only two types of polypeptide chain, one might expect, since the A chains of 7*S* γ-globulin and of γ-macroglobulin are antigenically different, that allotypic determinants A1, A2 and A3 would be lacking in the macroglobulins Our results leave this question still undecided Other macroglobulin preparations gave two lines with the goat anti-rabbit serum ('Koza') (which possessed both anti-7*S* γ- and anti-γ-macroglobulin specificity), one of which showed identity with 7*S* γ-globulin in these preparations, which we rejected as impure, the allotypic determinants A1, A2 and A3 were readily demonstrable

The results with the papain digest, Fraction I, show that the allotypic determinants are resistant to this type of degradation (see also refs 9 and 10) They indicate that the determinants A1, A2 and A3 must be present in the part of the A chain which is complexed with the B chain in papain fraction I, whether they are also present in the other part of the A chain, represented by the crystalline fraction III, could not be definitely established owing to the poor solubility and doubtful purity of these fractions

The study of the distribution of allotypic determinants between molecules is clearly only in a preliminary stage So far as our results go they are consistent with the genetic interpretation that in a homozygous A3,5 animal all the reacting molecules are A3,5, in an A2,3,6 animal they are either A2,6 or A3,6, in an A2,3,4,5 animal the situation is more complicated and we cannot exclude the occurrence of the combination A2,4 + A3,4 + A2,5 + A3,5 (see also ref 4) Further work along these lines with more suitable antisera is in progress

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IMMUNOLOGICAL TOLERANCE BETWEEN LITTER-MATES IN SHEEP

By Dr J. SLEE

Agricultural Research Council, Animal Breeding Research Organization, Edinburgh, 9

It is known that placental vascular anastomosis occurs in most twin pregnancies in cattle If the anastomosed twins are of unlike sex the female becomes a freemartin (intersex) It has also been shown[1] that most twin calves of all sexes show mutual tolerance of skin homografts, presumably as a result of embryonic vascular anastomosis In sheep, where twins are far more frequent, both embryonic vascular anastomosis and freemartinism have been regarded as rare[2–7] One ♂ ⚥ (freemartin) pair has been described[3] in which the twins were tolerant of reciprocal skin homografts At least one other case of skin homograft tolerance in sheep has been reported between two members of a triplet litter[4] Probably embryonic vascular anastomosis, which causes blood cell mosaicism[2], is a necessary pre-condition for all cases of freemartinism and tissue tolerance between litter-mates in cattle and sheep The frequency of embryonic anastomosis between sheep twins has been estimated at 5–10 per cent[2,4] There is independent evidence also that the frequency of blood mosaicism in twins may be about 5 per cent[2] It is possible that anastomosis of placental blood vessels does not always produce post-natal blood cell mosaicism Independent estimates of the frequency of immunological tolerance among sheep litter-mates are scarce (Table 1)

The foregoing results, if taken provisionally at their face value, suggest the possibility of implanting sheep embryos of widely different genotypes adjacently in the same uterus, so that in a certain small proportion

Table 1 FREQUENCY OF IMMUNOLOGICAL TOLERANCE AMONG SHEEP LITTER-MATES

Source	Twin litters No pairs tested	No pairs tolerant	Tolerant lambs (%)	Large litters (3–5 lambs) No litters tested	No lambs tested	No pairs tolerant*	Tolerant lambs (%)
Lampkin (ref 5)	2	0	0				
Hraba *et al* (ref 4)	9	0	0	1	3	1	—
Moore and Rowson (ref 3)	5	1	20				
Slee (1961–63)	7	0	0	18	52†	3	11
Total	23	1	4 3	19	55	4	14 6

* These numbers are doubled to assess the proportion of tolerant lambs

† Some lambs died before being grafted, survivors in these litters were grafted

of cases vascular anastomosis might occur, making such embryos immunologically tolerant If sheep of different breeds could be made mutually tissue-tolerant this would be of interest to (among others) the reproductive physiologist (transfer of ovaries), or the wool biologist (transfer of skin between sheep showing different wool growth patterns) Co-existing embryos of 50 per cent different breed genotype can be obtained by double mating ewes with rams of different breeds, so giving bi-paternal litters[8] Or, preferably, the techniques of transfer of ova[9,10] would allow fertilized eggs of 100 per cent different breed genotype to be implanted in the same uterus The most likely limiting factor would be the low frequency of embryonic vascular anastomosis It was therefore important (*a*) to assess the natural frequency more accurately, (*b*) to maximize it by experimental or other means For a relatively high frequency of anastomosis it was thought that large litters, causing uterine crowding, might be desirable It has been shown that uterine crowding increases the incidence of fused placentæ in mice[11]

The question of obtaining immunological tolerance between sheep litter-mates was therefore approached as follows To estimate the frequency of embryonic vascular anastomosis in large litters, lambs belonging to litters of three or more were tested by skin homografts from each other On each of the lambs, which were taken first at about 6 months of age, 3 homografts were accompanied by 3 control autografts All grafts were either 1 or 2 cm diameter, full thickness The criteria for tolerance were absence of homograft reaction, survival of homograft tissue for at least 6 months with the production of wool, and absence of a line scar Some of the large litters used were obtained naturally, some were produced by super-ovulation In some cases ewes were each mated to several rams of different breeds, producing bi- and tri-paternal litters From some litters placentæ were recovered intact at birth They were usually injected via the umbilical vessels with 'Neoprene' dye latex and examined for evidence of vascular anastomosis

The results of this work were briefly as follows Skin grafts were made on 66 lambs surviving from 7 sets of twins, 14 sets of triplets, 4 sets of quadruplets, and on 2 lambs surviving from a quintuplet litter Clear tolerance towards homografts was found between 3 pairs of lambs Two of these pairs were members of a quadruplet litter of Welsh Mountain lambs produced after super-ovulation of the ewe *A* ♂ and *D* ♀ were mutually tolerant, and *B* ♂ and *C* ♀ were likewise The other combinations showed no tolerance *D* was a freemartin (female external genitalia with enlarged clitoris, plus small undescended testes, male horn type and male sex behaviour) *C* was barren when mature but showed no external signs of freemartinism

First set skin grafts from *D* to *A* and *B* to *C* grew wool and were still present 1½ years after transplantation Second set grafts survived less well *D* to *A* second set grafts were rejected in 3 months although the first set grafts remained healthy during the same period Part of the second set grafts from *B* to *C* survived for more than a year, but the rest of the graft tissue appeared to be gradually lost The first set grafts again remained healthy First set reciprocal grafts of *A* to *D* and *C* to *B* survived for 6–9 months before being rejected

These sheep were tested for red blood cell mosaicism at about 1½ years of age by Dr Stormont of the University of California Mosaicism was detected between *A* and *D* (the freemartin), although in *A* one of the cell types had almost completely regressed No mosaicism was apparent in *B* and *C* but it could have existed at an earlier stage Two points emerge from these data First, homograft tolerance, which is probably caused or at least permitted by erythrocyte mosaicism, can persist after the mosaicism has regressed Secondly, first set grafts can be tolerated at the same time as second set grafts from the same donor appear to be slowly rejected Rejection of the second grafts could be related to the regression of the donor blood cells if this tells preferentially against the new grafts rather than the established ones The placenta was not recovered from this litter

The third pair of lambs showing mutual homograft tolerance were Merino–Blackface crosses (♂♂) surviving from a triplet litter produced after super-ovulation Here, first set grafts from *A* to *B* are still surviving now after 9 months Reciprocal homografts from *B* to *A* were less uniformly successful, but some of the donor tissue remains after 9 months The placenta, when recovered at birth, showed clear anastomosis of major veins between 2 of the original 3 embryos The lambs have not yet been tested for blood mosaicism Apart from this instance there have been two other cases of placental anastomosis of major blood vessels—both in triplet litters (Table 2) In the first of these (as in the case here) only 2 lambs survived, they showed only slight asymmetrical homograft tolerance Reciprocal grafts survived 4 and 7 weeks respectively, compared with the normal within-litter rejection time of 2–3 weeks Presumably one of these surviving lambs had been anastomosed with the dead lamb, and for that reason may have been tolerant of a wider than normal range of antigens The 3 lambs in the remaining litter showing placental vascular anastomosis have not yet been skin grafted In addition to these 3 cases of major vessel anastomosis, 2 other placentæ have shown fusion of minor capillary vessels derived from different embryos The significance of this is not known

Table 2 FREQUENCY OF PLACENTAL VASCULAR ANASTOMOSIS IN SHEEP LITTERS

	No Total	From twins	From triplets	From quads	From quins
Placentæ examined	20	11	5	3	1 (part only)
Cases of major vessel fusion between embryos	3	0	3	0	0
Cases of minor vessel fusion between embryos	3	2	0	1	0

General conclusions are as follows Embryonic vascular anastomosis does occur in large sheep litters with a frequency perhaps rather higher than might have been expected For the highest frequency of anastomosis large litters may be better than twins (Table 1), but the evidence for this is obviously not conclusive Embryonic anastomosis in sheep can be between major blood vessels, and then it appears to confer in some, perhaps all, cases a degree of post-natal immunological tolerance on the surviving lambs involved This may be accompanied by blood cell mosaicism for at least some time after birth Where anastomosis is between ♂ and ♀ embryos, freemartins may occur perhaps invariably but not always overtly The possibility that covert freemartinism causing female infertility may occur fairly frequently was examined on a statistical basis by comparing the fertility of 187 ewes which had been born twin to ♀♀ with the fertility of 167 ewes born twin to ♂♂ No difference was found The apparent rarity of freemartinism in twin litters could be connected with the high frequency of transuterine migration of ova in sheep[12] Nevertheless, the arrangement of the embryonic membranes and vascular system is such that anastomosis might occur even between embryos implanted in different horns of the uterus

The information discussed here, coupled with the proved technique of ova transfer, indicates that it would be difficult, but perhaps possible, to induce within-litter immunological tolerance in sheep and use it to transplant tissue between individuals of different breeds Although natural embryonic vascular anastomoses are not common, they may offer the best chance of success, since sheep embryos develop immunity early in gestation[13], thus making it difficult to produce tolerance experimentally[14] However, even if anastomosis can be induced sufficiently frequently between sheep embryos of different breeds, it remains to be seen whether the tolerance conferred is sufficient to bridge the between-breed immunological barrier

This work is now being extended in collaboration with Dr J L Hancock, whom I thank

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EFFECT OF METHAQUALONE HYDROCHLORIDE IN PREGNANT RABBITS AND RATS

By R G BOUGH, DR M R GURD, DR J E HALL and DR. B LESSEL

Research Department, Boots Pure Drug Co , Ltd , Nottingham

FOLLOWING the thalidomide disaster, attention has been directed to the need to test drugs for possible teratogenic activity Many drugs have recently been subjected to teratogenesis tests, in the laboratories of the pharmaceutical industry and elsewhere, but little of this work has yet been published, probably because of the difficulty of interpreting the results The picture which seems to be emerging is that there may be no drugs or chemical substances which are completely free from any effects on the fœtus, most substances which have so far been tested on experimental animals show toxic effects on the fœtus if they are administered to the mother in large enough doses This is not surprising, for if a substance were given to the mother in doses large enough to be toxic to her, it would be strange indeed if the fœtus was not affected

A possibly useful new drug is not rejected because it causes death in experimental animals when given in excessive doses; almost every drug does so Similarly, a drug need not necessarily be rejected because it causes death or abnormalities in the fœtuses of experimental animals when given to the mother in excessive doses Only experience can teach us to interpret the results of teratogenesis tests in experimental animals in terms of possible human hazards, and it is therefore important that work of this kind should be published, even if it does not at present answer all our questions

In the present state of knowledge a useful guide is the relation between the toxic dose of a drug to the fœtus and the toxic dose to the mother, if these two doses are about the same, that is, if the fœtus is no more susceptible to the toxic effects of the drug than is the mother, it is reasonable to suppose that the drug has no specific teratogenic effect

This article describes the results of animal experiments with methaqualone hydrochloride, and is intended both to give some guidance on the degree of risk attached to this drug, and to serve as an example of the kind of results which are likely to occur with many drugs in common use Methaqualone hydrochloride (2-methyl-3-*o*-tolyl-4-quinazolone hydrochloride, 'Melsedin', 'Tuazole') is a hypnotic drug, with a therapeutic dose up to 300 mg (about 5 mg/kg)

The first experiment we carried out was a comparison of the effects of methaqualone and thalidomide in rabbits, using the technique by which Somers[1] has successfully demonstrated the teratogenic action of the latter drug New Zealand White and Californian rabbits weighing 2 5–4 kg were used Each drug was given by mouth in a dose of 500 mg per rabbit daily, starting on the 8th day of pregnancy and continuing to the 13th day with thalidomide and the 15th or 16th day with methaqualone The mothers were killed the day before parturition was expected; each fœtus was examined internally and externally for abnormalities, radiographed and cleared and stained with alizarin red for detailed examination of the skeleton The results of this experiment are summarized in Table 1 Thalidomide treatment increased the proportion of fœtuses dying *in utero* and caused abnormalities in 80 per cent of the live fœtuses The abnormalities included skeletal defects such as those described by Somers[1] and others[2], and cerebral defects similar to those described by Giroud[3] and Felisati[4], in addition we observed five fœtuses with one kidney absent (four of them in one litter), which we believe has not been reported previously in experimental animals, although it has been described in man[5] Methaqualone treatment, in contrast, had no effect on the survival of fœtuses *in utero*, and no abnormalities were found

Table 1 EFFECT OF THALIDOMIDE AND METHAQUALONE IN PREGNANT RABBITS

	No of mothers*	No of fœtuses				Average live litter size	Implants dead or resorbed (%)
		Live normal	Live abnormal	Dead	Resorbed		
Controls	4	27	0	1	3	6 8	12 9
Methaqualone 500 mg/day							
Days 8–15	3	27	0	3	0	9 0	10 0
Days 8–16	3	33	0	0	4	11 0	10 8
Thalidomide 500 mg/day							
Days 8–13	7	5	22	1	30	3 9	53 4

* Animals originally included in the experimental groups but later found not to be pregnant have not been included in these four Tables

In the second experiment with methaqualone in rabbits three dose-levels were used, the doses were related to body weight, and dosing was continuous throughout pregnancy from the 1st to the 29th days, in other respects the technique was unchanged The results are summarized in the first part of Table 2 Two of the 79 fœtuses from animals treated with one or other dose of methaqualone showed abnormalities, and a third experiment was therefore performed using the same technique, but with larger groups of rabbits and one level only of methaqualone; the results of the third experiment are summarized in the second part of Table 2. Taking these two experiments together, there was no evidence that treatment with methaqualone, at 100 or 200 mg/kg/day, affected live litter size or increased the incidence of deaths *in utero*. Treatment at 400 mg/kg/day reduced the number of implants per animal, without affecting the survival of implanted fœtuses, which suggests interference with blastocyst development. Abnormalities of various kinds occurred in the fœtuses of both treated and control animals; these are listed in Table 3. We deal first with the least important condition, which we have described as 'hypoplasia of the gall bladder'. The gall bladder in rabbit fœtuses shows great variation in size, and is invisible to the naked eye in about 5 per cent of control animals; this condition we have noted as 'hypoplasia', although in every case the bile ducts are patent and there is no reason to believe that biliary function is abnormal. This condition appears to represent one extreme of the normal range of variation, and we do not regard it as a malformation. Next come the conditions which occurred with equal frequency in the treated and control groups and cannot therefore be attributed to treatment, that is, unilateral pelvic kidney, absence of a lobe of the lung, and minor abnormalities of the calcification centres in the sternum. Lastly, there are a few abnormalities which were observed in treated animals but not, in this experiment, in controls. Two of these conditions, rotation of a forelimb and deficient phalangeal development of a hind foot, have been observed in untreated rabbits in other experiments, and a third, fusion of rib heads, occurred only with a dose high enough to kill one of the adults. This leaves two abnormalities which occurred in rabbits receiving non-lethal doses of methaqualone, and which we have not so far seen in control fœtuses, that is, absence of a pollex and a jejunal diverticulum.

Table 2. EFFECT OF METHAQUALONE IN PREGNANT RABBITS

	No. of mothers	Adult deaths	No. of fœtuses Live	Dead	Re-sorbed	Average live litter size	Implants dead or resorbed (%)
Second Exp.							
Controls	4	0	34	1	1	8·5	5·5
Methaqualone 100 mg/kg/day	5	0	32	3	5	6·4	20·0
Methaqualone 200 mg/kg/day	4	0	30	1	7	7·5	22·2
Methaqualone 400 mg/kg/day Days 1–29	3	1	17	0	0	5·7	0
Third Exp.							
Controls	15	0	119	15	17	8·0	21·2
Methaqualone 100 mg/kg/day Days 1–29	18	0	139	17	16	7·7	18·1

Table 3. FŒTAL ABNORMALITIES IN RABBITS

	No. of fœtuses affected			
		Methaqualone: days 1–29		
	Controls	100 mg/kg/day	200 mg/kg/day	400 mg/kg/day
Skeletal abnormalities visible externally				
Rotation of a forelimb*		1		
Deficient phalangeal development of a hind foot*		1		
Absence of a pollex		1		
Skeletal abnormalities found by X-ray or staining				
Fusion of two rib heads on one side				1
Minor abnormalities of sternal calcification centres†	2	2		
Visceral abnormalities				
Jejunal diverticulum		1		
Unilateral pelvic kidney	1	1		
Absence of one lobe of lung	1	1		
"Hypoplasia" of gall bladder	7	14		
Total number of fœtuses	153	171	30	17
Number of fœtuses of which skeletons were stained†	119	139		

* These two conditions did not occur in control animals in these experiments, but have been found in control rabbits in other experiments.

† Minor abnormalities of sternal calcification centres would be detected only in stained skeletons.

The position in the rabbit is complicated by the occurrence of spontaneous abnormalities in 2–3 per cent of control fœtuses. The number of fœtuses showing abnormalities of one kind or another was slightly greater in the methaqualone-treated animals than in the controls, but the difference was far too small to have statistical significance, although more than 370 fœtuses were included in the comparison. A more important point is that each type of abnormality in the fœtuses from methaqualone-treated mothers occurred only once; there was no characteristic pattern of malformation. By contrast, thalidomide treatment increased the incidence of abnormality from about 3 to about 80 per cent of fœtuses, and gave a characteristic pattern of skeletal and cerebral defects.

Other experiments were done in rats. Newly mated female albino rats were given methaqualone hydrochloride daily by oral intubation at four dose-levels from the 1st to the 20th days of pregnancy; the animals were killed on the 21st day (the day before parturition was expected) and the fœtuses examined as previously described. The results are summarized in Table 4. The highest doses, 100 and 75 mg/kg/day, caused maternal deaths and increased the incidence of deaths *in utero*; among the 77 live fœtuses from animals receiving these doses three were abnormal; one with omphalocœle and one with complete cleft palate on 75 mg/kg, and one with extensive subcutaneous œdema on 100 mg/kg. The next lower dose, 50 mg/kg/day, caused neither maternal nor fœtal deaths, but reduced gain in body-weight in the mothers; no abnormalities were observed in the fœtuses from these animals. Examination of the stained skeletons showed apparent defects of calcification in the sternal centres and elsewhere in some animals on doses of 50 mg/kg/day upwards; further experiments in which rats given 50 mg/kg/day were allowed to survive to parturition showed that at this dose calcification was merely delayed for 24 or 48 h. The lowest dose, 25 mg/kg/day, had no effects of any kind, and no abnormalities occurred in the fœtuses from these animals or the controls.

Table 4. EFFECT OF METHAQUALONE IN PREGNANT RATS

	No. of mothers	Adult deaths	No. of fœtuses Live normal	Live abnormal	Dead	Re-sorbed	Average live litter size	Implants dead or resorbed (%)
Controls	12	0	101	0	0	7	8·4	6·5
Methaqualone 25 mg/kg/day	8	0	85	0	1	2	10·6	3·4
Methaqualone 50 mg/kg/day	9	0	79	0	0	7	8·8	8·1
Methaqualone 75 mg/kg/day	7	1	53	2*	3	15	7·6	25·5
Methaqualone 100 mg/kg/day	5	2	24	1†	7‡	6	4·8	35·2

* One omphalocœle and one cleft palate.

† Extensive subcutaneous œdema.

‡ All the dead fœtuses were in a single litter.

The threshold dose for methaqualone to produce minimal toxic effects in the rat mother and fœtus was about 50 mg/kg/day. These minimal toxic effects consisted of reduction of growth in the mother and delayed skeletal calcification in the fœtus. The important point is that the threshold dose was the same for mother and fœtus; at higher doses some of the mothers died, and the incidence of abnormal fœtuses increased from zero to about 4 per cent. The fact that the drug was toxic to the fœtus only in doses at which it was also toxic to the mother indicates that it lacks specific teratogenic activity.

The conclusion we draw from the results reported here is that methaqualone shows no specific teratogenic activity in rabbits and rats, and is therefore unlikely to present a hazard to the human fœtus in therapeutic use.

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HÆMOGLOBINS N AND P IN ITALIAN FAMILIES

By Prof. E. SILVESTRONI, I. BIANCO and C. BRANCATI

Istituto d'Igiene dell'Università di Roma,
Centro di Studi della Microcitemia e delle Anemia Microcitemiche, Roma

Frequency and Characterization of the Hæmoglobin N

IN our laboratory, four family stocks, carriers of hæmoglobin N, have been observed so far: the first one, already described[1], is of Sardinian origin, the other three originate from Calabria. The electrophoretic mobility and the other physical-chemical characteristics are identical in all the four stocks and fully correspond to those of hæmoglobin N: that is, at alkali *p*H (Fig. 1) the electrophoretic mobility is greater than that of hæmoglobin A, with a distinct separation from it; at acid *p*H the cathode mobility is slightly less than that of hæmoglobin A, so much so that it forms at the end of electrophoresis an oblong hæmoglobin spot; on agar gel at acid *p*H the mobility is the same as that of hæmoglobin A; on 'Amberlite *IRC*-50' chromatography at *p*H 6, the mobility is greater than that of hæmoglobin A with a distinct separation from it (Fig. 2). The hæmatologic characters are normal and the percentage of hæmoglobin N oscillates from 19 to 36.

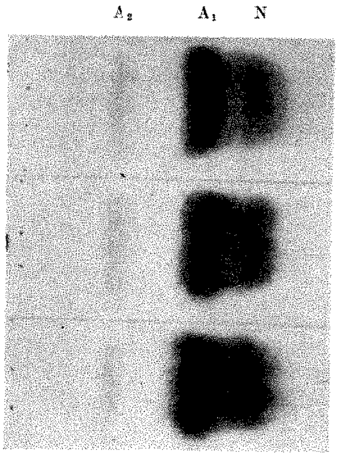

Fig. 1. Paper electrophoresis of hæmoglobin N belonging to three different families. Glycine buffer *p*H 8·8, ionic strength 0·04. Staining with bromphenol blue

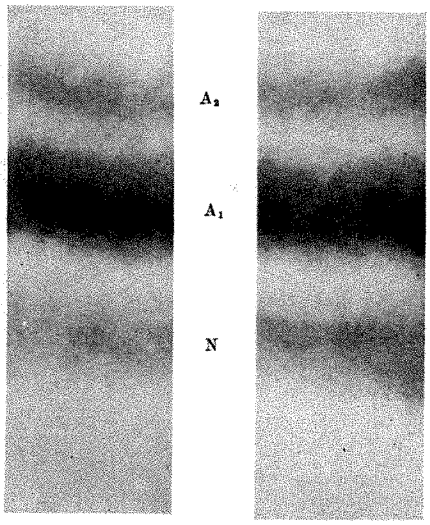

Fig. 2. Chromatography on columns of 'Amberlite *IRC*-50' of hæmoglobin N belonging to two different families. Citrate buffer *p*H 6, ionic strength 0·05. Hæmoglobin N precedes hæmoglobin A and separates clearly from it

Hybridization and recombination experiments carried out with the technique of Itano and Robinson[2], adapted to the micromethod by Gammack *et al.*[3], have revealed, in three of these stocks in which it has been possible to conduct the research, that the abnormal hæmoglobin is altered in the α-chain.

It has been ascertained that hybridization of hæmoglobin N with hæmoglobin L_{Ferrara} (which is altered in the α-chain[4]) does not produce any new hæmoglobin fraction (Fig. 3), while hybridization with hæmoglobin S (which is altered in the β-chain) produces an abundant portion of hæmoglobin A (Fig. 4), which appears also in hybridization of hæmoglobin N with hæmoglobin H. It is evident that in these hybridizations the products foreseen in the following equations are formed:

$$\begin{array}{ccccccc} \alpha_2^N\beta_2^A & + & \alpha_2^L\beta_2^A & = & \alpha_2^N\beta_2^A & + & \alpha_2^L\beta_2^A \\ \text{Hb N} & & \text{Hb L} & & \text{Hb N} & & \text{Hb L} \end{array}$$

$$\begin{array}{ccccccccccc} \alpha_2^N\beta_2^A & + & \alpha_2^A\beta_2^S & = & \alpha_2^N\beta_2^A & + & \alpha_2^A\beta_2^S & + & \alpha_2^A\beta_2^A & + & \alpha_2^N\beta_2^S \\ \text{Hb N} & & \text{Hb S} & & \text{Hb N} & & \text{Hb S} & & \text{Hb A} & & \text{Hb N/S} \end{array}$$

Hybridization of hæmoglobin N with canine hæmoglobin does not give rise to any new hæmoglobin fraction. This fact too shows that the structural alteration concerns the α-chain. In fact, if the alteration affected the β-chain, a hybrid ($\alpha_2^{can}\beta_2^N$) faster than hæmoglobin N and a hybrid ($\alpha_2^A\beta_2^{can}$) slower than canine hæmoglobin should appear; on the contrary, only two fractions are formed which correspond, in mobility, one to canine hæmoglobin and the other to hæmoglobin N: the first very probably contains, besides canine hæmoglobin, also the hybrid $\alpha_2^N\beta_2^{can}$ for which an electrophoretic position equal to that of canine hæmoglobin is expected and the second contains, besides hæmoglobin N, also the hybrid $\alpha_2^{can}\beta_2^A$, the

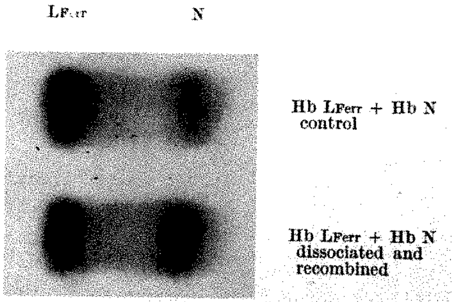

Fig. 3. Hybridization of hæmoglobin N with hæmoglobin L_{Ferrara}. Discontinuous buffer system, *p*H 8·2, ionic strength 0·06, *o*-dianisidine stain. No new hæmoglobin appears

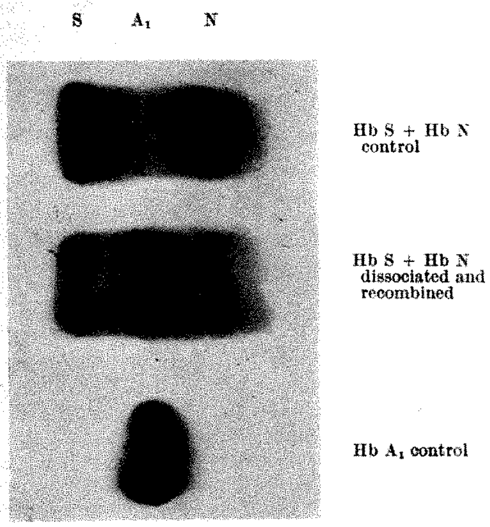

Fig. 4. Hybridization of hæmoglobin N with hæmoglobin S. Discontinuous buffer system, pH 8·2, ionic strength 0·06, o-dianisidine stain. In the dissociated and recombined mixture a new hæmoglobin appears, migrating at the same rate as hæmoglobin A

mobility of which is expected to be equal to that of hæmoglobin N. In this hybridization the products foreseen in the following equation are formed:

$$\underset{\text{Hb N}}{\alpha_2^N\beta_2^A} + \underset{\text{Hb}_{\text{can}}}{\alpha_2^{can}\beta_2^{can}} = \underset{\text{Hb N}}{\alpha_2^N\beta_2^A} + \underset{\text{Hb}_{\text{can}}}{\alpha_2^{can}\beta_2^{can}} + \alpha_2^N\beta_2^{can} + \alpha_2^{can}\beta_2^A$$

On the basis of these results, the conclusion can be drawn that the hæmoglobin N observed in various regions in Italy is characterized by a structural alteration of the α-chain, while the presence is reported, in literature, in other countries, of hæmoglobin N altered in the β-chain[3].

After hæmoglobin D, hæmoglobin L and hæmoglobin G, this is a new example of hæmoglobin, which, although having the same electrophoretic and physical-chemical characteristics, has its specific structural alteration once in α-chain, once in β-chain.

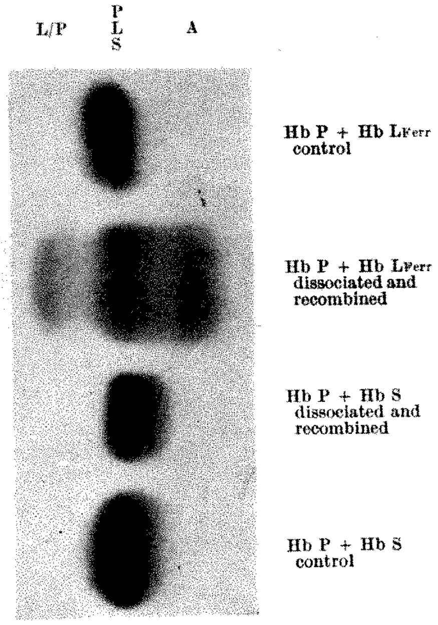
L/P
P
L
S
A
Hb P + Hb L_{Ferr} control
Hb P + Hb L Ferr dissociated and recombined
Hb P + Hb S dissociated and recombined
Hb P + Hb S control

Fig. 5. Hybridization of hæmoglobin P with hæmoglobin L$_{\text{Ferrara}}$ and with hæmoglobin S. Discontinuous buffer system, pH 8·2, ionic strength 0·06, o-dianisidine stain. The hybridization of hæmoglobin P with hæmoglobin L$_{\text{Ferrara}}$ produces two new hæmoglobins: hæmoglobin A and a slow hæmoglobin, which are lacking in the hybridization of hæmoglobin P with hæmoglobin S

Presence of Altered β-Chain in Hæmoglobin P

On the hæmoglobin P of the members of the Italian family, originating from Calabria, previously illustrated[5,6], experiments of hybridization have been conducted with the micromethod of Gammack *et al.*[3], for the purpose of identifying the abnormal chain. No new electrophoretic species were detected in hybridization of hæmoglobin P with hæmoglobin S (Fig. 5), but hybridization with hæmoglobin L$_{\text{Ferrara}}$ (which has an abnormal α-chain[4]) produced a new hæmoglobin species which migrates slower than hæmoglobin L and hæmoglobin P, and a very clear hæmoglobin fraction which migrates in the position of hæmoglobin A (Fig. 5).

These results are sufficient to show that the present hæmoglobin P has an abnormal β-chain. The fact is also confirmed by hybridization with canine hæmoglobin (Fig. 6), which produces only a new hæmoglobin species in the position of fraction $\alpha_2^A\beta_2^{can}$, while, as in the hybridization of hæmoglobin S with canine hæmoglobin (Fig. 6), the hæmoglobin fraction corresponding to the hybrid $\alpha_2^{can}\beta_2^A$ is completely lacking.

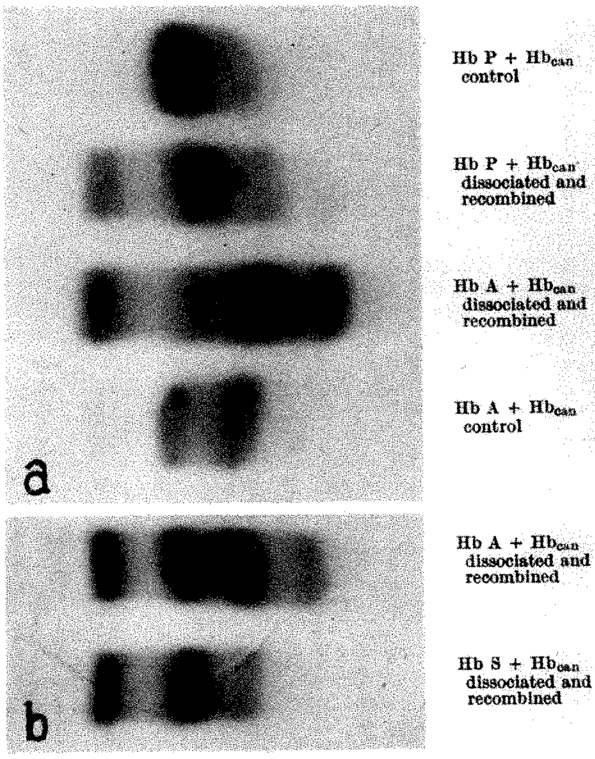
Hb P + Hb$_{can}$ control
Hb P + Hb$_{can}$ dissociated and recombined
Hb A + Hb$_{can}$ dissociated and recombined
Hb A + Hb$_{can}$ control
a
Hb A + Hb$_{can}$ dissociated and recombined
Hb S + Hb$_{can}$ dissociated and recombined
b

Fig. 6. *a*, Hybridization of hæmoglobin P with canine hæmoglobin, compared with hybridization of hæmoglobin A with canine hæmoglobin; *b*, hybridization of hæmoglobin A with canine hæmoglobin, compared with hybridization of hæmoglobin S with canine hæmoglobin. The slow hæmoglobin which appears from the hybridization of hæmoglobin P with canine hæmoglobin is identical to the slow hæmoglobin which appears from the hybridization of hæmoglobin S with canine hæmoglobin and to the hybrid $\alpha_2^A\beta_2^{can}$

The hæmoglobin P observed in Italy thus appears altered in the β-chain, as the hæmoglobin P observed by Dherte *et al.*[7] in Belgian Congo Negroes and analysed by Gammack *et al.*[3].

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A PHEROMONE FOR MELANIZATION OF LOCUSTS

By DR. D. J. NOLTE

Department of Zoology, University of the Witwatersrand, Johannesburg

THE theory of phase transformation of locusts, promulgated by Uvarov[1] in 1921, was placed on a firm basis by Faure[2], who demonstrated phase transformation and colour change in the four principal species of locusts of Africa. Characteristic of such change from the green or buff or fawn 'grasshopper' or solitaria phase is the melanization of the immature hopper stages or instars. This process is reversible during the instar stages on drastic reduction of population density. In the extreme gregaria phase the hoppers of *Schistocerca gregaria* Forsk., the desert locust, are almost totally black; hoppers of *Locusta migratoria migratorioides* R. and F., the tropical migratory locust, have the dorsal half of the body black; hoppers of *Locustana pardalina* Walk., the brown locust, are black laterally. In gregaria hoppers melanin constitutes the pattern pigment while background pigmentation is due mainly to tanned proteins, carotenoids and ommochrome, brownish, yellow and brown to red respectively.

In solitarization experiments in our Locust Research Unit, *Locusta* and *Locustana* hoppers of the second and third instars have been found to transform fairly readily to the green or fawn solitaria colours during later instars when kept in isolation in quart jars. During the past winter a biochemistry student had occasion to attempt such transformation for *Schistocerca* but produced only black succeeding instars with few exceptions. This phenomenon was also observed in a selection experiment for differences in colour transformation in strains of *Locusta* during the same period, whereas earlier in the year loss of melanin had been quite rapid. Since during the winter season the locust laboratory is rather poorly ventilated in order to attain the rather high temperatures required for locust breeding, it seemed as if the air of this room, which contains about 70 cages with the three species under crowded conditions, could be a contributory cause of retention of black coloration. An experiment was accordingly set up to test this hypothesis with the genetics laboratory as control room. This room houses *Drosophila* stocks and is also rather poorly ventilated during winter. The tests were run as three consecutive experiments.

In the first experiment, a series of ten isolated late second instar hoppers from each of crowded cages of *Schistocerca* and *Locustana*, thus with the characteristic black gregaria coloration, was placed in each of the laboratories. In the second experiment two hoppers were placed in each of five jars for the three species *Schistocerca*, *Locustana* and *Locusta* and duplicates housed in the two laboratories; for the first two species the hoppers were from different strains to those of the first experiment. *Locusta* was tested only in the pairs experiment since this species has been found to solitarize fairly readily in single cultures; for *Locustana*, females only were used since in the field males turn green much more rarely than females.

In the third experiment ten black hoppers of *Schistocerca* were isolated during early third instar and housed in the genetics laboratory. Immediately after the third moult the first three individuals which had turned fawn in colour were returned to the locust laboratory and placed slightly below the level of the crowded cage from which they had been obtained. For another three hoppers which turned fawn in colour at the third moult, the bottom of the jars were lined with strips torn from recently used filters of respirators worn as protection for workers in the locust laboratory against the allergenic substance which seems to be prevalent in locust laboratories; a strip of such a filter was also suspended in each jar for the hopper to roost on. These jars remained in the genetics laboratory where the other four hoppers were also retained as controls.

In all three experiments the quart jars were covered with gauze wire lids and the hoppers were kept constantly supplied with fresh green grass in tubes of water. The jars of a series were placed in a circle around a 40-W lamp and were separated from each other by cardboard partitions. Temperature and humidity conditions were kept as closely similar as possible in the two laboratories. For example, during the course of the first experiment the temperature range in the locust laboratory was 24°–31° C and the humidity range was 25–35 per cent, while for the genetics laboratory the ranges were 24°–30° C and 23–32 per cent; for the second experiment the ranges were 24°–32° C and 32–40 per cent for the locust laboratory and 23°–30° C and 30–40 per cent for the genetics laboratory.

Some fifth instar hoppers died during the final moult, but the adults which were obtained were measured for length of elytron (E), length of hind femur (F) and width of head (C) since from these data the most useful morphometric ratios differentiating the two phases may be calculated, namely, E/F and F/C.

The results obtained in the three series of experiments are given in Tables 1–3.

Table 1. COLOUR TYPES OF HOPPERS IN EXPERIMENTS 1 AND 2

Exp.	Species	Laboratory	Instar	Black (gregaria)	Blackish	Buff or fawn		Green	
					Greenish background	Some melanin	No melanin	Some melanin	No melanin
1·1	*Schistocerca*	Locust	III	10					
			IV	7	3				
			V	3	5	1		1	
		Genetics	III	7		3			
			IV	1			9		
			V			1	2	2	5
1·2	*Locustana*	Locust	III	10					
			IV			2		4	4
			V			3	1	1	5
		Genetics	III	10					
			IV	1		3			6
			V				3		7
2·1	*Schistocerca*	Locust	III	10					
			IV	10					
			V	10*					
		Genetics	III	10					
			IV	7		3			
			V	8		1			1
2·2	*Locustana*	Locust	III	10					
			IV	2	2	2	2		2
			V			4	4		2
		Genetics	III	10					
			IV	1	1		2	2	4
			V			1	4		5
2·3	*Locusta*	Locust	III	10					
			IV	2				7	1
			V	1			1	5	3
		Genetics	III	10					
			IV	1		1		3	5
			V	1			1	1	7

* Greater amount of black than in those of the same class in other laboratory.

Phase transformation in locusts is a process resulting in changes in behaviour, morphology, physiology and the biochemical processes of pigmentation. To a large extent, this transformation is dependent on density and is reversible in the immature stages or instars. Gregaria hoppers are more active, eat more, produce the typical melanin coloration, and in the adult the wings are relatively longer, that is, the morphometric ratio E/F is higher than in solitaria, while the head is broader so that the ratio F/C is smaller than in solitaria. The characteristic colour of hoppers of the solitaria phase is green, or varies from grey to buff or fawn. In the series of experiments described, there is quite clearly an external factor influencing loss of black pigmentation in hoppers reared under solitary conditions. Since the only variable factor

Table 2 Colour Types of Hoppers in Experiment 3

Treatment	Instar	Black (gregaria)	Blackish	Buff or fawn		Green	
			Greenish background	Some melanin	No melanin	Some melanin	No melanin
Genetics laboratory	III	4					
	IV	1	2		1		
	V					1	3
Genetics laboratory + filter	III	3					
	Early IV			1	2		
	Late IV					2	1
	V					1	2
Genetics laboratory	III	3					
	Early IV				3		
Locust laboratory	Late IV					3	
	V	1	2				

in the two breeding rooms appears to be the air it has to be postulated that some air-borne factor is affecting the rate of deposition of melanin in the cuticle This role could be filled by a pheromone, which Karlson and Butenandt[5] have defined as a substance secreted to the outside of the individual and received by a second individual of the same species in which it releases a specific action, for example, a definite behaviour or developmental process Whether this substance is species-specific is not evident in the present series of experiments since all three species are well represented in numbers in the locust laboratory, since the cages have gauze wire sides any volatile substance could diffuse readily There are, however, indications that the three species differ in their receptivity to the pheromone since, in the pairs experiment especially, *Schistocerca* was influenced to a greater extent than the other two species of which *Locusta* was perhaps slightly the more sensitive

The pairs experiment shows that once the gregarization process has been initiated, and thus the substance is being produced, it exerts a cumulative action so that even two individuals mutually affect each other Again, however, the locust room had a greater effect The pheromone discovered by Loher[6] in *Schistocerca* is probably of a different nature since it seems to exert only an accelerating influence on sexual maturity

It was thought that some connexion might exist between the postulated pheromone and the allergenic substance found in locust breeding rooms and which affects the mucous membranes of workers The results of the experiment in which the hoppers had continuous contact with the used respirator filter are, however, not conclusive since the substance may be fairly volatile and may dissipate easily

The recorded experiments have also demonstrated that the processes of melanization and production of green pigmentation are independent since hoppers under isolation may or may not build up green pigment while large amounts of melanin are still present Gunn and Hunter-Jones[7] have by means of divergent selection experiments for the *E*/*F* ratio been able to separate characters used in biometric estimates of phase status from each other and to some extent from colour The present series of experiments appears to demonstrate the fact that in a congregating group of hoppers the melanization process is triggered by the cumulation of pheromone in the atmosphere around the group To what extent this stimulation has a direct influence on morphometrical ratios is not quite clear. As shown in Table 3, the various treatments are not based on sufficient individuals to be statistically significant, and, in any case, all individuals spent part of their formative instar life in the gregaria phase In addition these ratios are most often not as extreme for cage-bred as for field populations In the case of *Locustana*, solitarization in both rooms has produced morphometric ratios of a more gregarious type in experiment 1 and of a more solitary type in experiment 2 For *Locusta* the figures are more or less equivalent for the two treatments and are of a more intermediate value. For these two species the grade of melanization seems to be an element separable from phase morphometrics For *Schistocerca*, however, it has lately been found in the Locust Research Unit that the *E*/*F* ratio of cage-bred populations is higher than the data given for swarms in the field[3] Furthermore, the results of Table 3 are indicative of more gregaria-like ratios in the individuals which were returned to the locust laboratory and which re-acquired darker coloration

Table 3 Morphometric Ratios of Adults obtained in the Three Experiments compared with Crowded Cages and Field Material

Species	Exp	Laboratory	Female		Male	
			E/*F*	*F*/*C*	*E*/*F*	*F*/*C*
Locustana	1 2	Locust	2 12	2 79		
		Genetics	2 10	2 78		
		Cage	2 20	2 60		
	2 2	Locust	1 98	2 82		
		Genetics	2 03	2 81		
		Cage	2 11	2 68		
		Field solitaria (ref 2)	2 02	2 98		
		Field gregaria (ref 2)	2 14	2 58		
Schistocerca	2 1	Locust	2 15	3 38	2 13	3 43
		Genetics	2 22	3 40	2 10	3 48
		Cage	2 32	3 30	2 29	3 23
	3	Locust	2 30	3 33	2 35	3 21
		Genetics	2 22	3 49	2 17	3 32
		Cage	2 35	3 32	2 30	3 29
		Field solitaria (ref 3)	2 07	3 93	2 04	3 86
		Field gregaria (ref 3)	2 29	3 18	2 24	3 11
Locusta	2 3	Locust	1 86	3 35	1 94	3 35
		Genetics	1 85	3 32	1 91	3 46
		Cage	2 00	3 17	1 96	3 21
		Field solitaria (ref 4)	1 81	3 46	1 78	3 67
		Field gregaria (ref 4)	2 18	2 86	2 13	2 96

Finally, it is suggested that the postulated pheromone could possibly be accumulated in the eggs of gregarious females, and in this sense it replaces the locustine suggested by Faure[2], this substance was supposed to accumulate in such eggs and give rise to the dark hatchlings produced by crowded females as compared with the light hatchlings from solitary females

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ABSENCE OF RIBONUCLEASE FROM THE RIBOSOMES OF *PSEUDOMONAS FLUORESCENS*

By H E WADE and H. K. ROBINSON

Microbiological Research Establishment, Porton, Salisbury, Wilts.

A CHARACTERISTIC of ribosomes is an instability towards ethylenediamine tetraacetate (EDTA)[1-3] which frequently terminates in the depolymerization of the ribonucleic acid (RNA)[4-6] This general observation, and the prominence given to an EDTA-activated ribonuclease which is associated with ribosomes from *Escherichia coli*[5-7], have led to the impression that a ribonuclease similar to pancreatic ribonuclease is associated with ribosomes from every source[2,6,8]. This impression has been fostered by the exclusive location of ribonuclease in the '30*S*' ribosomes of *E. coli*[6] and the observation that the ribonuclease content of living cells, in general, appears to vary like the concentration of ribosomes, with the rates of growth or protein synthesis[9].

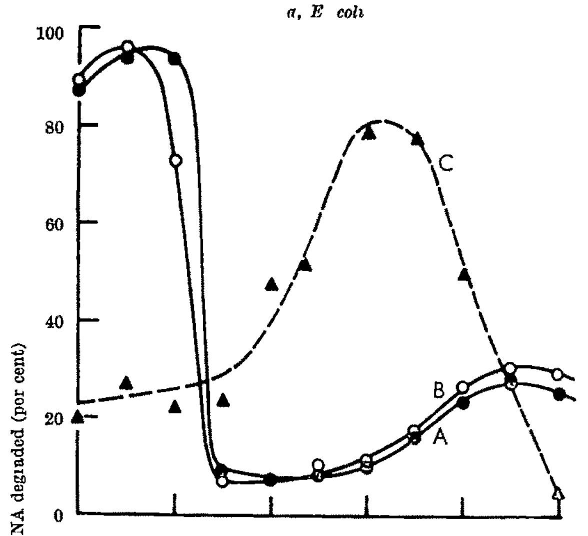
In the course of examining the ribosomes from other species of bacteria, it was found that the processes of ribosome autodegradation did not conform to a common pattern. In particular, ribosomes from *E. coli* and *Pseudomonas fluorescens* were markedly different, reflecting differences in their nuclease contents.

Escherichia coli, American type *B* (*E. coli, B*) was grown (division time 50 min) aerobically in a mineral salts–glucose–ammonium medium by continuous culture[5]. *Pseudomonas fluorescens* (N.C.T.C. 8248) was grown (division time 55 min) in a similar medium supplemented with glutamate.

The bacteria were disrupted in a press[10], suspended (10 per cent wet weight) in 0.145 M sodium chloride with 5 mM magnesium chloride at *p*H 6.5 (*SM* soln.) and centrifuged at 25,000*g* for 1 h at 2°–4° in the No. 30 rotor of the Spinco model *L* centrifuge to sediment heavy particulate material. The supernatant was centrifuged at 85,000*g* for 7.5 h at 2°–4° to sediment ribosomes. The ribosome pellets were inclined at an angle of 45° to allow loose gelatinous material on the surface to fall away, this material was transferred to the final supernatant fraction. The ribosomes were washed twice with 20 volumes of *SM* soln. and stored under toluene at 2°–4°.

The determinations of protein[11], RNA[12] and deoxyribonucleic acid (DNA)[13] showed that the distributions of these constituents between the fractions were very similar in the two species (Table 1). The ribosomes from *Ps. fluorescens*, however, had a greater proportion of protein to RNA than those from *E. coli, B*.

a, E. coli



b Ps. fluorescens

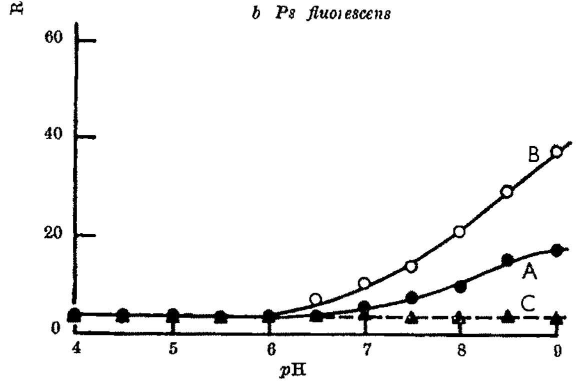


Fig. 1. The autodegradation of ribosomes from (*a*) *E. coli, B* and (*b*) *Ps. fluorescens* in 0.15 M Roth buffers in the presence of (*A*) mM Mg²⁺ (●), (*B*) mM Mg²⁺ and 10 mM orthophosphate (○), or (*C*) 20 mM EDTA (▲). Suspensions of ribosomes were incubated at 37° and samples taken at 2 h (*A* and *B*) or 10 min (*C*)

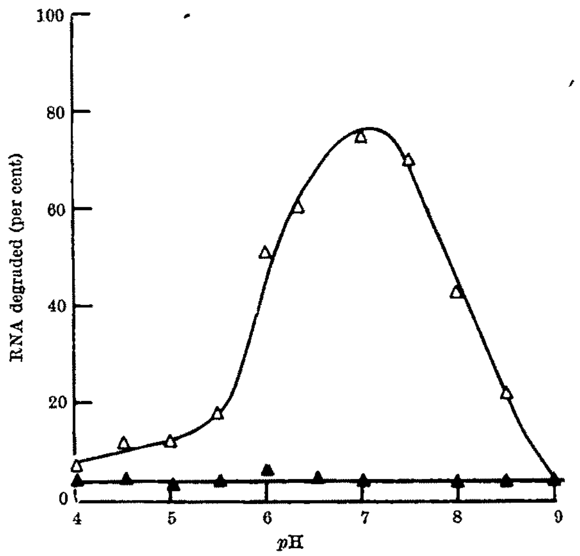


Fig. 2. The autodegradation of ribosomes in disrupted cells of *E. coli, B* (△), and *Ps. fluorescens* (▲). Suspensions were incubated at 37° in 0.15 M Roth buffers in the presence of 20 mM EDTA and samples were taken at 10 min (*E. coli, B*) or 6 h (*Ps. fluorescens*)

The ribosomes were suspended (20 mg dry wt./ml.) in *SM* soln. at 2°–4° and diluted with (*a*) 4 vol. of 0.15 M Roth buffer[14], (*b*) 4 vol. of 0.15 M Roth buffer containing 12.5 mM sodium orthophosphate, or (*c*) 2.32 vol. of 0.15 M Roth buffer containing 28.5 mM EDTA. The buffers were adjusted to values between *p*H 4 and *p*H 9 at intervals of 0.5. The diluted ribosome suspensions (0.5 ml.) were incubated at 37° and samples were extracted with 0.1 N perchloric acid at regular intervals to determine the extent of RNA degradation[5]. The results revealed differences between the ribosomes from *E. coli, B* (Fig. 1*a*), and *Ps. fluorescens* (Fig. 1*b*).

In the presence of mM magnesium chloride, there was rapid breakdown of RNA at *p*H 4–5 in *E. coli, B*, but none in *Ps. fluorescens* (Figs. 1*A* and *B*). Above *p*H 7.5 the rates of breakdown were similar in the absence of orthophosphate (Fig. 1*A*) but, in its presence (Fig. 1*B*), the breakdown was stimulated to a greater extent in *Ps. fluorescens* than in *E. coli, B*. This suggests that ribosomes from the *Pseudomonas* have a higher content of polynucleotide phosphorylase[15]. In this respect, *Ps. fluorescens* more closely resembles *E. coli* N.C.T.C. 1100 (ref. 15).

In the presence of 20 mM EDTA, the differences between *E. coli, B*, and *Ps. fluorescens* were very marked (Fig. 1,*C*). The ribosomal ribonuclease revealed by this treatment was very prominent in *E. coli, B*, and undetectable in *Ps. fluorescens* even after continued incubation for 2 h. The degradation at *p*H 4–5 observed in the presence of mM magnesium chloride (Fig. 1*a*, *A* and *B*) could also be attributed to the ribonuclease since an alkali degradation of the primary acid-soluble products, produced only nucleoside 2′(3′)-phosphates, which were identified by

Table 1. DISTRIBUTION OF NUCLEIC ACIDS AND PROTEIN BETWEEN SEDIMENTATION FRACTIONS FROM *Escherichia coli, B*, AND *Pseudomonas fluorescens*

Fraction		*E. coli, B*	*Ps. fluorescens*
Heavy particulate	Protein	4.77	5.3
	RNA	0.86	0.66
	DNA	0.19	0.15
Ribosome	Protein	1.78	2.25
	RNA	2.20	2.38
	DNA	0.001	0.005
Soluble	Protein	8.4	7.4
	RNA	0.98	1.00
	DNA	0.50	0.54

The results are expressed as mg/ml. of initial, unfractionated suspension of disrupted bacteria their elution pattern from ion-exchange resin[16] and their resistance to periodate[17].

Although ribonuclease appears to be located exclusively in the ribosome fraction of *E. coli*[6], this did not preclude the possibility that, in *Ps. fluorescens*, the enzyme is present in some other fraction of the cell. To examine this point, a suspension of unfractionated disrupted bacteria was incubated with 20 mM EDTA between *p*H 4 and *p*H 9 (Fig. 2). In *E. coli, B*, the RNA was broken down at about the same rate as in the isolated ribosome fraction; in *Ps. fluorescens* there was no significant breakdown even after incubation for 6 h, suggesting the absence of the enzyme from this species.

The inability to detect ribonuclease in *Ps. fluorescens* may have a simple explanation. It is possible that the enzyme is present but tightly bound to an inhibitor and that, in other circumstances, it would have been detected. If the enzyme is in fact absent, its absence may reflect some feature of growth rather than a genetic inability to synthesize the enzyme. Whatever the explanation, the ribosomes from *Ps. fluorescens* more closely resemble certain ribosome preparations from higher cells in having negligible ribonuclease activity; in these preparations the ribosomes are not degraded by EDTA beyond smaller sedimentable particles[2,3,18-22]. Sedimentation analyses were carried out to determine whether or not similar changes could be observed with ribosomes from *Ps. fluorescens*.

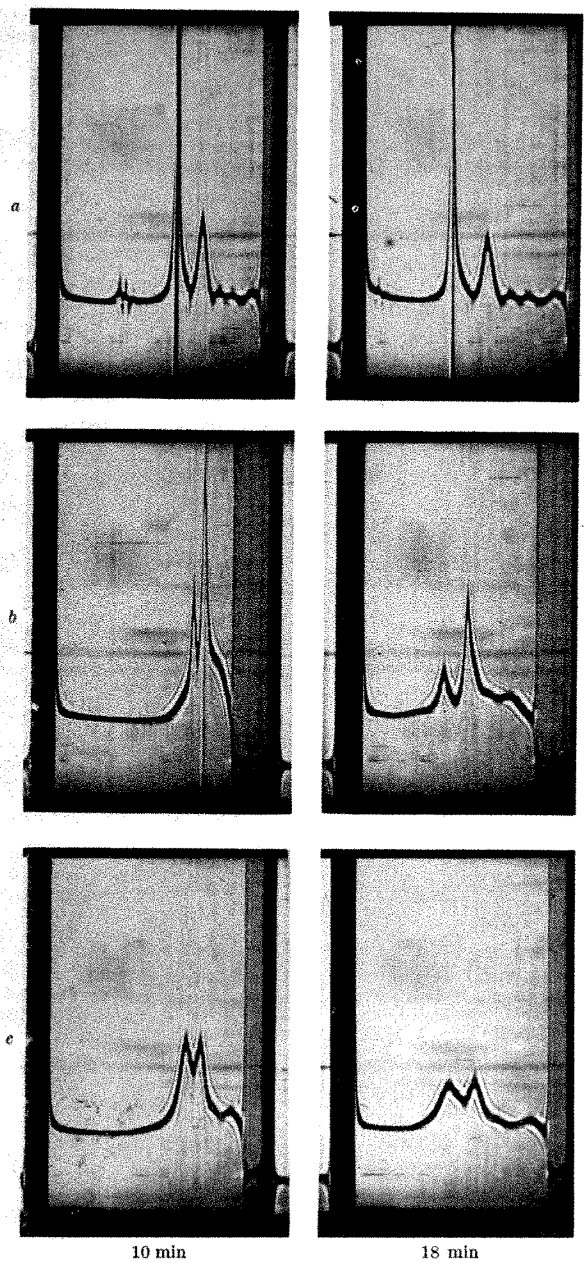


Fig. 3. The sedimentation analysis of ribosomes from *Ps. fluorescens*. (*a*) A suspension (12 mg dry weight/ml.) in 0·145 M NaCl with 5 mM $MgCl_2$ at *p*H 6·5; (*b*) after dialysing for two days against 0·145 M NaCl with mM EDTA; (*c*) after dialysing for two days against 0·145 M NaCl with mM EDTA and two days against 0·145 M NaCl with 5 mM $MgCl_2$. Photographs were taken at 10 min and 18 min after reaching full speed (47,660 r.p.m.) at 25° (R.T.I.C. unit); phase angle 55°

A suspension of ribosomes in *SM* soln. gave a typical sedimentation pattern in the analytical centrifuge (Spinco model *E*), with a major 45·4*S* (S_{w20}) peak and a minor 31·9*S* peak, corresponding to the '50*S*' and '30*S*' ribosomes[23] respectively, and also traces of a '70*S*' and two slow sedimenting peaks (Fig. 3*a*). When this suspension was dialysed against 0·145 M sodium chloride with mM EDTA at *p*H 6, conditions under which the RNA in *E. coli* ribosomes breaks down into acid-soluble substances, dissociation into more slowly sedimenting components occurred with major peaks at 15·4*S* and 20·3*S* (Fig. 3*b*). When the reversibility of these changes was tested by dialysing back into *SM* soln., non-specific aggregation occurred with the formation of peaks at 23·1*S* and 31·2*S* (Fig. 3*c*), similar to the aggregation which occurs with EDTA-treated ribosomes from higher cells[18,19].

As in other ribosome preparations which exhibit negligible ribonuclease activity, the ribosomes from *Ps. fluorescens* produce two, more slowly sedimenting components when exposed to EDTA. In pea seedlings[18], thymus nuclei[20], rat liver microsomes[19] and Novikoff hepatoma[22], these components sediment at 23–30*S* and 40–46*S*, the heavier component being about twice the concentration of the ligher component. In contrast to this, the two most prominent components from *Ps. fluorescens* sediment more slowly (15·4*S* and 20·3*S*) and the lighter one is the more prominent (Fig. 3*b*) suggesting that the untreated ribosomes react differently towards EDTA. It is tempting to speculate, from the sedimentation coefficients and the relative concentrations, that in *Ps. fluorescens* these components are ribosomal RNA[23]; however, there is at present no other experimental evidence to support this.

We thank Mr. K. H. Grinstead for carrying out the sedimentation analyses and Dr. K. A. Cammack for his advice.

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INSECTICIDE RESISTANCE IN THE CABBAGE ROOT FLY IN BRITAIN

By T. H. COAKER, D. J. MOWAT and G. A. WHEATLEY

National Vegetable Research Station, Wellesbourne, Warwick

ALDRIN and dieldrin are the principal insecticides used in Britain to control the cabbage root fly (*Erioischia brassicae* (Bouché)) attacking brassica crops. If the recommended methods for using these insecticides are followed, that is, a root dip for transplants or treatment of the soil around the plants after transplanting, the amount of active ingredient applied does not usually exceed 0·4 lb. per acre and this is adequate to give a high degree of control[1]. There is no evidence that resistance to these insecticides has developed in Britain when these recommendations have been followed.

Early in June 1963, a case of a failure to control this pest with aldrin was brought to our notice. On a farm near Great Rollright, Oxfordshire, the infestation by the first generation of the root fly was very severe with up to 50 or more larvæ present on many of the plant root systems. About 140 acres of the early brussels sprout crop were destroyed and several more acres were severely damaged, despite the copious use of aldrin as a broadcast treatment to the soil before transplanting and also as a spot treatment after planting out; either treatment by itself is normally sufficient to effect control. Furthermore, enquiries revealed that most fields on the farm had been intensively cropped with brassicas (at least five crops of cauliflowers or brussels sprouts in eight years) and that both broadcast and spot treatments with aldrin had been used on each crop. The brassica crop on this farm was relatively isolated from those on neighbouring farms, and a consideration of these factors suggested that the root fly population on this farm was local and had developed resistance to aldrin.

In order to confirm that large amounts of aldrin had been used in the past, soil samples were obtained from one field where the crop had been destroyed by the root fly. Two samples were taken from the inter-plant spaces and a further two samples from within about 5 cm of the plant stems in the region which would normally have been spot-treated. Each sample comprised 25 cores × 2·59 mm diam.; those from the interplant spaces were taken to the maximum estimated depth of cultivation (22 cm), while those from near the plants were restricted to 15 cm in depth. The samples were thoroughly mixed and sub-samples were taken and kept at $-8°$ C prior to analysis. 50 g of soil was extracted with hot acetone and, after suitable clean-up, the extracts were transferred to *n*-hexane, concentrated and made up to 5 ml. Aliquots of the extracts were analysed qualitatively and quantitatively by reversed phase paper partition chromatography[2], the identities of the residue components being confirmed by gas/liquid chromatography using an electron capture detector. The results in Table 1 show that there was indeed a high concentration of aldrin in the soil next to the plant roots at the time when large numbers of root fly larvæ were present. Aldrin was also present in the soil taken from between the plants and there was a high general level of dieldrin in both types of sample, the dieldrin having been formed by epoxidation of aldrin used on previous crops. DDT was known to have been used occasionally to control certain foliage pests, and the several components of technical DDT, and the degradation product DDE, were detected in the samples, thus confirming previous observations that foliage treatments with this insecticide lead to appreciable residues in the soil[2].

Table 1. RESIDUES IN SOIL SAMPLES TAKEN ON JUNE 13, 1963; ASSESSED BY PAPER CHROMATOGRAPHY

Means of duplicate measurements on duplicate samples

Insecticide	Position of sample			
	Near plant stem		Inter-plant space	
	p.p.m.	lb./acre	p.p.m.	lb./acre
Aldrin	13	20	0·70	1·7
Dieldrin	1·8	2·7	0·82	2·0
p,p'-DDT	—	—	0·77	1·8
o,p'-DDT	—	—	0·16	0·38
DDE	—	—	0·28	0·68
DDD	Small amount detected—not measured			
γ-BHC	—	—	< 0·008	< 0·018
Standard error (%)	± 19·0	± 18·9	± 5·6	± 5·4

Table 2. RESIDUES IN SOIL SAMPLES TAKEN AUGUST 20, 1963, AFTER THE FIELD HAD BEEN CULTIVATED

Means of duplicate measurements on four samples

Insecticide	Chromatographic system			
	Paper partition		Gas/liquid	
	p.p.m.	lb./acre	p.p.m.	lb./acre
Aldrin	0·75	1·7	0·71	1·6
Dieldrin	0·72	1·7	0·58	1·3
p,p'-DDT	0·74	1·7	0·56	1·3
o,p'-DDT	0·15	0·35	0·13	0·29
DDE	0·22	0·51	0·13	0·29
DDD	Small amounts detected—not measured			
γ-BHC	< 0·008	< 0·018	0·004	0·009
Standard error (%)	± 15·2	± 15·0	± 13·1	± 13·1

The remains of the crop were afterwards destroyed and four further soil samples were obtained from the same field on August 20, 1963, after the soil had been cultivated. The residues were assessed by paper and gas/liquid chromatography, and the results (Table 2) from the two methods are mutually confirmatory. The levels of residues found were similar to those in the inter-plant soil samples taken in June.

Laboratory tests. The susceptibility to dieldrin of adult cabbage root flies obtained from pupæ collected at Great Rollright was compared with that of flies collected similarly from a field population at Wellesbourne. The root fly population at Wellesbourne is still readily controlled by normal applications of aldrin and dieldrin although these insecticides have been regularly used during the past ten years.

Batches of flies comprising equal numbers of newly emerged males and females were used for the tests. They were anæsthetized with nitrogen, and technical dieldrin dissolved in Shell 'Risella 17' oil was applied to the coxal region after which the flies were allowed to recover and were held at 18° C during the tests. The mortalities were assessed at suitable intervals and the time/response curves constructed, their average slopes being 0·98 and 1·40 for the Rollright and Wellesbourne strains respectively. From the relationship, dose versus time, taken for 50 per cent response (T_{50}, Fig. 1) derived from the time/

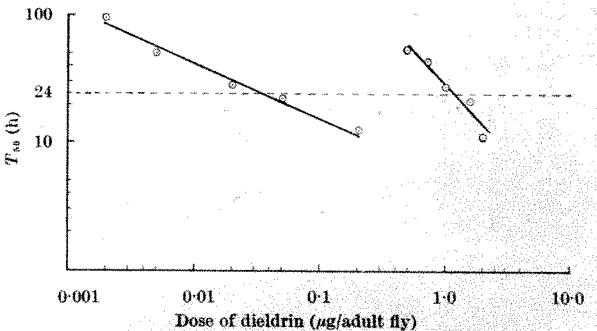

Fig. 1. Relationship of time taken for a 50 per cent response with several doses of dieldrin for Wellesbourne and Rollright strains of adult *E. brassicae*

Table 3 DEGREE OF CONTROL OF THE CABBAGE ROOT FLY POPULATION AT ROLLRIGHT BY SPOT TREATMENTS OF INSECTICIDES

	Active ingredient (per cent)	No larvæ and pupæ/10 plants	Root damage index (per cent)
Dieldrin	0 03	146	68
Dieldrin	0 15	141	63
Dieldrin	0 30	127	51
BHC	0 02	84	41
Diazinon	0 05	22	18
Untreated	—	119	56
	S E (diff)	±19 6	± 7 6
	L S D (*P* = 0 05)	±43 7	±16 9

response curves, 1 23 μg dieldrin per fly was required for 50 per cent response at 24 h for the Rollright strain compared with 0 035 μg dieldrin per fly for the Wellesbourne flies Further tests using aldrin and γ-BHC indicated that the Rollright strain was resistant to these insecticides also

Field tests To test the susceptibility of the larvæ attacking brussels sprout plants at Rollright, drench treatments of three commercially available insecticides (dieldrin, γ-BHC and diazinon) known to control the root fly under normal conditions were applied to young plants on June 26, one week after transplanting Two months later, at the end of the attack by the second generation of the root fly, the plants were sampled and the numbers of larvæ and pupæ and the amount of root damage were assessed (Table 3) Plants treated with dieldrin, even at ten times the amount normally required to kill susceptible larvæ, were as severely damaged as the untreated ones and the numbers of larvæ present were similar Gamma-BHC gave some degree of control, and it was only the organophosphorus insecticide, diazinon, which was highly effective in reducing both the number of larvæ and root damage.

It is also interesting that no parasitism was recorded in a total of about 2,000 pupæ collected from Rollright whereas in the Wellesbourne population 40–60 per cent of the pupæ are normally parasitized by *Aleochara* spp (Col , Staphylinidae) and *Idiomorpha rapae* (Westwood) (Hymen , Cynipidae)

The ability of larvæ of the Rollright strain of root flies to survive in the presence of high concentrations of aldrin and dieldrin in the soil, and the widely differing susceptibilities of the adult flies from Rollright and Wellesbourne to aldrin, dieldrin and γ-BHC, indicated that the former strain of flies had developed resistance to these insecticides This is further confirmed by the satisfactory control obtained in the field by diazinon, a result which also suggests that diazinon may be used as a possible alternative means of controlling root fly which is resistant to the *cyclo*diene-type insecticides

We should like to thank Mr K Coghill for bringing this problem to our attention

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POLYDISPERSITY

By R. A GIBBONS

National Institute for Research in Dairying, Shinfield, Reading

IT seems desirable to attempt to clarify the concept of polydispersity in biological macromolecular preparations The term 'polydisperse' has often been used as a synonym for heterogeneous, nevertheless there is a general sense, as a rule, that the former implies a less-impure preparation than the latter It appears to me that there is, or should be, a valid distinction between these two terms which it is useful to maintain

A preparation which is monodisperse with respect to all its properties, that is, homogeneous *sensu strictori*, consists of an assemblage of identical molecules, having the same electrophoretic mobility, frictional coefficient, chemical composition and structure, immunological reactivity, etc The variance of all measurable parameters throughout the sample is zero If this is not so, and one or more parameters show some variability within the sample, two types of variability may be usefully distinguished Where n_i is the number of molecules in the sample possessing amount p_i of a measurable property, p_i may be (1) a unimodal or (2) a bi- or poly-modal function of n_i A preparation in which p_i varies unimodally with n_i for all measurable properties p (that is, the distribution curves have one maximum value and two points of inflexion only) may in most contexts be considered a pure or homogeneous preparation, provided that the variance is not great The molecules in such a sample constitute statistically a single population, and this should be the criterion of a polydisperse preparation Where the variability of any parameter can be shown to be bi- or poly-modal, the preparation should be characterized as heterogeneous

In this way a preparation containing molecules which are all the same type, but which vary only in chain-length or in some other structural detail, is distinguished from a mixture of two different molecular species Thus pure samples of chondroitin sulphates *A* and *B* would each be homogeneous but polydisperse (with respect to chain-length), but a mixture of the two would be heterogeneous even if the molecular weight distributions for each component happened to be the same, for the distribution of other parameters (amount of glucuronic acid per molecule, optical rotation) would be bimodal Clearly, a preparation may be monodisperse with respect to one parameter, polydisperse with respect to a second and heterogeneous with respect to a third, the foregoing terms are only meaningful if the parameter measured is implicitly or explicitly specified

It is assumed here that biological macromolecules may be produced having the type of variability in physical and chemical properties which may be approximated to a continuous distribution, as for example the chain-length of a synthetic polymer This assumption is not compatible with present-day views on the very high degree of precision with which proteins are synthesized[1], but there is no reason to believe that all macromolecules, or even all proteins, arise by primary gene-directed synthesis. Proteins are indeed something of a special case among naturally occurring macromolecules in that the primary structure is specified by the gene DNA, and any variability is limited to the loss of information in transmission between gene and microsome Present evidence suggests this is slight[2] Genetically determined variants of what is functionally the same protein exist in some profusion[3]

Where a protein is made up of a number of sub-units, themselves polymorphic in the genetic sense, the number of distinguishable 'isoproteins' may be quite large, even in one individual In these circumstances, it may sometimes be convenient to regard the material as polydisperse in the sense defined with respect to some of its properties, for example, sedimentation coefficient or electrophoretic mobility Even so, this type of variability is generally likely to lack sufficient of the random element for any molecular parameter to be usefully considered as continuously variable, but it has been suggested that some degree of random variability in secondary or tertiary structure may confer polydispersity with respect to frictional coefficient on some proteins[4] The position is otherwise with regard to substances the synthesis of which is not directly gene-controlled Genetic control is exercised here only in the sense that the gene (or genes) produces an enzyme which participates in the biosynthetic system Glycogen is a well-studied macromolecule which is known to be produced in this way[5] At least two enzymes, themselves the products of different genes, are involved co-operatively in its synthesis, and, while these enzymes are quite specific in the type of chemical linkage which they introduce, the length of the poly-glucoside chains and the precise position of the 1→6 branch points are subject to a limited amount of random variability[5,6]

Thus, glycogen is not a unique chemical individual, any preparation contains molecules which vary both in molecular weight and in detailed chemical structure Another material which is similarly produced is the human blood-group substance This may seem surprising since the simple Mendelian inheritance of the blood-group characteristic suggests a close relation of the blood-group substance and the gene, but this is not so The ABO blood-group antibodies react with rather small specific oligosaccharide groupings[7] which are introduced quite late in the synthesis of the macromolecule[8] The enzymes which effect this introduction are themselves presumably the products of the blood-group genes. The blood-group substance molecule, as present in mucous secretions, is the product of many genes, and the evidence suggests that, like glycogen, there is considerable natural variation in the size and structural detail of these substances[9] It is likely that many macromolecular substances, particularly when they are largely or entirely non-protein in character, arise in this way Thus epithelial mucus contains a number of mucosubstances chemically similar to the blood-group substances[10], and their biosynthesis is unlikely to be radically different The composition of one of these substances varies with the hormonal state of the animal[11], this indicates that polydispersity with respect to one aspect of the structure of this molecule (amount of terminal neuraminic acid present) is of biosynthetic origin Some proteins may likewise be modified by other gene products before reaching their final functional state[12] In very large molecules, the extent of variability arising from a quite small degree of imprecision in biosynthesis may be very great—for example the number of theoretically distinguishable glycogen molecules of molecular weight 10^7 is greater than 10^{2000} A sample of this polysaccharide can never be other than polydisperse, but a pure or homogeneous preparation of glycogen is nevertheless a meaningful concept This is the justification for the distinction between 'heterogeneous' and 'polydisperse' which is proposed

It is necessary to reconsider the customary criteria of homogeneity to distinguish criteria of homogeneity from criteria of monodispersity Thus the observation of a single boundary in a sedimentation velocity or electrophoretic experiment is evidence of homogeneity within the limits of sensitivity of the optical system, the more rigorous ultracentrifugal test of Creeth[13] or electrophoretic test of Alberty[14] distinguish monodisperse from polydisperse materials Furthermore, it is desirable to characterize a preparation which is polydisperse with respect to any given property by the variance of this property (or better its distribution function) in addition to its mean value This is now possible, under favourable conditions, for sedimentation coefficients[15] and electrophoretic mobilities[16]

It is always theoretically possible to subfractionate a polydisperse material Where this is also possible in practice, the subfractions may, indeed should, be distinguishable chemically This is to be interpreted as evidence of polydispersity rather than, necessarily, of inhomogeneity Furthermore, if the naturally occurring material is considerably polydisperse, small variations in isolative procedure will select a slightly different assemblage from an initially polydisperse starting material Different preparations of what is allegedly the same substance will then show small but significant physical chemical and possibly biological differences from one to another, a circumstance which has caused confusion The blood-group substances and the submaxillary mucoproteins would appear to be instances where this has occurred

In considering the properties of a polydisperse assemblage to be continuously variable it has been tacitly assumed that the quantities of all variable properties considered, when expressed as amount per molecule, are large This is tantamount to considering only high-molecular-weight material Where relatively small macromolecules are concerned, it is necessary to take into account the fact that all differences between molecules are ultimately discrete differences It may then be better to represent the distribution as a discontinuous histogram rather than a smooth curve, for it may prove possible to separate adjacent members of a polydisperse assemblage into discrete subfractions A recent examination of human α_1-glycoprotein suggests that this has been achieved in this instance[17] In an extreme case, such as that of ovalbumin, which may have none, one or two phosphate residues only per molecule[18], the statistical concept of molecular variability outlined here is no longer helpful

Molecular variability has been discussed at length by a number of authors[19] It is submitted that confusion has arisen because it has been tacitly assumed in many cases that the system which is now being revealed as responsible for the primary DNA-directed synthesis of proteins (or sub-units of proteins) is the only important biosynthetic mechanism for macromolecular substances This is clearly not true for a number of polysaccharides and glycoproteins, and may not be true of all proteins The distinction between primary and secondary gene-products is a useful one, though probably not absolute, thus the protein portion of a glycoprotein may be a primary gene product whereas the saccharide portion may not be

When the substance under investigation is a protein which is believed to be directly derived from the gene or genes which control its synthesis, it may be justifiable to regard evidence of polydispersity as indicating the presence of either impurities or degradation products This view has, however, served to obscure a distinction which assumes some importance when biosynthesis is not directly gene-controlled and consequently the material being examined does not necessarily consist of an assemblage of identical replicates

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LETTERS TO THE EDITOR

PHYSICS

Ultra-violet Gas Laser at Room Temperature

ULTRA-VIOLET coherent light has been generated directly at room temperature in a pulsed nitrogen-gas laser A group of 20 strong simultaneous lines have been observed in the ultra-violet Thirty lines have been identified These range in the ultra-violet spectrum from 3000 Å to 4000 Å The strongest line has been observed at 3371 Å, the strongest visible line is at 3400 Å The output radiation is believed to be produced as the result of inversion in the triplet state of nitrogen, resulting in subsequent emission in the second positive group, $C^3\pi_u \rightarrow B^3\pi_g$ The beam has a full angular width of 1 milliradian, as measured 100 ft from the confocal laser cavity

The plasma is excited with a 100–150-kV pulse of sub-μsec duration The coherent-light pulse width is as short as 20 nsec Despite the short pulse duration the output spot of the laser is exceedingly brilliant The output spot for the confocal Fabry–Perot cavity does not contain the usual mode structure associated with single line operation, a characteristic that is noted when several sets of transitions compete simultaneously

Output powers of the order of 10-W peak have been obtained summing over the lines The plasma tube has a 48-in discharge-length Cold cathode excitation is used The silver-coated flint glass end mirrors are spaced at 150 cm The radius of curvature of the mirrors is approximately 400 cm One mirror is heavily silvered, whereas the other transmits 2 5 per cent

The infra-red nitrogen band spectra[1], which can be produced with 40-kV, 30 amp pulses of 1 μsec duration, are not observed when high-voltage pulses are applied

H G HEARD

Energy Systems Inc
Palo Alto, California

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Exchange Currents at Liquid Electrodes

RATES of non-equilibrium processes cannot be calculated *a priori* without quantitative hypotheses as to energies (heats) of activation I emphasize this truism for electrochemical interface phenomena because ions are field particles in the sense that the potential barrier, over which they pass between the bound state and the solvated state, is itself changed by their passage Only in thermodynamic equilibrium do ions become mere test particles, and it is perhaps for this reason that reversible thermodynamics does not always appear fruitful in this field

In a recent communication[1] a model of the potential barrier, separating ions of a solid metal from solution, has been put forward and compared with known observations I now reconstruct that model for interfaces between liquid metals and polar (aqueous) electrolytes The basis of the model is retained· the height of the potential barrier (activation energy for dissolution) is the corresponding height, V_0, with respect to *vacuo* (the real potential) divided by the dielectric constant, $\varepsilon(\omega)$, of the electrolyte taken at the vibration frequency, ω, of the bound ions in accordance with Debye's theory[2] of the dispersion of the dielectric constant Thus the electrolyte is viewed as a continuous dielectric medium with regard to the weakening of electrostatic interactions, but as a set of molecular dipoles with regard to its temperature-dependent response to the local electrical perturbations caused by the oscillations of ions bound at the interface

The barrier has, therefore, a different height for each vibration frequency, the probability of dissolution per ion and unit time being again[1] $(\omega/2\pi)\exp[-V_0/\varepsilon(\omega)kT]$ We thus need to know the distribution of bound surface ions over frequencies, and for this purpose the specific heat model of Debye[3] has been adapted[1] for solid electrodes Its counterpart for the vibrations of liquid metal surfaces has been given by Frenkel[4], who considered surface capillary waves rather than elastic volume waves and obtained a cut-off frequency by regarding twice the mean particle separation as the shortest admissible wave-length, corresponding to a maximum wave number $K = K_{\max}$ The dispersion of capillary waves is given by

$$\omega_{u} = 2\pi K^{3/2}(\sigma/\rho)^{1/2} \qquad (1)$$

σ being the surface tension and ρ the density of the metal The number of vibration modes per unit surface area (which may be equated to three times the surface concentration of ions, in the manner of Debye[3]) between K and $K + \mathrm{d}K$ is $2\pi K\mathrm{d}K$, so that the absolute dissolution rate is

$$R = \frac{2\pi}{3}\left(\frac{\sigma}{\rho}\right)^{1/2} \int_0^{K_{\max}} \exp[-V_0/\varepsilon(K)kT]K^{5/2}\mathrm{d}K \qquad (2)$$

Wave numbers in the region of $K_{\max}$ correspond by equation (1) to frequencies larger than the inverse Debye relaxation time $1/\tau$ of water molecules At such frequencies the orientational part of the polarizability vanishes and the activation energy becomes prohibitive at room temperatures (about $V_0/3$) It is for this reason that a good first approximation for R is obtained by putting $K_{\max} \sim \infty$ together with $V_0/\varepsilon \approx (V_0/\varepsilon_s)\ (1+\omega^2\tau^2)$, where $\varepsilon_s \approx 80$ is the static dielectric constant of a dilute aqueous solution

$$R \approx \frac{\Gamma(7/6)}{9(2\pi)^{7/3}}\left(\frac{\rho^2}{\tau^7\sigma^2}\right)^{1/3} \frac{\exp(-V_0/\varepsilon_s kT)}{(V_0/\varepsilon_s kT)^{7/6}} \qquad (3)$$

$\Gamma(7/6) \approx 0\ 93$ being a Γ-function The correspondence between equations (2) or (3) and the corresponding expressions for solid metals[1] may be brought out by comparing Frenkel's cut-off frequency with Debye's

When the liquid electrode comes to equilibrium with a solution of its ions, the surface tension σ is connected with the electrode potential according to Lippmann's equation[5] (note that R is the smallest at the potential of the electro-capillary maximum) We may then identify[6] zeR (ze is the ionic charge) with the exchange current density obtained by extrapolating Tafel's overvoltage relations to zero net current[5] It can be shown that Hg^{++} ions are dissolved in monovalent form ($V_0 \approx 6\ 5$ eV from a well-known cycle) and are ionized further by a fast redox step, and if we disregard the slight electrocapillary effect of the Hg^{++} concentration in solution on σ, equation (3) yields some 1 4 amp cm^{-2} at room temperature (not counting the electron current, if we count only one degree of freedom per surface ion, this result is to be multiplied by 3) This agrees with the observation[5] that the exchange current density should exceed 0 4 amp cm^{-2}

On the other hand, from the expression for R of solid metals[1] we obtain for Cu^+ at room temperature the exchange current density of $3\ 8 \times 10^{-5}$ amp cm^{-2} as compared with the observed[5] value 2×10^{-5} amp cm^{-2} Considering the complexity of the physical situation, the calculated values for the liquid as well as the solid electrodes agree well with measurements The two appropriate frequency distributions being very different from each other, the method of constructing the barrier appears to be successful.

L BASS

Department of Mathematics,
Lanchester College of Technology,
Coventry

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Fluorescence Spectrum of Di-cæsium Uranyl Nitrate

IN the course of our investigations on radiation effects on cæsium uranyl nitrate ($CsUO_2(NO_3)_3$) it was found that the spectrum of di-cæsium uranyl nitrate is not known although the spectra of the corresponding di-rubidium and di-potassium uranyl nitrates are known Attempts to prepare di-cæsium uranyl nitrate ($Cs_2UO_2(NO_3)_4$) do not seem to have been successful so far[1] It has, however, been possible to prepare the salt and examine its fluorescence spectrum which is recorded here

If an aqueous solution containing cæsium nitrate and uranyl nitrate hexahydrate in the molar ratio of 2 1, or slightly higher, is allowed to evaporate it is possible to get either mono-cæsium uranyl nitrate or di-cæsium uranyl nitrate by controlling the temperature at which evaporation occurs. Thus at 26° C the crystals which separate out are mostly the well-known pale green crystals of mono-cæsium uranyl nitrate mixed with a few yellowish-green crystals which can be easily picked out from the bulk of the crystals If, however, the evaporation is carried out at 10° C it is found that only the yellowish-green crystals are obtained. Chemical analysis and X-ray diffraction studies of the yellowish-green crystals thus obtained prove conclusively that they are the di-cæsium uranyl nitrate crystals

The fluorescence spectrum of the di-cæsium salt at 77° K which is shown in Fig 1 consists of an extended system of bands The structure of the spectrum as indicated in Fig 1 is in general similar to that of the mono-cæsium salt The symmetric vibration *A* occurs in several quanta (observed up to four) alone and in combination with a single quantum separately of each of the antisymmetric vibration *B*, the bending vibration *C* as well as several of the NO_3 and the so-called lattice frequencies Thus *B*, *C*, NO_3 and the lattice vibrations occur only in single quantum either alone or each in combination with one or several quanta of *A* The *B* band is more intense than *A* and the strongest are *A* + *B*, 2*A* + *B*, 3*A* + *B*, and 4*A* + *B* combination bands The frequencies of vibration of the UO_2^{++} ion are, however, different from those of mono-cæsium uranyl nitrate and as derived from the fluorescence spectrum are 857 2 cm^{-1} (sym *A*), 936 1 cm^{-1} (antisym *B*) and 259 0 cm^{-1} (bending *C*) The resonance band, *R*, is at 20,751 cm^{-1} (4817 7 Å). Certain relevant relations between frequencies of the mono- and di-alkali salts are given in Table 1 The values of vibration frequencies for the various uranyl salts except those of di-cæsium uranyl nitrate are taken from Dieke and Duncan[1]

Table 1

Ratio of vibration frequencies Salt	$\frac{B\text{(antisym)}}{A\text{(sym)}}$ Ratio
$Cs_2UO_2(NO_3)_4$	1 09$_2$
$Rb_2UO_2(NO_3)_4$	1 08$_9$
$K_2UO_2(NO_3)_4$	1 09$_1$

Ratio of vibration frequencies of mono- and di-alkali uranyl nitrates

Vibration	$\frac{\text{mono-}}{\text{di-}}$ Cs	$\frac{\text{mono-}}{\text{di-}}$ Rb	$\frac{\text{mono-}}{\text{di-}}$ K
A (sym)	1 03$_1$	1 03$_1$	1 00$_5$
B (antisym)	1 02$_8$	1 02$_6$	1 00$_4$

R K ASUNDI
R. M DIXIT

Spectroscopy Division,
Atomic Energy Establishment Trombay,
Bombay 28

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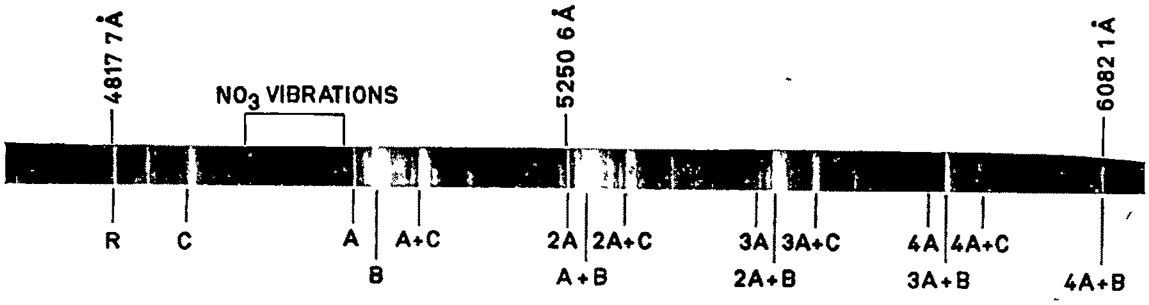

Fig 1

Run-off of Cæsium-137 and Strontium-90 from Rivers

THE factor of weathering must be taken into consideration in the prediction of future radiation dose, either external or internal, caused by the accumulated ground deposits of fission products

Measurements have been made since 1960 on the concentrations of cæsium-137 and strontium-90 in the River Tonegawa, which has the largest catchment area in Japan Monthly deposits of these radionuclides were also measured at Kiriu City, which is situated in the vicinity of the sampling station of river water The results are summarized in Table 1 The concentrations in rain water were calculated by dividing the monthly deposits by the monthly rainfall

An attempt was made to evaluate the run-off of a radionuclide in terms of the cumulative deposition and the deposition rate on the basis of an expression of the following general form

$$C = C_d + p_r C_r$$

where *C* is the concentration of a radionuclide in the river water (μμc./l), C_r is the concentration of the radionuclide in the rain water (μμc /l) corrected for the evaporation (30 per cent is assumed), p_r is a proportionality factor and C_d is the concentration of the radionuclide in the river water which was derived from the cumulative deposition C_d may be assumed to be a function of the cumulative deposition but since its dependence on time during the period of this observation was rather small as compared with that of C_r, it has been temporarily assumed to be constant

Table 1. CONCENTRATIONS OF CÆSIUM-137 AND STRONTIUM-90 IN RIVER- AND RAIN-WATERS (FEBRUARY 1960–JULY 1962)

Time of collection	^{137}Cs $\mu\mu$c./l.		^{90}Sr $\mu\mu$c./l.	
	River*	Rain†	River*	Rain†
Feb. 1960	0·18	7·9	—	—
May 1960	0·070	2·8	—	—
Sept. 1960	0·063	1·2	0·10	0·41
July 1961	0·066	1·1	0·15	0·27
May 1962	0·24	11	0·51	4·8
July 1962	0·32	15	—	—

* At Kurihashi, River Tonegawa.
† At Kiriu City, Gunma Pref.

Regression analyses by use of the data shown in Table 1 showed the values of p_r for cæsium-137 and strontium-90 as 0·013 and 0·072, respectively. This implies that 1·3 per cent of cæsium-137 and 7·2 per cent of strontium-90 from the land surface which originated from fall-out would be carried by river to the sea. We have also obtained the values of 0·035 and 0·064 $\mu\mu$c./l. for C_d respectively for cæsium-137 and strontium-90; these concentrations indicate the run-off of the cumulative ground deposits as the result of weathering.

To check the geographical variation of these values, which may be recognized between different water sheds, measurements were made on the river waters collected from several localities in Japan during August 1962. The results shown in Table 2 indicate an approximate constancy between different rivers in the concentrations of both cæsium-137 and strontium-90. This suggests the universal validity of the proportionality factors p_r for Japanese rivers.

Table 2. CONCENTRATIONS OF CÆSIUM-137 AND STRONTIUM-90 IN RIVER WATERS IN JAPAN (AUGUST 1962)

River	Day of collection (August)	^{137}Cs ($\mu\mu$c./l.)	^{90}Sr ($\mu\mu$c./l.)
Ōyodogawa	1	0·32	0·19
Shirakawa	3	0·32	0·39
Sendaigawa	8	0·37	0·37
Kuzuryūgawa	10	0·35	0·49
Aganogawa	19	0·21	—
Shinanogawa	19	0·45	0·46
Tonegawa	20	0·23	0·32
	Av.	0·32 ± 0·08	0·37 ± 0·11

The validity of the values of C_d for different rivers is also supported by the work of Miyake and Tsubota[1], who reported a similar constancy for river waters collected in Japan during July–August 1961 when the deposition rate was the smallest and the contribution of the ground deposits through leaching was the largest.

The cumulative ground deposit (D) caused by the continuous and constant deposition rate (R) can be shown by the following equation:

$$D = \frac{(1 - p_r)R}{\lambda_e}(1 - e^{-\lambda_e t})$$

where p_r has been given earlier and λ_e is the effective decay constant on the ground, which can be expressed as $\lambda_p + \lambda_w$, where λ_p is the radioactive decay constant and λ_w is the weathering constant.

The run-off by river of the ground deposits of cæsium-137 and strontium-90 can be estimated as 17·5 c. and 32 c. respectively per annum on the basis of the mean concentrations (C_d) as given earlier and the total discharge of 5×10^{14} l. of river water in Japan. By assuming the cumulative depositions of cæsium-137 and strontium-90 as 80 and 28 mc./km^2 respectively in the spring of 1962 and the total land area as $3·65 \times 10^5$ km^2, the weathering constant λ_w would be calculated as $6·0 \times 10^{-4}$ and $3·1 \times 10^{-3}$ respectively for these radionuclides. These values are much lower than those of the radioactive decay constants by factors of 8 for strontium-90 and 40 for cæsium-137. Half the ground deposits of cæsium-137 and strontium-90 would only be leached out after 1,150 and 220 years, respectively. Hence, no weathering factors need to be considered, especially for cæsium-137, in the future prediction of cumulative deposition. However, the slow penetration of the radionuclides into undisturbed soil and the mixing due to cultivation would result in the attenuation of the external exposure and the extent to which the radionuclides may enter the food chain.

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NOBORU YAMAGATA
Institute of Public Health,
Tokyo.

SHUNJI MATSUDA
Kiriu College of Technology,
Gunma University, Kiriu.

KIYOSHI KODAIRA
National Institute of Agricultural Sciences,
Tokyo.

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METALLURGY

Grain Boundary Cavitation in Molybdenum

THE formation of cavities at grain boundaries under creep conditions had been observed in many metals and may lead to intercrystalline failure[1]. We have observed such cavitation in molybdenum wires of 0·010 mm diam. heated by electron bombardment to temperatures between 1,500° and 2,500° C (that is, above 60 per cent of the absolute melting point) and strained a few per cent at rates of 1·5–2·5 per cent elongation per hour. These cavities are of the two types identified by McLean in Nimonic-type alloys[2], wedge-shaped and ellipsoidal. Zener[3] suggested that wedge-shaped cavities are formed by the stress concentration at a grain boundary junction when sliding occurs on boundaries meeting there.

Table 1

	Stresses (lb./in.^{2}) on the molybdenum wire up to but not exceeding		
	1,000	2,000	3,000
No. of experiments	14	31	43
Percentage developing cavities	28	42	50
Percentage developing wedge cavities	7	26	36

Ellipsoidal cavities form at any point along a boundary which is roughly perpendicular to the strain axis. These cavities may link together, forming intercrystalline cracks. The proportion of experiments leading to the formation of cavities increases with the temperature of the molybdenum and with the total strain. The proportion of samples developing cavities, and the proportion of cavitated samples in which the cavities are of wedge type increase with the stress (Table 1). McLean reported the same relationship between cavity type and stress in alloys of Nimonic type[2].

P. J. BOWLES
G. A. GEACH
Associated Electrical Industries, Ltd.,
Aldermaston Court,
Berkshire.

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Enhanced Age-hardening in Aluminium–Magnesium Alloys

ALTHOUGH the solubility of magnesium in aluminium is as high as 16·8 per cent (alloy contents are expressed as atomic percentages) at 450° C (ref. 1), and decreases to an estimated value of 1 per cent at room temperature, the alloys show an abnormally small response to age-hardening. Furthermore, there is no evidence to indicate that the ageing characteristics of these alloys are changed by small additions of third elements, as happens in other binary alloys such as aluminium–copper[2] and aluminium–zinc[3]. For example, the addition of 0·45 per cent zinc to aluminium–8 per cent magnesium causes only a small increase in

hardening[4] even though age hardening is very marked in a wide range of aluminium–zinc–magnesium alloys.

Polmear[5] has shown that trace additions (<0·1 per cent) of silver have a unique effect on the response of many aluminium–zinc–magnesium alloys to ageing in the medium temperature range (120°–220° C). Nucleation of the phase formed at these temperatures is facilitated so that the precipitate is very finely dispersed. Because of these structural changes, higher hardening is achieved. Possible effects of silver are now being sought in other alloy systems, and some interesting results have been obtained for several aluminium–magnesium alloys.

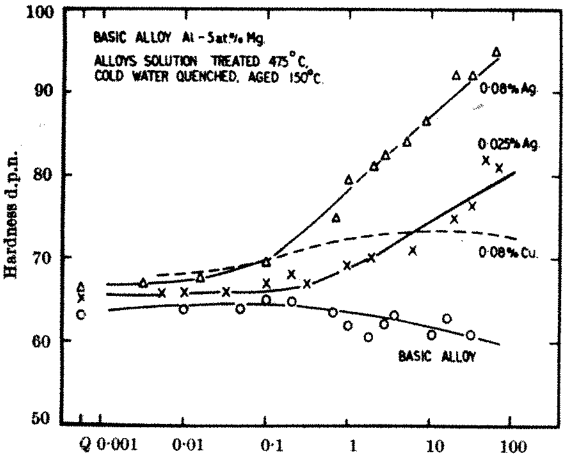
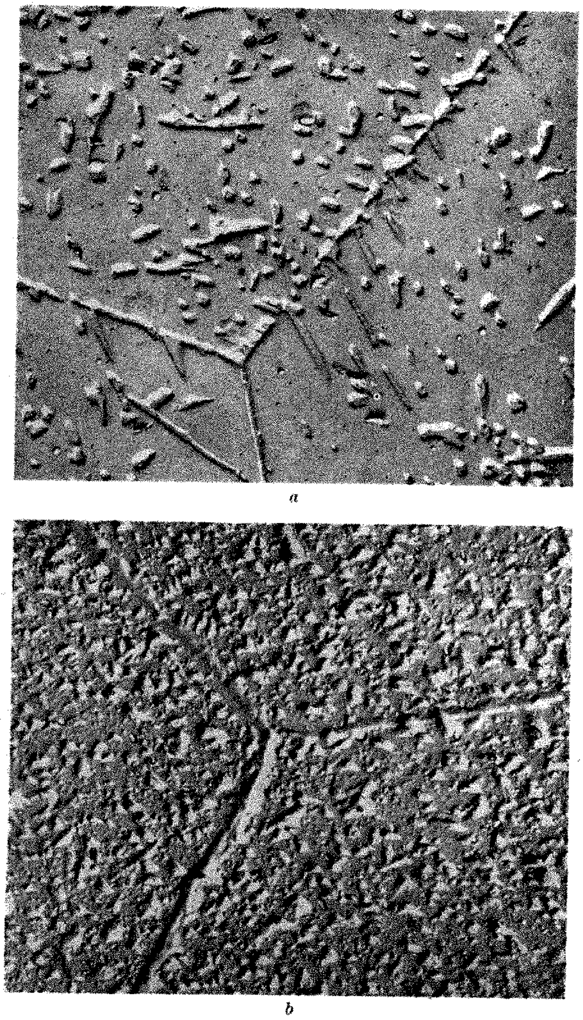


Fig. 1. Effects of small additions of silver and copper on the hardness–time curves for aluminium – 5 per cent magnesium aged at 150° C


a

b

Fig. 2. Replica electron micrographs showing the effect of silver on the microstructure of aluminium–7 per cent magnesium solution treated 475° C, cold water quenched, and aged 10 days at 175° C. *a*, No silver; *b*, 0·12 per cent silver. (× 3,000)

It has been found that quite small amounts of silver may induce hardening in several wrought aluminium–magnesium alloys which normally do not age-harden. These effects were again evident in the medium temperature range, and Fig. 1 shows an example of this for the alloy aluminium–5 per cent magnesium aged at 150° C. Some hardening occurred on adding 0·025 per cent silver, and this became quite pronounced for the higher silver content of 0·08 per cent. The effect of this latter amount of silver was notably greater than that obtained on adding an equivalent amount of copper.

Binary alloys containing higher magnesium contents show some response to artificial ageing, and in these alloys silver both accelerated ageing and increased hardening. An examination was made of the effects of silver on the microstructure of one such alloy, aluminium–7 per cent magnesium aged at 175° C, and the results are shown in Fig. 2. It is clear that silver has again caused the precipitate to be finer and more uniformly dispersed.

These results suggest that silver may also have a unique effect on ageing in aluminium–magnesium alloys. The magnitude of these effects is less than those observed on adding silver to aluminium–zinc–magnesium alloys; but it is significant to note that each alloy system behaved in a similar way. This observation may contribute to the understanding of the mechanism by which silver modifies ageing in certain aluminium alloys. For example, it now seems possible that an interaction between magnesium and silver atoms may be important in this regard. The present results may also be of practical interest since wrought aluminium–magnesium alloys are used for many applications in which increased strength could be an advantage.

Both the theoretical and practical aspects are being studied in more detail, and results will be reported elsewhere.

We thank D. W. Glanvill and P. Curcio, who assisted in the experimental work.

I. J. POLMEAR
K. R. SARGANT

Aeronautical Research Laboratories,
Australian Defence Scientific Service,
Department of Supply,
Melbourne, Australia.

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CHEMISTRY

Negative Degrees of Freedom and the Phase Rule

IN a recent paper, Brynestad[1] states that the occurrence of negative degrees of freedom "is entirely improbable, in opposition to the statement of Halliwell and Nyburg[2]".

Most of Brynestad's paper is not concerned with this particular issue but with suggesting that S (the total entropy), V (the total volume) and the masses of each atomic species present in a system be used to discuss the phase rule rather than T, P and these masses. Lest there be confusion about this we should say we agree with Brynestad that this choice of variables has advantages particularly in defining the masses of each phase present, a feature missing from the T, P scheme.

The question at issue can be illustrated by a simple example[2]. Suppose we were to find two substances, A and

B, the triple points of which were so close that when placed in the same vessel the mutual, but limited, miscibility of the liquid phases brought the triple points into exact coincidence In this case we should have five co-existent phases. solid *A*, solid *B*, *A*-rich liquid, *B*-rich liquid and mixed vapour Then $F = -1$ The significance is clear the degree of freedom lost is that, having chosen *A*, not any *B* will suffice, *B* has to have specific physical properties

This conclusion is, to us, irrefutable A subsidiary issue, and one which seems to preoccupy Brynestad, is "How likely is this to happen?" According to Brynestad it is entirely improbable, a curious phrase, the meaning of which is made clearer by his comparison with the chance of failure of the second law of thermodynamics on a macroscopic scale We do not know how this estimate was arrived at and we do not believe that one can seriously discuss the chances of finding materials with the necessary physical properties in terms of normal probability theory In the strict application of the theory one would be forced to admit that no two people had the same height or weight

The issue resolves into a question as to how accurately can triple points be specified as to remain physically meaningful ? The accuracy cannot exceed the inevitable statistical fluctuations of the system Within these limits we do not see why, if one was patient enough to search, one would not find substances falling within the required limits We do not regard the practical issue of importance We do insist, however, that if a system having $F = -1$ were found the physical significance would be that which we have given

H F HALLIWELL

Nuffield Foundation Science Teaching Project,
5-7 Tavistock Place,
London, W C 1

S C NYBURG

Department of Chemistry,
University of Keele

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Formation of Peroxidic Precipitate in the Radiolysis of Uranyl Nitrate Ketone Solutions

It has been found that in the radiolysis of moist uranyl nitrate methyl isobutyl ketone solutions saturated with oxygen a yellow insoluble substance is formed This remains clinging to the walls of the irradiation vessel According to the weight analysis of the precipitate the most suitable formula appears to be UO_4 $4H_2O$

Gamma-radiation of cobalt-60 at the dose rate $0\ 6 \times 10^{16}$ to $4\ 0 \times 10^{16}$ eV/ml h was used Yield $G(U)$ is given by the number of uranium atoms in the precipitate formed due to the absorption of 100 eV in solution

The dose dependence of the precipitate formation is given in Fig 1 In the concentration range of 0 001–0 1 M $UO_2(NO_3)_2$ $6H_2O$ the initial yield $G(U) = 2\ 7$ At concentrations of 0 001 and 0 01 M the precipitate formation is stopped at corresponding doses as a consequence of the exhaustion of uranium from the solution At higher concentrations than 0 1 M the yield $G(U)$ is smaller and an induction period appears At concentrations above 0 1 M the precipitate is no longer formed

The water concentration has a noticeable influence on the precipitate formation At the concentration of water given only by the crystallinic water of $UO_2(NO_3)_2$ $6H_2O$, the precipitate is not formed at all With increasing concentration of water the precipitate yield is increased and reaches a value of 2 7 when the solution is saturated (about 1·3 M H_2O)

The influence of nitric acid is evident from Table 1 As nitric acid is also formed during the radiolytic decomposition of uranyl nitrate the decrease in $G(U)$ at doses above 2×10^{20} eV/ml may also be due to the action of that acid At concentrations higher than 0 1 M $UO_2(NO_3)_2$ and in the presence of water, some hydrolysis of uranyl nitrate evidently occurs The nitric acid formed in this way can also be responsible for the decrease of the precipitate yield at these higher concentrations and eventually for the enhancement of the induction period

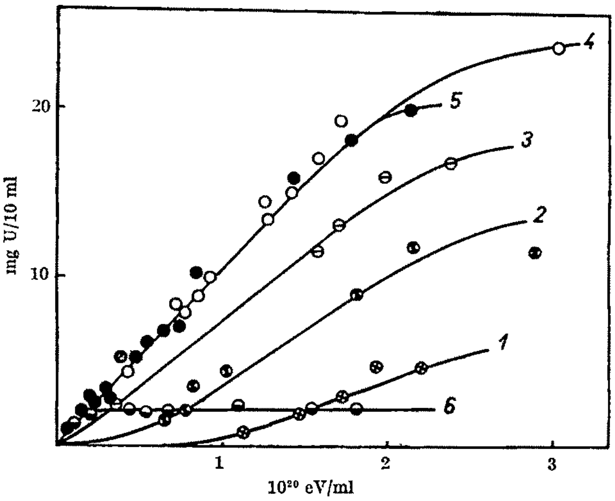

Fig 1 Dose dependence of precipitate formation in methyl isobutyl ketone saturated with water and oxygen 1, 0 5 M, 2, 0 2 M, 3, 0 15 M, 4, 0 1 M, 5, 0 01 M, 6, 0 001 M $UO_2(NO_3)_2$ $6H_2O$

Replacement of the oxygen atmosphere by air causes the appearance of the induction period even in solutions of 0 1 M $UO_2(NO_3)_2$ concentration and the yield $G(U)$ to decrease to 2 4 Consumption of the aerial oxygen is achieved at corresponding doses and the precipitate formation is stopped In a nitrogen atmosphere the precipitate is not formed at all

The precipitate formation in the two-phase systems (5 ml of water + 5 ml of ketone solution) is demonstrated by Fig 2 According to the distribution coefficients the uranyl nitrate concentration in the water phase is much greater than that in the ketone phase[1] From visual observation it is evident that the precipitate is formed predominantly at the interface In this interface a diaphragm, which is characterized by some mechanical strength, is gradually built Its existence impedes any further precipitate formation (Fig 2), only in the case of its mechanical destruction is the precipitate formation partially restored This remarkable mode of precipitate formation indicates that the most favourable reaction conditions are at the interface But by the building of the mechanical membrane these conditions gradually deteriorate, the mutual diffusion of reactants is suppressed and the reactions leading to the precipitate formation are stopped

Similar results were also obtained with methyl *cyclo*hexanone solutions The precipitate is formed only when the concentration is less than 0 2 M $UO_2(NO_3)_2$, at 0 05 M concentration $G(U)$ attains a constant value of 3 5 The same salient effect of water concentration was observed Addition of nitric acid inhibits the precipitate formation and even at a concentration of 0 028 M HNO_3 in 0 1 M $UO_2(NO_3)_2$ solution the suppression is complete

The mechanism of formation of the uranium peroxidic precipitate in these systems is apparently not a simple one One can assume that the precipitate is formed by reaction of the uranyl nitrate with peroxidic radicals

Table 1 0 1 M $UO_2(NO_3)_2$ SATURATED WITH WATER AND OXYGEN

Concentration of added HNO_3 mole/l 10^2	$G(U)$
0 0	2 7
2 8	2 0
7 0	1 2
14 0	0 0

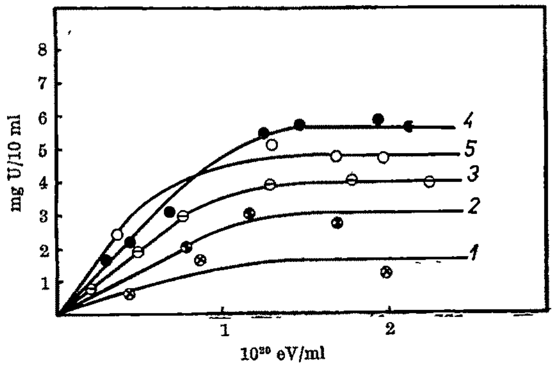

Fig 2 Dose dependence of precipitate formation in two-phase systems saturated with oxygen The total dose is the sum of doses absorbed in aqueous and in organic phase, the concentration of $UO_2(NO_3)_2\,2H_2O$ in aqueous phase 1, 1 M, 2, 0 5 M, 3, 0 2 M, 4, 0 1 M, 5, 0 01 M

and/or with hydrogen peroxide So long as the uranium peroxidic compound remains in solution it is decomposed by the radiolytic process As soon as its concentration exceeds the solubility limit, it is precipitated and so protected against further decomposition The existence of the induction period may be understood in a similar way The solubility of the uranium peroxidic compound can be affected by the nitric acid and consequently the yields *G*(U) and length of induction periods changed

JIŘÍ TEPLÝ
VÁCLAV ŠTULÍK

Nuclear Research Institute,
Czechoslovak Academy of Sciences,
Řež by Prague

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New Fluorine Compounds of Technetium

IN contrast to the many fluorides, oxide fluorides and complex fluorides known for rhenium, only a few compounds of technetium have been reported, namely, the hexafluoride TcF_6 (ref 1), the trioxide fluoride[2], and salts of the quadrivalent $TcF_6^{=}$ ion[3]

Preliminary results in these laboratories indicate that the blue oxide tetrafluoride $TcOF_4$ (m p 134°) and the yellow pentafluoride (m p 50°) are formed as by-products in the direct fluorination of technetium metal The oxide tetrafluoride is much like the rhenium analogue[4,5] in appearance and in physical properties, and the magnetic moment (μ_{eff} (25°) = 1·76 Bohr magnetons, θ = 9°) is of the expected order for a d_1 compound The pentafluoride is isostructural with chromium pentafluoride and has orthorhombic symmetry (a_0 = 7 6, b_0 = 5 8, c_0 = 16 6 Å) It is more stable than rhenium pentafluoride[4,5], but begins to decompose in glass at 60°

Technetium hexafluoride can be reduced more easily than rhenium hexafluoride The action of a solution of potassium iodide in iodine pentafluoride (that is, of $I_2 + IF_4^-$) on the compound leads to a quadrivalent species, probably $KTcF_5$ (cf $KMnF_5$ (refs 8 and 9)), the corresponding reduction of ReF, gives Re(V) as the salt $KReF_6$ However, the action of solutions of alkali chlorides on TcF_6 in IF_5 gives Tc(V), and the salts $NaTcF_6$ and $KTcF_6$ have been prepared and characterized These yellow compounds are isostructural with the corresponding ruthenium derivatives[10,11] and differ from the rhenium salts[12] (Table 1) They obey approximately the Kotani rules of magnetic behaviour[13] for $d\varepsilon_2$ compounds, for example, for $NaTcF_6$ μ_{eff} = 2 25 Bohr magnetons at 25° and θ = 130°

We thank the University of Birmingham for a grant for technetium, Imperial Chemical Industries, General Chemicals Division, for the fluorine cell, and the Royal Society for a magnetic balance and for X-ray equipment The research was carried out during the tenure of a Department of Scientific and Industrial Research fellowship (to A J E) and a D S I R studentship (to D H)

Table 1 LATTICE CONSTANTS OF SOME COMPLEX FLUORIDES

$NaReF_6$ (ref 12)	Cubic	a_0 = 8 18 Å
$NaTcF_6$	Rhombohedral	a_0 = 5 77, α = 55 8°
$NaRuF_6$ (ref 11)	Rhombohedral	a_0 = 5 80, α = 54 5°
$KReF_6$ (ref 12)	Tetragonal	a_0 = 10 01, c_0 = 5 13
$KTcF_6$	Rhombohedral	a_0 = 4 97, α = 97 0°
$KRuF_6$ (ref 11)	Rhombohedral	a_0 = 4 96, α = 97 5°

A J EDWARDS
D HUGILL
R D PEACOCK

Department of Chemistry,
University of Birmingham, 15

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Formation of 2-Iminothiazolidine-4-carboxylic Acid in the Cyanobromination of Lanthionine

Sullivan and Folk[1] found that the symmetrical thioether, lanthionine, is converted quantitatively into cysteine by the combined action of cyanogen bromide and sodium cyanide Although we could confirm the presence of cysteine in the reaction products we were unable to substantiate the quantitative nature of this conversion The heterocyclic compound 2-iminothiazolidine-4-carboxylic acid, which we detected in the mixture after reaction, could have been formed by further reaction of cysteine on the cyanogen halide and could account for our low cysteine yields

Initially, the method described by Sullivan and Folk[1] was followed meticulously, namely, 5 ml of a solution of lanthionine in 0 1 N hydrochloric acid (0 1 mg/ml) was added to 5 ml of an aqueous NaCN solution (5 per cent) One ml CNBr solution, prepared by adding 5 per cent NaCN to a saturated solution of bromine until the mixture was just colourless, was added After three consecutive boilings for 30 sec and coolings for 3 min the thiol content was estimated colorimetrically using either naphthoquinone sulphonate[2], phosphotungstic acid[3], or brucine[4] It soon became evident that the boiling period was insufficient to expel all CNBr which interferes with the estimation of thiol by any of these methods The lanthionine/CNBr reaction mixtures were then consequently boiled continuously for 10 min Positive tests for thiols were obtained by the nitroprusside test as well as by the methods referred to here The quantity of thiol formed was not consistent with that expected from the amount of lanthionine used as starting material and reproducibility of results was found to be poor A typical set of results is shown in Fig 1

It is known that thiazoline and thiazolidine derivatives are formed when sulphydryls are reacted on by cyanogen halides[5] The presence of 2-iminothiazolidine-4-carboxylic acid in the reaction mixture was verified by descending chromatography utilizing a solvent consisting of *sec* butanol, formic acid, and water (75 15 10)

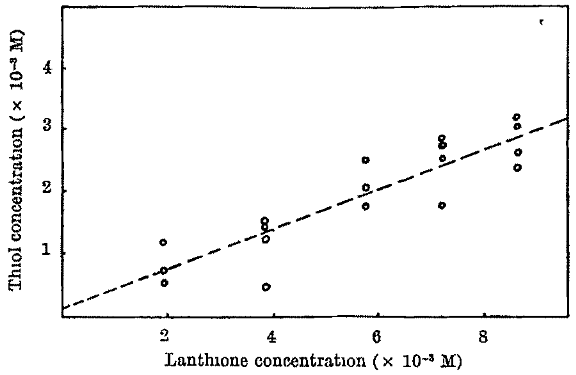

Fig 1 Original lanthionine concentration versus thiol concentration found after boiling a mixture of lanthionine, cyanogen bromide and sodium cyanide for 10 min

Dann *et al*[6] showed that lanthionine is attacked by alkali with the formation of thiazolidine derivatives In our experiments the solution rapidly turned alkaline as the hydrochloric acid was converted into HCN and expelled by boiling The resulting high *p*H of the solution, due to residual NaCN, could well be responsible for the conversion of some thio-ether into the heterocyclic compound

We conclude that under our reaction conditions conversion of thio-ether to sulphydryl by a boiling mixture of CNBr and NaCN, as claimed by Sullivan and Folk, does occur The quantitative value of the method is, however, impaired by the formation of 2-iminothiazolidine-4-carboxylic acid, which may be due either to cyclization of some of the liberated cysteine by the cyanogen bromide or to direct attack of alkali on the thio-ether

N J J van Rensburg

South African Wool Textile Research Institute,
Grahamstown,
South Africa

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BIOCHEMISTRY

Sarcoplasmic Proteins in Pale, Exudative Pig Muscles

Among the histological aspects of the so-called white muscle disease or 'Muskeldegeneration' (pale, exudative muscle)[1] is the presence of dark bands which cross the muscle fibres when these are seen in longitudinal section[2,3] Bendall and Wismer-Pedersen[3] presented evidence that these bands consisted of denatured sarcoplasmic protein The denatured proteins appeared to be precipitated on to the myofibrils under the conditions of relatively low *p*H and high temperature, which obtain during post-mortem glycolysis in this disorder It has recently been shown, using starch-gel electrophoresis, that various glycolytic enzymes, including creatine kinase, are among the sarcoplasmic proteins which precipitate in exudative pork[4] These proteins remain soluble if the temperature during post-mortem glycolysis is relatively low or, irrespective of temperatures up to 37° C, if the ultimate *p*H is high

It would have been inferred from these findings that no bands of denatured sarcoplasmic proteins would form in muscle of high ultimate *p*H, but that in muscle of abnormally low ultimate *p*H (ref 2) such bands would have been especially marked In the course of our present investigation, however, we have observed (*a*) bands apparently of denatured sarcoplasmic protein in longitudinal sections of muscle fibres of ultimate *p*H 6 94; (*b*) the relative freedom from such bands in sections from muscle of ultimate *p*H 4·74, compared with sections from muscle of ultimate *p*H 5 40 Recent work by Scopes[6] on the solubility of sarcoplasmic proteins under various *p*H/temperature combinations affords an explanation of these anomalies, and also supports the view that the bands are in fact of sarcoplasmic origin He found that, whereas a high ultimate *p*H appreciably protects sarcoplasmic proteins against precipitation at temperatures up to 37° C, denaturation occurs almost independently of *p*H at 45° C It is known that the temperature of pig muscles may rise above 37°/38° C immediately *post mortem*[3], and in such cases it would appear that bands of precipitated sarcoplasmic proteins might form even although the *p*H is about 7 0 In this connexion it is of interest that the holding of pigs at 45° C for a period before slaughter causes a pale, exudative musculature to develop[7]

Scopes[6] also found that sarcoplasmic proteins were more soluble at *p*H 4 7 than at *p*H 5 4, although they were denatured to a greater extent at the former value, and it has been found that the percentage of sarcoplasmic protein which is extractable (at the *p*H of the muscle) attains a minimum when the ultimate *p*H is about 5 3 (ref 8) These findings could explain the prevalence of bands in muscles having an ultimate *p*H of 5 4 and their relative absence in muscles of abnormally low ultimate *p*H

R A Lawrie
I F Penny
R K Scopes
C A Voyle

Low Temperature Research Station,
Downing Street,
Cambridge

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Conversion of DPNH Dehydrogenase to DPNH-cytochrome Reductase by Thiourea

Investigations in this laboratory have shown that the various DPNH-cytochrome reductase preparations from heart and liver mitochondria described in the literature are fragments of the respiratory chain-linked DPNH dehydrogenase produced by the extraction method (acid-ethanol-heat or thermal degradation) used in the isolation of cytochrome reductases[1-5] By the action of these agents as well as various proteolytic enzymes, urea and under a number of other experimental conditions the purified dehydrogenase is also degraded to lower molecular weight cytochrome-reducing fragments This conversion involves major changes in many of the molecular and catalytic properties of the enzyme of which the emergence of cytochrome (and DCIP) reductase activity is but one feature The observations on thermal and proteolytic degradation have been confirmed and extended by Kaniuga[7,8], who has independently discovered the urea-catalysed transformation of the enzyme Acid-ethanol degraded DPNH dehydrogenase is not distinguishable from the preparations of Mahler *et al*[9], de Bernard[10], and Mackler[11], while the thermally degraded enzyme is in every respect identical with the preparation isolated by King and Howard[12] of which the major component is the Mahler enzyme[9] Although these investigations have directed attention to the fact that DPNH dehydrogenase is unstable under a variety of conditions[13] and readily breaks down to fragments with very different properties, very recently yet another DPNH oxidizing preparation derived from heart mitochondria has been described[14] which appears to be a breakdown product of the respiratory chain-linked DPNH dehydrogenase It is the purpose of this communication to show that, in terms of available evidence, the "DPNH dehydrogenase" of Chapman and Jagannathan[14] is not a naturally occurring enzyme but a fragment produced by the action of thiourea on DPNH dehydrogenase

The preparation of Chapman and Jagannathan is isolated from ETP by repeated incubation with thiourea at 0° As judged by its flavin content, it is a much lower molecular weight unit than the dehydrogenase isolated by Ringler *et al*[15] Further, its reported activity, even at the arbitrary fixed electron acceptor concentrations used, is much lower with ferricyanide and much higher with cytochrome *c* menadione, and other oxidants than that of the respiratory chain-linked enzyme For these reasons it appeared likely that thiourea (a known protein denaturing agent) degrades DPNH dehydrogenase to a modified fragment That this is indeed the case is shown in Table 1 Although thiourea modifies soluble DPNH dehydrogenase more rapidly than it does the particle-bound enzyme, the destruction of ferricyanide and emergence of cytochrome reductase activities are fast enough even in the case of ETP preparations to account for the formation of the derivative described by the Wisconsin group Further illustration of this fact is given in Fig 1, which shows the balance of DPNH dehydrogenase (as measured with ferricyanide) and of the thiourea product (as measured with cytochrome *c*) on sequential application of thiourea and phospholipase extractions and vice versa Treatment with thiourea under the exact conditions of Chapman and Jagannathan results in more than 99 per cent destruction of the dehydrogenase, as may be ascertained by direct assay as well as subsequent phospholipase digestion at 30° Prior extraction of the dehydrogenase with phospholipase *A* removes the material from which the reductase fragment originates thus subsequent incubation of the residue with thiourea yields very little cytochrome reductase

The differences between the thiourea product and the Mahler enzyme are explained by the fact that while acid-ethanol, thermal, and subtilisin degradations yield highly similar products, the action of low concentrations of urea and of thiourea produces fragments with different properties An example of this is the fact that urea and thiourea under the conditions tested do not increase DCIP reductase activity significantly. It should be emphasized that at equal concentrations thiourea is an even more effective transforming agent than urea itself Details of these observations will be published elsewhere[6].

Table 1 DEGRADATION OF DPNH DEHYDROGENASE BY THIOUREA

Exp	Conditions	Time of incubation (min)	Activities (μmoles DPNH/min/ml)		
			$Fe(CN)_6^{\equiv}$	Cytochrome *c*	DCIP
1	ETP, 0°, 1 19 M thiourea*	0	630	2 05	
		120	450	4 3	
		285	75	8 0	
		375	31	8 5	
2	ETP, 10°, 1 19 M thiourea	0	630	2 05	
		70	589	7 1	
		120	254	14 5	
		180	95	7 3	
3	DPNH dehydrogenase 10°, 0 5 M thiourea	0	4,000	1 15	2 4
		7	2,000	3 38	2 5
		15	1,360	9 25	2 5
		30	682	10 5	2 9

* This was the concentration of thiourea used by Chapman and Jagannathan (ref 14) in the isolation of a "DPNH dehydrogenase" from ETP Although the temperature of their experiments is stated to have been 0°, this concentration of thiourea exceeds the limits of solubility at 0° but is in solution at 10°

All activities are V_{max} values with respect to the electron acceptor at 30° Assays were as previously described (refs 5, 13 and 15) In Exps 1 and 2 the conditions of Chapman and Jagannathan (ref 14) were used 1 ml each of ETP (66 mg/ml , specific activity = 30 5 in $Fe(CN)_6^{\equiv}$ assay), 0 25 M sucrose, 1 M phosphate, *p*H 7 5, 0 15 ml 1 M *tris* buffer, *p*H 7 5, and 0 03 ml 0 05 M ethylenediamine tetraacetate were mixed Thiourea (200 mg) was suspended in the reagents and then the ETP was added In Exp 3 equal volumes of the highly purified dehydrogenase (specific activity = 347, 23 1 mg/ml) in 0 03 M phosphate, *p*H 7 8, and 1 M thiourea in 0 2 M phosphate, *p*H 7 8, were mixed

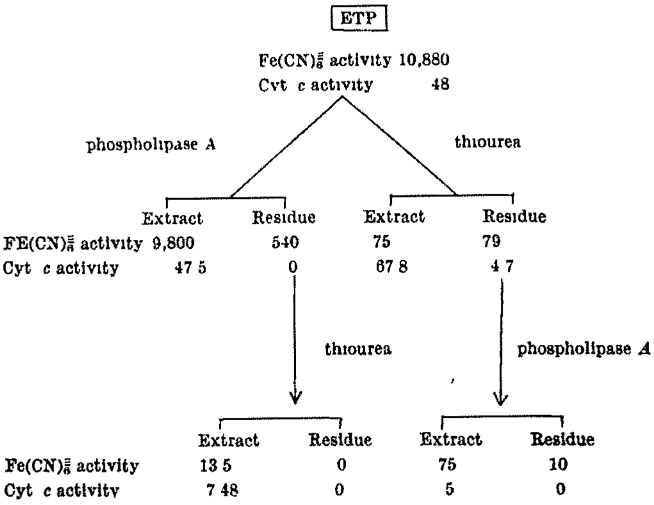

Fig 1 Balance of DPNH dehydrogenase and cytochrome reductase activities on sequential application of thiourea and phospholipase *A* Assays are total activities (μmoles DPNH/min) at V_{max} and refer to 316 mg ETP (biuret basis) Right side thiourea extraction under the conditions of Chapman and Jagannathan, followed by phospholipase extraction ETP (316 mg in 5 ml 0 25 M sucrose–3 mM phosphate, *p*H 7 4) was mixed with 5 ml 0 25 M sucrose, 5 ml M phosphate, *p*H 7 5, 0 75 ml M *tris* buffer, *p*H 7 5, 0 15 ml 0 05 M EDTA, and 1 g thiourea at 0° and the suspension was frozen rapidly After 1 h it was thawed slowly at 0° and centrifuged for 30 min at 140,000*g* The sediment was suspended in 6 ml 0 25 M sucrose containing 0 25g of thiourea and was kept for 2 h at 0°, was frozen and thawed as before, and centrifuged at 140,000*g* for 30 min The supernatant fluid was the extract Thiourea was removed from the residue by passage of the suspension through 'Sephadex *G*-25' (equilibrated with 0 25 M sucrose–30 mM phosphate, *p*H 7 4) and phospholipase digestion was performed with 0 003 mg purified phospholipase *A*/mg protein for 2 h at 30° following the method of Ringler *et al* (ref 15) Left side phospholipase extraction as above, followed by thiourea treatment In the latter step the proportion of reagents remained as in the previous experiment, but the insoluble protein after phospholipase digestion was made to 65 mg/ml and the volume of other reagents added was adjusted proportionately to the volume of this material

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TERENZIO CREMONA*
EDNA B KEARNEY
G. VALENTINE

Edsel B Ford Institute for Medical Research,
Henry Ford Hospital,
Detroit

* On leave of absence from the International Institute of Genetics and Biophysics, Naples

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Inhibition of Protein Synthesis *in vitro* by Cycloheximide

CYCLOHEXIMIDE ('Actidione'), an antifungal antibiotic, isolated from *Streptomyces griseus*, was reported by Kerridge[1] to inhibit protein synthesis in *Saccharomyces carlsbergenesis* These observations have been substantiated by other workers[2,4] Recent investigations indicate that the changes produced by cycloheximide in intermediate metabolism, such as amino-acid, organic acid, adenosine triphosphate (ATP), and , other nucleotide production, is not responsible for the inhibition of protein synthesis[3,4] The data presented here will show that incorporation of leucine labelled with carbon-14 into protein in a cell-free system from *Saccharomyces pastorianus* is inhibited by low concentrations of cycloheximide

S pastorianus was grown as previously described[3] and cells were collected in the log phase of growth Approximately 12–15 gm of packed cells were suspended in 20–30 ml of a solution consisting of 0 05 M *tris*(hydroxymethyl) aminomethane-hydrochloric acid (*tris*-hydrochloric acid), *p*H 7 8, 0 005 M $Mg(OAc)_2$, 0 005 M potassium chloride; and 0 001 M mercaptoethanol Cells were broken in a French pressure cell (15,000–24,000 lb /in ^{2}) at 1° C Whole cells and cellular debris were removed by centrifugation at 20,000*g* in the cold and the extract was adjusted to *p*H 7 5 with 1 0 N sodium hydroxide The extract containing supernatant enzymes and microsomes necessary for protein synthesis was incubated for 15 min at 37° C with leucine labelled with carbon-14 and appropriate co-factors The reaction was terminated by the addition of 1 2 volumes of cold 10 per cent trichloroacetic acid (TCA) containing 0 1 M ^{12}C-DL-leucine The protein precipitate was washed and prepared for counting essentially by the method of Siekevitz[5] The protein precipitate was dissolved in 88 per cent formic acid and aliquots were added to 1-in planchets and counts were made in a windowless flow counter Protein was determined by the method of Lowry *et al* [6]

Table 1 REQUIREMENTS FOR INCORPORATION OF LEUCINE LABELLED WITH CARBON-14 INTO YEAST PROTEIN *in vitro*

Conditions	Incorporation of ^{14}C-leucine into protein (c p m)
Complete	532
+ 10 per cent TCA at zero time	10
+ 100 μg RNase	36
+ 100 μg cycloheximide *	90
+ 0 003 M $Mg(OAc)_2$	462
− 0 005 M $Mg(OAc)_2$	158
− 0 007 M $Mg(OAc)_2$	64
− KCl	290
− GTP	392
− ATP, PEP and pyruvic kinase	12

* 84 per cent inhibition

The complete reaction mixture contained 0 125 M *tris*-HCl buffer *p*H 7 5, 0 0095 M $Mg(OAc)_2$, 0 0075 M KCl, 0 001 M ATP, 0 0001 M guanosine triphosphate, 0 005 M phosphoenol pyruvic acid, 200 μg pyruvic kinase, 0 25 μc L-leucine-1-^{14}C specific activity 8 mc /mM, and 10 mg of protein (2 5 mg microsomal protein and 7 5 mg supernatant protein) in a volume of 2 ml Tubes were incubated for 15 min at 37° C and the reaction was stopped with 1 2 vol of 10 per cent cold TCA containing 0 1 M ^{12}C-DL-leucine RNase and cycloheximide were mixed with other reactants prior to the addition of the cell-free extract

The results in Table 1 show that protein synthesis *in vitro* is dependent on the presence of various co-factors and is inhibited by ribonuclease (RNase) and 50 µg/ml of cycloheximide In order to determine the effectiveness of cycloheximide, varying concentrations, from 0 1 µg/ml to 250 µg/ml , were mixed with other reactants prior to the addition of supernatant enzymes and microsomes The data in Fig 1 show that cycloheximide is a potent inhibitor of carbon-14 amino-acid incorporation into protein The 50 per cent inhibitory dose (ID_{50}) of the antibiotic for leucine incorporation was 0 2 µg/ml Thus, this process is of the same order of sensitivity to cycloheximide as is growth of cells of *S pastorianus*

A cell-free extract was pretreated with 25 µg/ml of cycloheximide and compared with the control and samples treated at zero time Pretreatment of the cell-free extract, containing microsomes, with cycloheximide before addition to the other reactants had very little, if any, effect on the levels of inhibition when compared with the samples treated at zero time (Fig 2) The inhibition ranged from 89 to 80 per cent at 2 5 and 10 min for non-pretreated samples and 90 to 86 per cent for pretreated samples for the same time-intervals Under these experimental conditions the maximum inhibition never exceeded 90 per cent and consistently ranged from 80 to 90 per cent in all the tests Whether the inability of high concentrations of the antibiotic to inhibit completely protein synthesis *in vitro* is due to the experimental conditions used or to the true nature of the antibiotic action has not been determined

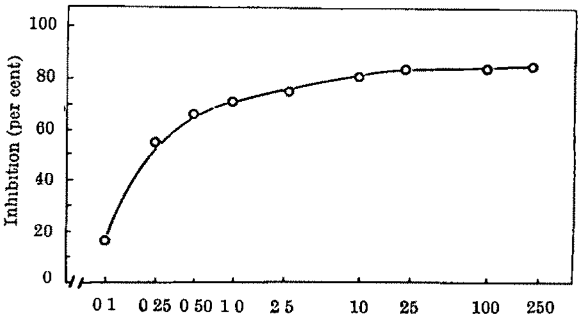

Fig 1 Effect of various concentrations of cycloheximide (0 1–250 µg/ml) on incorporation of ^{14}C-leucine into protein (see Table 1 for conditions) Cycloheximide was mixed with other reactants before addition of microsomes and supernatant Results for complete and treated samples were corrected for ^{14}C-leucine incorporated into the blank (complete + 10 per cent TCA at zero time)

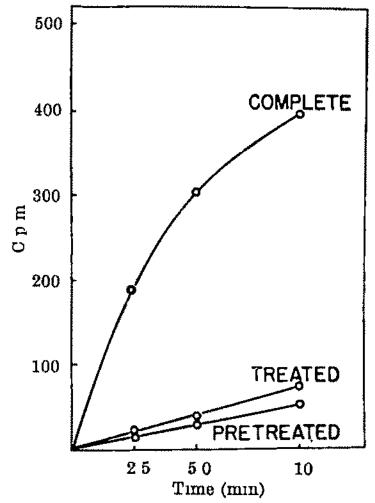

Fig 2 Effect of pretreatment of supernatant and microsomes with cycloheximide Conditions for this experiment were the same as those in Table 1 except the reaction mixture was contained in 1 ml and 5 0 mg of protein (supernatant and microsomes) and 0 5 μc DL-leucine-1-^{14}C, specific activity 54 6 mc /mM, was added to each tube The microsomal and supernatant mixture was pretreated with 25 μg/ml of cycloheximide 10 min before mixing with other reactants The reaction was stopped at various time-intervals by addition of cold 10 per cent TCA to each tube The ^{14}C-leucine incorporated into the blank (complete minus ATP, PEP and pyruvic kinase) was subtracted from that incorporated into complete, treated and pretreated samples

The site of action of the antibiotics chloramphenicol[7] and puromycin[8,9] has been traced to protein synthesis Specifically, they inhibit the polymerization of amino-acids to form protein on the microsomes Previous investigations[4] showed that cycloheximide does not affect the first two enzymatic steps in protein synthesis, that is, amino-acid activation and transfer of amino-acids to soluble ribonucleic acid (*s*–RNA) Thus the evidence indicates that the antibiotic affects the third enzymatic step either by inhibiting the transfer of amino-acids from amino acyl *s*–RNA to the microsomes or the polymerization of amino-acids into protein The effects of cycloheximide on yeast metabolism resemble those of chloramphenicol in bacterial systems Both antibiotics inhibit protein synthesis, stimulate ribonucleic acid synthesis, and partially inhibit deoxyribonucleic acid synthesis

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MALCOLM R SIEGEL
HUGH D SISLER

Department of Botany,
University of Maryland,
College Park

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Deoxyribonucleic Acids of Marine Mollusca

RECENT investigations have demonstrated a wide range of deoxyribonucleic acid (DNA) base compositions which correlate well with other taxonomic criteria in both bacteria[1-4] and protozoa[5,6] The available data for metazoans are less extensive[4,7] However, a considerable variation in DNA base compositions is found among the porifera[8], and the published analyses for three species of molluscs[4,9] indicate the probability of a range of compositions in that phylum. This communication describes work on several other species of molluscs DNA was isolated from sperm on testes using a modification[10] of Marmur's procedure[11] Base compositions were estimated from density gradient centrifugation studies[13] and from thermal denaturation temperatures[12] The results are recorded in Table 1

Table 1 PROPERTIES OF MOLLUSC DNA's

Class and species	Tm (°C)	% GC*	Density (g/ml)†	% GC§
Lamellibranchia				
Crassostrea gigas (Thunberg)	80 7	28	1 693	34
(Pacific oyster)			1 708‡	
Saxidomas giganteus Deshayes	82 5	32	1 693	34
(Butter clam)				
Protothaca staminea (Conrad)	82 8	33	1 694	35
(Native littleneck clam)				
Clinocardium nuttalli (Conrad)	82 8	33	1 696	37
(Basket cockle)				
Gastropoda				
Haliotis kamtschatkana Jonas	84 5	37	1 702	43
(B C abalone)			1 714‡	
Polinices lewisii Gould	85 6	40	1 704	45
(Moon snail)				

* Percentage guanine-cytosine calculated from thermal denaturation temperature, Tm (12)
† Relative to *E coli* buoyant density, 1 710 g/ml (ref 13)
‡ Heat denatured DNA
§ Percentage guanine-cytosine calculated from buoyant density

All DNA samples were unimodal with respect to buoyant density and thermal denaturation temperature Representative lamellibranch (*Crassostrea gigas*) and gastropod (*Haliotis kamtschatkana*) DNA's were unimodal in the cæsium chloride gradient after heat denaturation All the mollusc DNA's were rich in adenine-thymine, those of the lamellibranchia containing the largest amount of these bases The range of guanine-cytosine (GC) contents was 11–12 per cent In common with other nucleic acids of extreme base composition, there is some divergence between GC content calculated from thermal denaturation temperatures and from buoyant densities[12,13] There is reasonable correlation of the foregoing results with earlier data Thus Sueoka, using the buoyant density technique, obtained a GC content of 37 per cent (38 per cent using the method of calculation of Marmur and Doty) of DNA in the quahog, *Venus* (*Mercenaria*) *mercenaria* L [4], and Lee and Barbu, by chemical analysis, found 31 per cent GC in the blue mussel, *Mytilus edulis* L , DNA[9]

The two prosobranch gastropods have a mean GC content 7–8 per cent higher than the mean value of the lamellibranchs and none of one class intrudes into the region of the other It is of interest that the GC content, 35 per cent, of a cephalopod, *Octopus vulgaris lam* , DNA[9] lies in the mid-region of the range bounded by the lamellibranchs and the prosobranch gastropods

We thank Mr J C Smith for samples of *P staminea*, *C nuttalli* and *P lewisii* testes and *H kamtschatkana* sperm, and C E Woelke for *C gigas*, *S giganteus* and *P staminea* sperm

MICHAEL SMITH

Technological Station,
Vancouver 8, B C

D B QUAYLE

Biological Station,
Fisheries Research Board of Canada,
Nanaimo, B C

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Metabolism of 3-Hydroxyanthranilic Acid labelled with Carbon-14 Carboxyl in Normal and Neoplastic Mice

THE *o*-aminophenol, 3-hydroxyanthranilic acid (3-OHAA) is a metabolite of tryptophan normally present in urine in trace quantities Recent observations have been concerned both with the carcinogenic property of 3-OHAA to induce cancer of the bladder and with elevated levels of urinary excretion of 3-OHAA in neoplastic diseases[1]

A limited amount of carboxyl-^{14}C-labelled-3-OHAA (^{14}C-3-OHAA) was available for experiments with a few animals The carboxyl-^{14}C-labelled-3-OHAA was given to control mice and mice with transplantable tumours of mouse origin in an attempt to examine the influence of these tumours on metabolism of 3-OHAA. These preliminary observations indicate a lowered rate of metabolism of 3-OHAA in the ascites mice accompanied by a reduced level of urinary quinolinic acid labelled with carbon-14 (^{14}C-QA)

Female Swiss albino mice were used in these experiments Three homologous transplantable tumours were used in this work The mammary adenocarcinoma appears spontaneously in about 3 per cent of our virgin females when 6–8 months of age The Ehrlich ascites tumour has been maintained in our mice in ascites form for several years by intra-abdominal injection of a suspension of tumour cells at intervals of 10–15 days A transplantable brain tumour (glioblastoma multiforme) was induced by intracranial implantation of methylcholanthrene pellets[2] Tumour mice were injected with ^{14}C-3-OHAA 5 days after intracranial transfer of the brain tumour cells and 9 days after intra-abdominal transfer of the ascites cells Previous experiments demonstrated active growth of tumours at this time A moribund condition usually developed 5–7 days later

The carboxyl-^{14}C-labelled-3-OHAA was from a preparation previously described[3] It was injected by intra-abdominal route in doses of 0 0011 mM containing 0 024 µc /g body-wt Respiratory carbon dioxide and urine were collected and quinolinic acid (QA) values determined on all urines by microbiological assay[4] Crystalline QA was isolated by column chromatography[5] Small samples of QA were combusted to carbon dioxide and the carbon-14 measured by the method of Van Slyke, Steele and Plazin[6] The tumour from a mammary tumour mouse was excised and homogenized. Aliquots were dried in Van Slyke combustion tubes, and analysed for total carbon and for carbon-14 content[6]

The observed initial rapid oxidative metabolism of ^{14}C-3-OHAA in the normal mice (Fig 1) may account for the rather low level of ^{14}C-QA (13 per cent) found in the urine This is in contrast to the relatively higher percentage (46 per cent) conversion of ^{14}C-3-OHAA to carbon-14 by mouse liver cell free extracts[7] A small store of QA is indicated in the normal mice (Table 1) from the 84 per cent ratio of specific activity of isolated ^{14}C-QA to the administered dose of ^{14}C-3-OHAA A change in the ratio of the specific activity of isolated urinary ^{14}C-QA to the injected dose of ^{14}C-3-OHAA indicated an alteration of the metabolism of 3-OHAA in the tumour mice since the test dose (mg/g of body-wt) was the same in these experiments

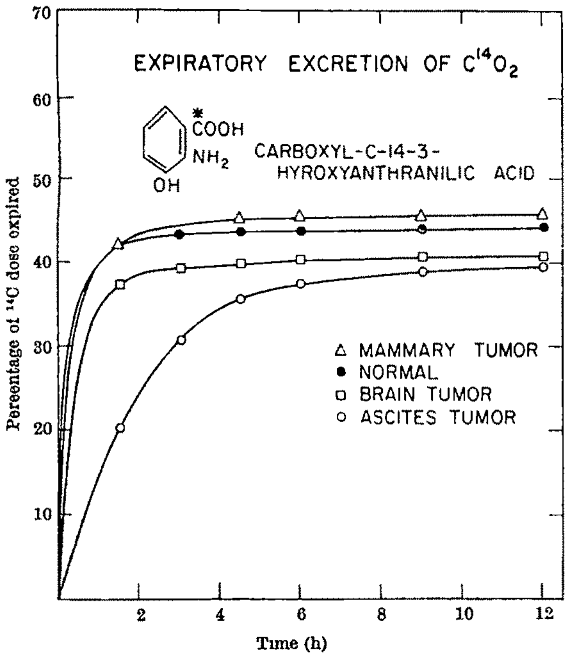

Fig 1 Twelve-hour excretion of $^{14}CO_2$ by mice after carboxyl-^{14}C-3-hydroxyanthranilic acid administration

The reduced amount of carbon-14 (6 7 per cent) present as QA in the urine of the mammary tumour mice as compared with the level (13 per cent) found in normal urine may be accounted for by several mechanisms (1) an increased rate of metabolism of ^{14}C-QA into ^{14}C-nicotinic acid, ^{14}C-N^1-methylnicotinamide[8] or ^{14}C-N^1-methyl-2-pyridone-5-carboxamide; (2) increased conversion of compound I into glutarate or acetate which leads to formation of $^{14}CO_2$ (ref 9), (3) free or conjugated ^{14}C-3-OHAA Since the carbon-14-label is in the carboxyl position, none of the observed activity of carbon-14 could be present as urinary picolinic acid Portions of a homogenate of the excised mammary tumour were analysed for total carbon-14 to determine if a major amount of the injected dose of ^{14}C-3-OHAA was bound by the tumour. The carbon-14 analysis accounted for only 0 5 per cent of the injected dose

Table 1 12-Hour Urinary Excretion of Quinolinic Acid after Injection of Carboxyl-^{14}C-3-Hydroxyanthranilic Acid

Type of tumour		Normal	Mammary	Brain	Ascites
Sp act of inj 3-OHAA, µc /m mole	a	21 985	21 985	21 985	21 985
3-OHAA, inj m mole*		0 038	0 043	0 031	0 032
C-14 inj , µc	b	0 842	0 940	0 666	0 704
^{14}C content of urine per cent of dose		29 85	26 57	20 35	21 78
Quinolinic acid					
Q-Acid excreted (in mg)		0 964	0 478	0 370	0 116
Idem (m mole)	c	0 006	0 003	0 002	0 001
Sp act of excreted Q-acid, µc /m mole	d	18 487	20 955	20 026	23 78
C-14 excreted in Q-acid µc $= c \times d$	e	0 111	0 063	0 040	0 024
Per cent of injected $^{14}C = \frac{100e}{b}$		13 18	6 70	6 01	3 41
Ratio $= \frac{\text{Sp act of excreted Q-acid}}{\text{Sp act of inj 3-OHAA}} = d/a$		0 841	0 953	0 911	1 082

* Injected 0 011 mM/10 g mouse

The slower rate of metabolism of 3-OHAA into $^{14}CO_2$ by the brain tumour animals as well as the lower levels of carbon-14 in their urines assumes greater interest when we consider the relative size of the brain tumour mass (175 mg) as compared with the excised mammary tumour (6 5 g) In view of the small size of the excised brain tumour, it is difficult to assume that the lowered rate of metabolism of ^{14}C-3-OHAA is due to endogenous metabolism by the tumour tissue

Pal and Christensen[10] have shown cell transport and binding of pyridoxal and pyridoxine occurred in ascites tumour cells Their *in vitro* study indicated pyridoxal binding through amino groups of cellular proteins and amino-acids through the aldehyde group of pyridoxal. It should be considered that pyridoxal binding may occur through hydrogen binding of the hydroxyl group on the pyridoxal ring In view of the foregoing findings obtained with tritium-labelled pyridoxal, several mechanisms may be considered to account for the slower metabolism of ^{14}C-3-OHAA observed in the ascites tumour animals Initial uptake of ^{14}C-3-OHAA by the tumour cells may have occurred with hydrogen binding through the hydroxyl group to cellular components The weak nature of the hydrogen binding would allow a return of the ^{14}C-3-OHAA to body fluids for subsequent metabolism into $^{14}CO_2$ via the acetate pathway[9] The ^{14}C-3-OHAA in the ascites cells may also be split into the straight-chain compound (1-amino-4-formyl-buta-1 3-diene-dicarboxylic acid) and afterwards bound to amino-groups through the terminal aldehyde group This mechanism would be similar to pyridoxyl binding with the aldehyde compound being metabolized into $^{14}CO_2$ and ^{14}C-QA It is also possible that the aldehyde compound formed by host metabolism may be externally bound to cells through the aldehyde group and afterwards metabolized into $^{14}CO_2$ and ^{14}C-QA The lower ^{14}C-QA values (Table 1) obtained in the urines of the tumour mice as compared with normal mice may possibly be accounted for through accelerated activity of enzyme II[7] This enzyme decarboxylates the aldehyde compound to form picolinic acid, which would leave less substrate available for ^{14}C-QA formation

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Lawrence V Hankes
M Schmaeler
Richard D Stoner

Medical Research Center,
Brookhaven National Laboratory,
Upton, Long Island,
New York

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Preparation of 2-Isopropylthiamorpholin-3-one-5-carboxylic Acid

ISOVALTHINE is a new sulphur-containing amino-acid which is isolated from the urine of hypercholesterolæmic patients[1,2] A molecular model of isovalthine suggests a possibility of forming a thiamorpholinone derivative by the intramolecular cyclization Such thiamorpholinone derivative may also be produced in the animal body and have some biological effects From these considerations, 2-isopropylthiamorpholin-3-one-5-carboxylic acid was prepared from L-isovalthine and its biological effect was examined

L-Isovalthine (30 mg, decomposition point 186° C)[3] was heated in a small flask immersed in an oil-bath at about 190° C for 15 min During this operation a vacuum was applied to the flask to remove water which was produced during cyclization After cooling the flask to room temperature, the brown residue was dissolved in a small amount of 2 N ammonia and filtered The filtrate was made weakly acidic with acetic acid, and transferred on a column containing strong cation exchanger (*H*-form) The effluent and water washings were collected and evaporated to dryness in vacuum The residue was taken up in ether and filtered The ether solution was concentrated to a proper volume and left standing at room temperature Needles obtained were recrystallized from ether, giving 18 mg, m p 140° C $[\alpha]_D^{17} = +4\ 1°$ in ethanol It was slightly soluble in water, soluble in ether, and easily soluble in methanol, ethanol, and alkali

Anal calc for $C_8H_{13}O_3NS$ C, 47 27, H, 6 45, N, 6 89, found C, 47 18, H, 6 44, N, 6 89 The molecular weight (calculated, 203) was found to be 186 and 217 by the Rast method The ultra-violet absorption spectrum of the compound is shown in Fig 1 $\lambda_{max} = 202\ 8$ mμ ($\varepsilon = 13\ 000$) Since another synthetic sample of thiamorpholin-3-one-5-carboxylic acid prepared from *S*-(carboxymethyl)-L-cysteine[4] also showed a similar absorption, the $\lambda_{max} =$ 202 8 mμ band is thought to be specific for the thiamorpholinone ring The infra-red spectrum of the compound is shown in Fig 2

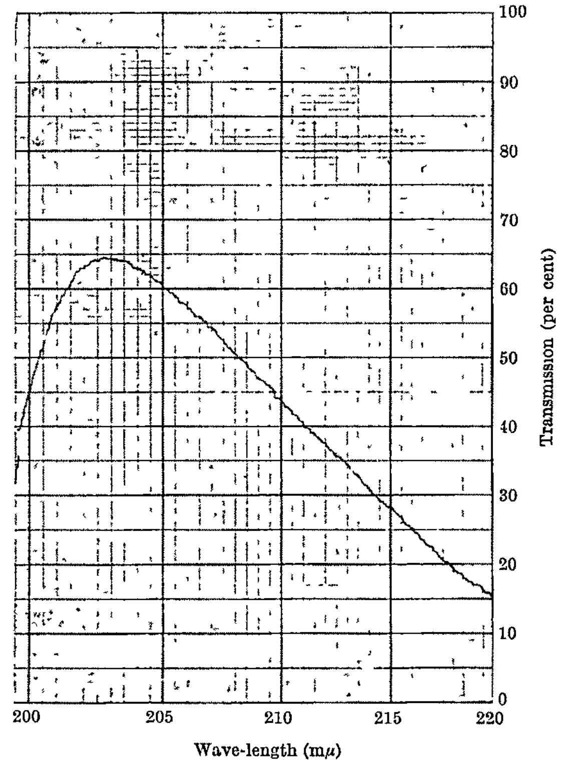

Fig 1 Ultra-violet absorption spectrum of 2-isopropylthiamorpholin-3-one-5-carboxylic acid in methanol solution

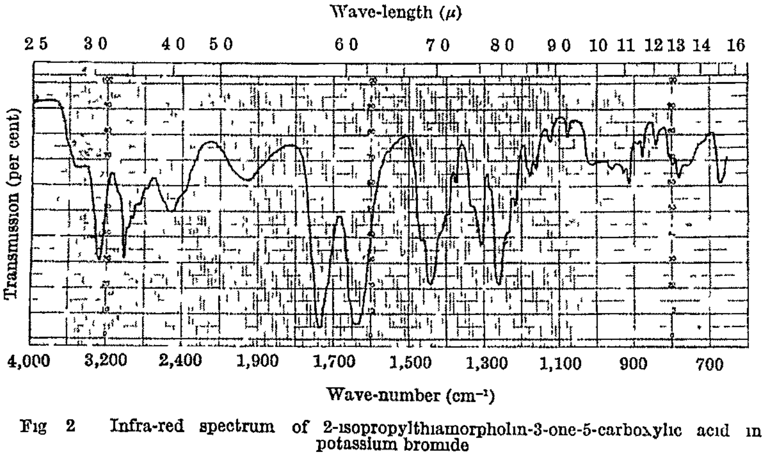

Fig 2 Infra-red spectrum of 2-isopropylthiamorpholin-3-one-5-carboxylic acid in potassium bromide

Although the thiamorpholinone derivative prepared as described here is similar in chemical structure with amytal, it has neither hypnotic effect on mice nor inhibitory effect on the electron transport system of isolated rat liver mitochondria

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S MIZUHARA
S OHMORI
K HORIUCHI
T KUWAKI
T UBUKA

Department of Biochemistry,
Okayama University Medical School,
Okayama, Japan

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Configuration of Urinary Isovalthine

ISOVALTHINE crystals first isolated from urine were rhombic plates which gave DL-alanine after treatment with Raney nickel Needle-shaped crystals were obtained by condensation of L-cysteine and (±)-α-bromoisovaleric acid They were a mixture of L- and L-allo-isovalthine, and readily converted into the rhombic form by heating in hydrochloric acid[1,2]

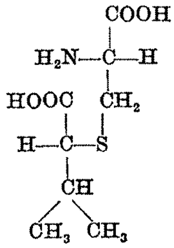
Recently we have synthesized L- and L-allo-isovalthine separately by using (+)- and (−)-α-bromoisovaleric acid The resolution of (±)-α-bromoisovaleric acid into each of optical antipodes was achieved by using ephedrine Optical rotation of the isomer synthesized from L-cysteine and (+)-α-bromoisovaleric acid was $-52\ 4°$ ($[\alpha]_D^{17}$ in 2 N hydrochloric acid) and that of the other isomer synthesized from (−)-α-bromo acid was $+82°$ ($[\alpha]_D^{17}$ in 2 N hydrochloric acid) The decomposition point of the isomer of $[\alpha]_D = -52\ 4°$ was 186° C and that of $[\alpha]_D = +82°$ was 185° C The elemental analyses of these isomers were correct, and these single isomers gave L-alanine after treatment with Raney nickel

On an amino-acid analyser (Beckman model 120 *B*), the needle-shaped crystals yield two peaks between serine and proline at the effluent ml of 183 and 196 (30° C, *p*H 3 24, 0 2 N sodium citrate buffer, 150 cm column) The isomer of $[\alpha]_D = -52$ 4° yields one peak ($C_{HW} = 20$ 5) and overlaps 196 ml peak of the needle The other isomer of $[\alpha]_D = +82°$ yields also one peak ($C_{HW} = 19$ 2) and overlaps the 183 ml peak of the needle The rhombic plates yield two peaks with the same effluent ml as that of needles, and the mixture of these two racemic compounds gives just two peaks The calculated ratio of 183 ml peak to 196 ml peak was as follows needle, 4 6, rhombic plate, 9 1

Isovalthine in urinary acidic amino-acid fraction yields one peak at 196 effluent ml and the peak overlaps the isomer of $[\alpha]_D = -52$ 4° The urinary isovalthine fraction is prepared as follows 250 ml of urine is made weak acidic and transferred on a column containing 200 g of the acetate form of 'Amberlite *AG*-45' The column is washed with 600 ml of 0 1 N acetic acid and then eluted with 2 l of 2 N acetic acid The 2 N acetic acid eluant is transferred on a column containing 100 g of strong cation exchanger (*H*-form) The column was washed with water and eluted with 500 ml of 2 N ammonia The ammonia solution is evaporated to dryness in vacuum at 40° C The residue is dissolved in 10 ml water and 2 ml of the filtrate is analysed on amino-acid analyser The recovery of urinary isovalthine was about 75 per cent Further treatment for the purification changes urinary isovalthine gradually into the mixture of L- and L-allo-isovalthine Therefore, it is difficult to isolate urinary isovalthine as a single isomer without changing its configuration

On the paper electropherogram prepared by Ubuka's method[3], the needle-shaped crystals yield also two bands with almost equal concentrations Urinary isovalthine and the isomer of $[\alpha]_D = -52$ 4° overlap the slower-moving band of the needle, and the isomer of $[\alpha]_D = +82°$ overlaps the faster-moving band of the needle

For these reasons, it may be concluded that urinary isovalthine has the same configuration with the compound synthesized from L-cysteine and (+)-α-bromoisovaleric acid, this may be called L-isovalthine (+)-α-Bromoisovaleric acid is known to be derived from D-valine by the treatment with nitrosyl bromide and to be converted into D-valine by the treatment with ammonia If (+)-α-bromoisovaleric acid is assumed not to change its configuration during condensation with L-cysteine, the configuration of L-isovalthine may be written as follows



It requires some effort to find out the patients who excrete isovalthine in their urine Experimental isovalthinuria, however, can readily be induced in the guinea pig by the administration of isovaleric acid, dehydrocholic acid, cholic acid, or 22,25-diazacholestanol ('*SC*-11952') These inducers take effect on the following times after administration 200 mg/kg/day of isovaleric acid, second day, 12 5 mg/kg/day of dehydrocholic acid, 5–7 days, 12 5 mg/kg/day of cholic acid, third week, 5 mg/kg/day of 22,25-diazacholestanol, second week Although isovaleric acid is the best inducer for some animals, isovaleric acid-1-^{14}C has not significantly incorporated into urinary isovalthine[4] The administration of cholesterol alone does not induce isovalthinuria up to the end of the fourth week

Table 1 EXPERIMENTAL ISOVALTHINURIA INDUCED BY BILE ACID AND CHOLESTEROL IN GUINEA PIG

Inducers	Isovalthine excreted µmoles/100 ml urine 5–7 days	Second week	Third week	Fourth week
Dehydrocholic acid (12 5 mg/kg/day)	0 38	0 76	0 55	0 45
Dehydrocholic acid (12 5 mg/kg/day) + cholesterol (250 mg/kg/day)	0 75	1 98	0 85	0 28

But its administration with dehydrocholic acid appears to enhance isovalthine excretion as shown in Table 1

Isovalthine is also obtained by using liver homogenate of guinea pig from isovaleric acid or α-ketoisocaproic acid as substrates Glutathione and air appear to be necessary for this reaction As cysteine is less effective than glutathione, the first product synthesized in the reaction mixture is assumed to be a glutathione-isovaleric acid conjugate The assumption, however, has not so far been verified

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S OHMORI
T UBUKA
T KUWAKI
K HORIUCHI
S MIZUHARA

Department of Biochemistry,
Okayama University Medical School,
Okayama, Japan

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A Comparison of Plant Cell Division Inducers from Coconut Milk and Apple Fruitlets

COCONUT milk is recognized as a rich source of plant cell division inducers, which have become known as kinins[1–5] Recently, apple fruitlets were also shown to be a rich source[6], and the kinins have been extracted from this tissue and concentrated[7] The results showed that the extract contains at least four and probably five independently active kinins as revealed by the tobacco stem pith test[8] In this test, blocks of tobacco pith are cultured singly in small tubes on a sucrose–mineral salt medium which also contains the auxin, indolyl-3-acetic acid An 'active' extract, added as a supplement to this medium, induces cell division and the formation of microscopically visible clusters of new cells after 14 days In control cultures the cells enlarge but do not divide Reduced nitrogen compounds and hexitols are not required in this assay, nor do they enhance the effect of active extracts as they do in the carrot root phloem test[9], which has been used in many studies on coconut milk

The kinins obtained from coconut milk and from apples were compared by subjecting coconut milk to the extraction, purification and fractionation procedures developed for apple fruitlets Volatile solvents added in the course of purification procedures were removed by evaporation under reduced pressure before assaying the activity of fractions by the tobacco stem pith test

Coconut milk (500 ml) from fresh, nearly mature coconuts was subjected to the purification procedure developed for apple extract[7] The most efficient purification steps in this scheme are those which use 'Dowex–50–H$^+$' and carbon columns The purified coconut milk, so prepared, showed perceptible cell division activity at a concentration of 0 1 mg dry weight/l

Except for slight activity in the water effluent from 'Dowex–50–H$^+$', which was found for coconut milk but not

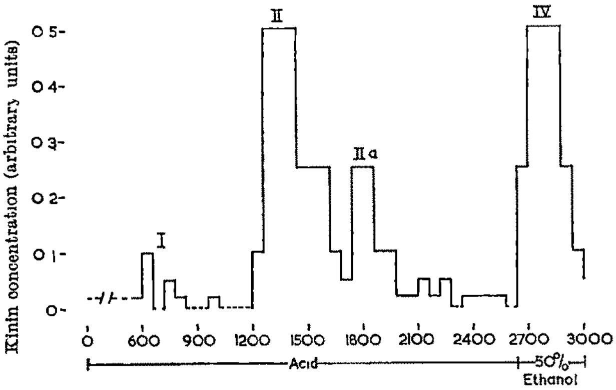

Fig 1 Chromatography on a 'Chromax' rolled-paper column of partially purified coconut milk kinins The eluting solvents were *n* butanol/glacial acetic acid/water (4 1 1), and 50 per cent ethanol

apple extract, the behaviour of the kinins from the two sources was the same

The purified extract was chromatographed on a 'Chromax' 3502 paper roll column (L K B Produktor, Stockholm) in *n*-butanol/glacial acetic acid/water (4 1 1, v/v) and then in 50 per cent aqueous ethanol Fifty-four × 60 ml fractions of acid eluate were collected and seven of aqueous ethanol Each fraction was assayed at five dilutions as previously described for apple extract[8]

The greatest dilution giving perceptible activity was plotted against the volume of eluate (Fig 1) This revealed four major peaks of activity compared with five given by apple extract[7]. A sample of each of the peaks was chromatographed on a paper strip in methyl ethyl ketone/acetic acid/water (4 : 1 1, v/v) The R_F values of these peaks as well as those of the peaks found in apple extract are shown in Table 1

Table 1 PAPER CHROMATOGRAPHY OF KININS OBTAINED FROM COCONUT MILK AND FROM APPLE FRUITLETS IN METHYL ETHYL KETONE/ACETIC ACID/WATER (4 1 1, v/v)

Peak	R_F values Coconut milk	Apple extract
I	0 71–0 79	0 76
II	0 42–0 56	0 46–0 50
IIa	0 44–0 53	0 46–0 50
III	Absent	0 27–0 33
IV	0 05–0 27	0 00–0 15

But for the absence of peak III in coconut milk, there is a close correspondence between the R_F values of the kinins from the two sources Although peak II*a* has been observed several times in eluates from 'Chromax' columns it has invariably given the same R_F as peak II on paper strips Presumably the resolution on the 'Chromax' column developed with *n*-butanol–glacial acetic acid–water is superior to that of paper strips developed with methyl ethyl ketone–acetic acid–water

It was reported previously[7] that treatment of a mixture of peaks III and IV with normal hydrochloric acid gave rise to a peak with the R_F of peak II It has now been found that treatment of peak IV from both apple fruitlets and coconut milk on 'Dowex–50–H$^+$' produces peaks which have R_F values corresponding to I, II and III Perhaps, therefore, peak IV is the native kinin in each case and the other peaks are artefacts produced by the 'Dowex–50–H$^+$' step in the purification procedure However, the appearance of III after 'Dowex–50–H$^+$' treatment of coconut milk peak IV contrasts with its apparent absence in purified coconut milk No satisfactory explanation of this discrepancy can be given

From both sources, kinins which have (with the exception of peak III) corresponding R_F values can be obtained Further, degradation of peak IV gives an identical result in each case These two facts suggest, but do not prove, that the chemical nature of the kinin or kinins from the two sources are very similar or even identical This view is supported by the corresponding behaviour of the kinins from both sources in the purification steps

J A ZWAR
N P KEFFORD
W BOTTOMLEY
M I BRUCE

Commonwealth Scientific and Industrial
Research Organization,
Division of Plant Industry,
Canberra, Australia

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PHYSIOLOGY

Electrical Activity of the Parturient Human Uterus

FUNCTIONAL uterine disorders cannot be diagnosed with certainty and the progress of labour is doubtful even in those instances when the patients are labelled clinically 'normal' The fact that most women eventually deliver a normal new-born without severe complications is of no comfort to the attending physician at the time of the onset of labour, for he has no guarantee, except a statistical one, that his given patient will have a quick and uncomplicated delivery His uncertainty is the more disturbing, for the outcome of labour will to a great extent depend on his diagnostic and prognostic skill in recognizing functional uterine disorders and his choice of management

This article reports preliminary work on a new technique of apparent diagnostic and prognostic value The new method yields a tracing of the electric activity, of the parturient human uterus Naturally it can be applied to the non-pregnant uterus as well The method, when combined with the 'extra-ovular pressure' technique[1], allows the simultaneous recording of the electric and mechanic activity of the uterus for hours or repeatedly during several days in non-anæsthetized patients The significance of knowing the character of the electric activity is the following

The amniotic pressure tracing describes accurately the quantity and to a large extent the quality of the uterine activity[1] It informs us about the resting and active pressure, the frequency and duration of cyclic activity, and by the shape of the pressure curve about the extent of propagation together with the synchrony of activity However, all this valuable information, no matter how accurate, is insufficient for predicting the course of labour even in those patients who are labelled as clinically 'normal' Not infrequently we observed that quantitatively and qualitatively excellent activity failed in securing normal progress

Those clinicians for whom intra-amniotic pressure tracings were not available concluded that, although the clinical symptoms of effective uterine activity were apparent in a given patient, she failed in progress most likely because the quantity or quality (or both) of uterine effort must have been inadequate Those obstetricians who did have the evidence in the tracings of a quantitatively and qualitatively excellent uterine activity concluded that the reasons for slow or no progress lay in the cervix, resisting dilation Several hundred pressure tracings now allow us to state with certainty that clinically perfectly 'normal' patients may completely

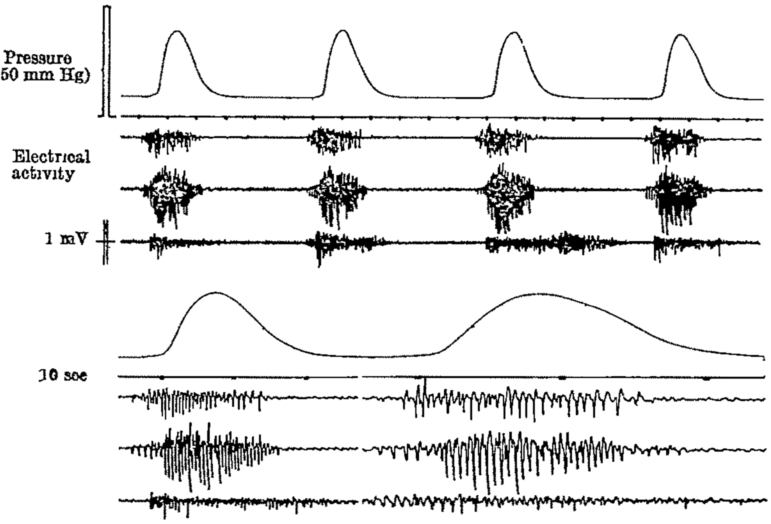

Fig 1. Electrical and mechanical activity of the parturient rabbit uterus. Note the synchrony in electrical activity at the ovarian, mid and cervical uterine portions. Note the uncomplicated shape of the pressure curves

fail in their progress of labour, not only for hours but for days, in spite of excessive uterine activity. More than that, patients may fail in their progress even in those instances when the cervix has all the clinical qualities which permit normal or rapid progress.

It is apparent, therefore, that the classic clinical concepts concerning prolonged labour, uterine inertia, cervical distocia, the arrest of labour, etc., are to a large extent unsatisfactory and must be revised. The more so because it has been shown[2] that when patients with fœtal death or with missed abortion are induced to deliver by hypertonic solutions under the most unfavourable conditions, that is, poor spontaneous activity, closed, hard and long cervix, they deliver during a very short period of 2·7 h (on the average) after the clinical onset of labour. The intra-amniotic injection of hypertonic solutions has two known effects: the suppression of placental function (and most likely that of the amniotic membranes) and the increase in uterine volume. It is significant that these effects create an obstetrical situation in which patients deliver quickly and predictably. We are forced to conclude that the unpredictability of normal labour lies in our incomplete understanding of placental function.

The concepts of the local effect of placental progesterone[3], the consequent endocrine and functional asymmetry[4] of the uterus permit a reasonable explanation of slow progress (or no progress) in those patients whose uterine activity is normal or excessive. A clinically not apparent unfavourable condition is that when the placenta has a fundal or partly fundal implantation. In this condition, activity would seem to begin at the lower uterine segment and will then propagate in the wrong, upward, rather than the downward direction. The pressure tracing does not reveal the direction of the activity wave.

It is the recording of the electrical activity at different portions which shows clearly not only the character but also the direction in the spread of the electrical activity. If electrical activity is recorded together with the amniotic pressure, uterine activity is described in full detail.

We developed a new method[5] for the simultaneous recording of the electrical and mechanical activity of both the pregnant and non-pregnant uterus. A characteristic tracing is illustrated by Fig. 1. Several hundred such model experiments in rabbits provided us with information which now allows us to predict rather closely the pattern of electrical activity from the pressure tracing. However, the tracing alone does not inform us about the direction in the spread of electrical activity. In order to obtain this information we had to adopt our new method of electro-uterography to the human uterus.

Fig. 2 illustrates schematically the technique of how electrical activity of the human uterus is recorded. The technique itself as applied to animals is described in detail in an earlier paper[5]. In women the insertion of the recording electrodes had to be modified. The myometrium was approached as if the patient would undergo amniocentesis[6]. Instead of traversing the myometrium the point of the needle (No. 18) was only immersed into its surface layers, allowing the insertion of an electrode through its lumen. The electrode was enamel-coated copper wire, 0·45 mm diameter, recoated at its naked tip (in contact with the myometrial cells) by silver or gold. The needle itself served as the indifferent electrode.

It is apparent that the electrical activity of parturient patients can be recorded, the tracings being very similar to those obtained by model experiments in rabbits. At present we are investigating the question of what additional information can be gained by the application of a set of electrodes, rather than one.

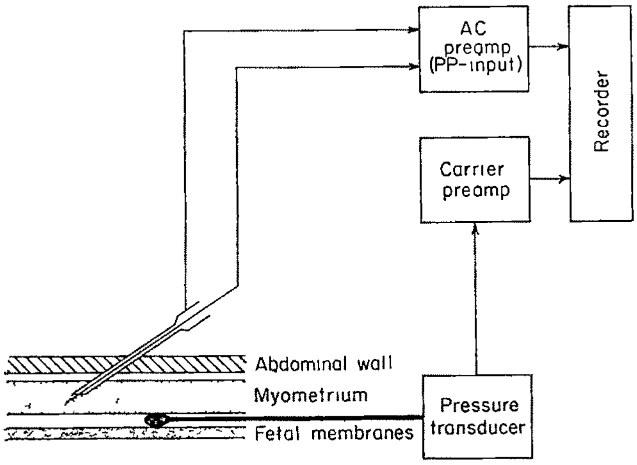

Fig 2. Schematic illustration of the technique, used in human patients. The needle inserted into the myometrium serves as indifferent electrode

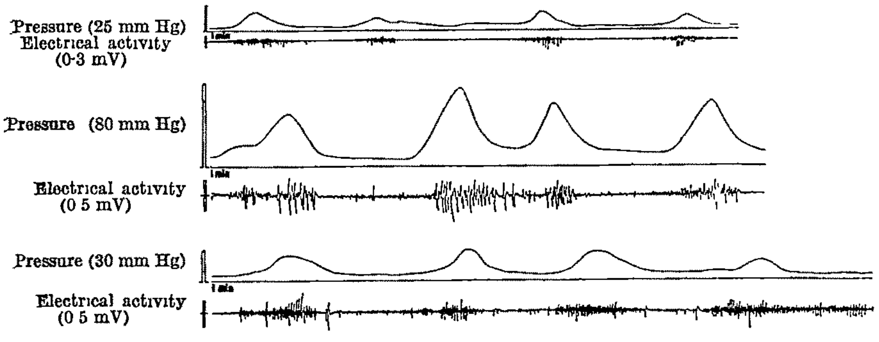

Fig 3. Electrical and mechanical activity of three patients in early labour. Note the similarity in the character of electrical activity of the rabbit and human uterus

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ARPAD I. CSAPO
HIROSHI TAKEDA

The Rockefeller Institute,
New York.
Washington University and
University of Bahia, Brazil.

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In vitro Stimulation by Insulin of α-Aminoisobutyric Acid Transport in the Absence of Protein Synthesis

SINCE the demonstration by Kipnis and Noall[1] that insulin stimulates the accumulation of the non-utilizable amino-acid α-amino-isobutyric acid (AIB) by cells of the intact rat diaphragm *in vitro*, attempts have been made to relate this phenomenon to the action of insulin on protein synthesis. A review of the available evidence led Manchester and Young[2] to the conclusion that the action of insulin on accumulation of amino-acid by the diaphragm of the rat is probably limited to non-utilizable amino-acids and that it may represent an effect secondary to a direct stimulation of protein synthesis. In their view, AIB accumulation "can be regarded as filling the gap left by amino-acids used up in the synthesis of extra protein which occurs in the presence of insulin". The more recent observation[3] that insulin increases the transport of a number of naturally occurring amino-acids into muscle cells prompted us to re-examine the possibility that the effect of insulin on AIB accumulation may be a direct action of the hormone on an amino-acid transport system.

The conditions used for the investigation of AIB-^{14}C transport and amino-acid incorporation into protein in the 'intact' hemidiaphragm of the rat have been described in detail[4,5]. Briefly, normal female rats (Charles River strain) weighing 60–70 g were killed by dislocation of the cervical vertebræ, their diaphragms excised with rib cage attached and bisected through the central ligament and through the insertions to the xiphoid process and spine. The 'intact' hemidiaphragms thus obtained were incubated in Krebs bicarbonate buffer gassed with 5 per cent carbon dioxide and 95 per cent oxygen and containing glucose (10 mM). At the termination of incubation the diaphragms were dissected from surrounding tissue, blotted, weighed and the radioactivity of the tissue water and the purified proteins determined by liquid scintillation spectrometry. The extracellular space was measured by the distribution of insulin-1-^{14}C. Extracellular space of the tissue was determined for each control and experimental situation utilized in this investigation and the results expressed as the concentration ratio of AIB-^{14}C

$$\left(\frac{\text{c.p.m./ml intracellular water}}{\text{c.p.m./ml incubation medium}}\right)$$

Bovine insulin (Lilly *PJ*-4609, 23.8 U/mg) was added to the incubation medium at a concentration of 0.1 U/ml. Protein synthesis in the isolated diaphragm was blocked by the addition of puromycin[6] (500 μg/ml.). This concentration was chosen because it maximally depressed the incorporation of uniformly labelled ^{14}C-valine into diaphragm protein (Table 1). Similar findings have been obtained with ^{14}C-histidine, ^{14}C-alanine and ^{14}C-leucine in diaphragms of normal and hypophysectomized rats.

The results are presented in Table 2. It may be seen that puromycin at a concentration which essentially abolishes amino-acid incorporation into protein did not depress the accumulation of AIB in the intracellular compartment of control diaphragm muscle nor did it influence in any way the marked stimulation of uptake of AIB effected by the simultaneous addition of insulin to the medium.

Table 1. EFFECT OF PUROMYCIN ON THE INCORPORATION OF ^{14}C-VALINE* INTO PROTEIN OF 'INTACT' RAT HEMIDIAPHRAGM *in vitro*

	Time of incubation (min.)			
	45	90	120	180
	Specific activity (c.p.m./mg protein)			
Control	189.1 ± 25.9 (3)†	229.3 ± 7.1 (11)	331.0 ± 22.3 (3)	482.0 ± 81.0 (3)
Puromycin (500 μg/ml.)	3.5 ± 0.1 (6)	3.5 ± 0.2 (12)	3.3 ± 0.2 (6)	3.6 ± 0.2 (6)

* 0.1 mM (0.05 μc./ml.).
† Mean ± *S.E.* No. of observations in parentheses.

Table 2. EFFECT OF INSULIN AND PUROMYCIN ON THE ACCUMULATION OF ^{14}C-AIB* IN THE INTRACELLULAR WATER OF 'INTACT' RAT HEMIDIAPHRAGM *in vitro*

Additions		Time of incubation (min.)		
Insulin	Puromycin	90	120	180
		Concentration ratio		
–	–	1.48 ± 0.10 (12)†	1.60 ± 0.09 (13)	2.10 ± 0.20 (12)
–	+	1.53 ± 0.09 (6)	1.62 ± 0.19 (7)	1.81 ± 0.05 (6)
+	–	2.63 ± 0.09 (12)	3.55 ± 0.16 (12)	4.16 ± 0.17 (12)
+	+	2.50 ± 0.09 (6)	3.34 ± 0.22 (6)	4.11 ± 0.15 (6)

* 0.05 mM, 0.025 μc./ml.
† Mean ± *S.E.* No. of observations in parentheses.

These experiments clearly demonstrate that the stimulation by insulin of AIB accumulation in muscle cells of the isolated intact rat diaphragm is not secondary to the stimulation of protein synthesis and that it may represent a primary action of the hormone. Whether this conclusion is also applicable to naturally occurring amino-acids remains to be determined. It may be of interest that the stimulation of AIB transport by growth hormone in diaphragms of hypophysectomized rats is similarly not abolished by the action of puromycin[7].

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G. R. FRITZ*
E. KNOBIL

Department of Physiology,
University of Pittsburgh School of Medicine,
Pittsburgh 13, Pennsylvania.

* Postdoctoral Research Fellow of the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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Precocious Organic Iodine Secretion in the Blood by the Thyroid Gland

WHILE examining the variation in time of plasmatic PB^{131}I (normal human subject) after ingestion of an Na^{131}I as a tracer, we have found, in some cases, the existence of a radioactivity 'peak' during the first few hours after ingestion. The phenomenon is repeated, whatever the method of separation of the plasmatic proteins: precipitation with trichloroacetic acid, fractioning by ion-exchanger resin[1], or in a 'Sephadex' column[2]. Fig. 1 illustrates a typical case.

Assuming that the occurrence is not due to some form of artefact, it would appear logical to assume that it indicates the secretion of iodinated organic substances of

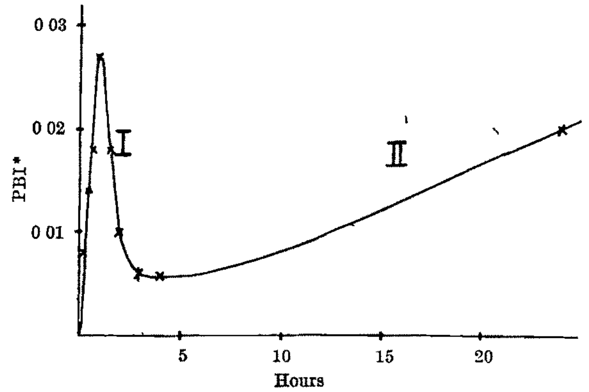

Fig 1

rapid metabolism (phase I) in addition to the usual hormonal secretion (phase II)

An argument in favour of this assumption is the effect on the occurrence of thyroid stimulating hormone and perchlorate, which conforms with the well-known effects of these agents on thyroid metabolism A further and stronger argument being the separation of a radioactive iodinated organic fraction, varying in concentration in the plasma with time, and in parallel with phase I of the curve, we have experimented on the rat, the demonstration involving the administration of too great a dose of radioactivity for a human subject

A typical experiment consisted of administering 40 μc by intraperitoneal injection and killing the rats in groups of three at intervals varying from the time of injection The curve in Fig 2*a* shows the variation of concentration of organic iodine in plasma, separated by ion exchanger resin, versus time Again, a curve is plotted which appears to demonstrate the existence of two phases of metabolism Fig 2*b* shows the same variation during a similar experiment, but after injection of 5 U H L thyroid-stimulating hormone 'Endopancrine' to each rat 1 h before administration of the iodine isotope

Comparison of curves in Figs 2*a* and 2*b* reveals the highly significant effect of the thyroid-stimulating hormone on the concentration of organic radioactive iodine in plasma, and particularly on the maximum appearing at about the third hour As already stated concerning the human subject, this appears to confirm the thyroid origin of phase I, and the possibility of the existence of an organic iodine secretion of rapid metabolism Chromatograms according to the Partridge method[3] have been carried out on the butanolic extract of sera obtained at various intervals after injection of the tracer Fig 3 is a radiochromatogram on a sample obtained after 3 h The presence of an iodinated fraction distinct from the iodide but with a very closed R_F may be noted On the contrary, chromatograms on samples obtained after 24 h reveal the usual iodinated components only—iodothyronine and iodide—but the fraction with an R_F close to that of the iodide has completely disappeared

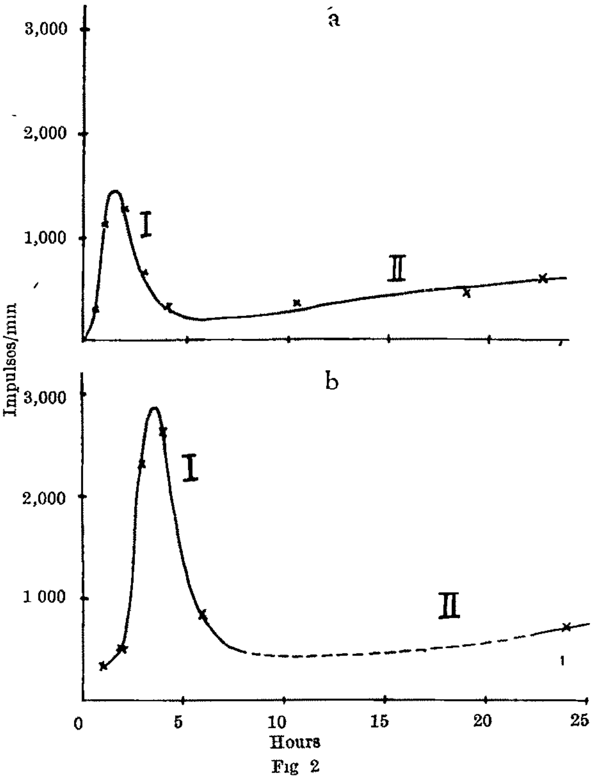

Fig 2

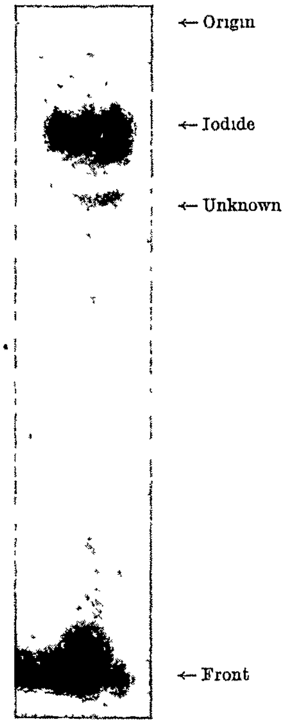

Fig 3

Taken together, these experimental results appear to us to indicate the existence of secretion by the thyroid of iodinated organic substances renewed much more rapidly than the hormones What is the nature of these substances, and what is their physiological role ? Regarding the first question, our preliminary investigation seems to indicate that the substances are iodinated peptides of thyroid origin

The existence of rapid metabolism in addition to the usual metabolism of thyroid hormones could perhaps explain the apparent contradictions liable to appear in the results obtained with iodine-127 and iodine-131, in the case of samples obtained after the 24 h

R RIVIERE
D COMAR
P BLANQUET
C KELLERSHOHN

Commissariat à l'Energie Atomique,
Département de Biologie,
Service Hospitalier Frédéric Joliot,
Orsay, Seine et Oise

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Hyperlipidæmia : a Cause of Decreased Oxygen Saturation

THE induction of hyperlipidæmia by the intravenous infusion of a fat emulsion in human beings produces a fall in arterial oxygen saturation While the phenomena were observed earlier by Kuo[1] in human beings and in dogs by Stutman[2] and Cain[3], the observations have not been extremely pursued nor has the mechanism of such a decrease in arterial oxygen saturation been fully explained

Eleven male volunteer prisoners (age range 20–30 years) were investigated in the supine fasting state An indwelling Cournand needle was placed in the brachial artery and control blood samples taken for blood oxygen content and capacity measurements All measurements were carried out by the manometric method of Van Slyke and Neill[4]. 500 ml of fat emulsion ('Lipomul' supplied by courtesy of the Upjohn Co , Kalamazoo, Michigan) containing 15 per cent cottonseed oil was infused into an anticubital vein over a 2-h period The infusion of 500 ml of 'Lipomul' was found to raise indirect triglyceride-levels from a control range of 2 2–7 m equiv /l to 43 m equiv /l and TEFA levels from 250–400 mg to 1.500 mg/per cent Samples for arterial oxygen saturation were obtained at the end of the infusion period The addition of 'Lipomul' in appropriate quantities to blood samples was shown not to interfere with the determination of blood oxygen by the manometric technique The increase in fat concentration does, however, alter the amount of physically dissolved oxygen which must be subtracted from the oxygen capacity and content before calculating the oxygen saturation The solubility coefficient for oxygen in cottonseed oil is approximately 5 times that in blood[5] The addition of 500 c c of 'Lipomul' is equivalent to the addition of 75 c c of cottonseed oil All oxygen measurements from the bloods containing 'Lipomul' were corrected for the amount of physically dissolved oxygen which would have normally been present plus the additional amount which was present because of the added fat before saturation values were calculated

Table 1 EFFECT OF 'LIPOMUL' ON OXYGEN SATURATION

Subject	% Arterial oxygen saturation Control	% Arterial oxygen saturation 'Lipomul'	% Arterial oxygen saturation 40% O_2 and 'Lipomul'
A A	96 4	90 6	96 6
A G	95 5	90 1	94 1
A L	97 3	91 7	99 0
A W	94 5	90 2	93 9
B W	95 0	92 2	95 4
C W	97 0	93 1	98 0
B L	96 0	93 1	95 9
A J	95 2	96 8	—
B J	96 0	91 8	—
D W	96 2	90 3	—
A H	97 5	90 3	—
Average	96 1	91 9	96 1

From Table 1 it may be seen that arterial oxygen saturation fell from an average of 96 1 to 91 9 per cent following the production of hyperlipidæmia In order to help determine if this fall in oxygen saturation was caused by a shunt or by a diffusion defect, the subjects were given 40 per cent oxygen to breathe for 5 min and arterial samples again obtained The rise in saturation to pre-infusion levels would indicate that the fall in oxygen saturation might well be due, at least in part, to a diffusion defect Preliminary measurements of pulmonary diffusing capacity for carbon monoxide by the single breath method of Ogilvie *et al* [6] on 4 of the subjects before and after 'Lipomul' indicate a decrease from a control value of 35 6 ml CO/min/mm mercury to 30 2 ml CO/min/mm mercury in the post-infusion period These observations do not rule out the possibility of changes in ventilation-perfusion relationships in the lung being a contributing factor in the production of the arterial oxygen unsaturation The failure of the saturation values to rise above the pre-infusion levels does indeed suggest that some degree of shunting may be present

The significance of these observations particularly as they relate to the deprivation of oxygen to cellular metabolic systems represents an intriguing investigative field at the present time While it is widely appreciated that hyperlipidæmia may be associated with atherosclerosis and profound changes in the blood coagulation systems, the establishment of significant decreases in arterial oxygen saturation in normally occurring hyperlipæmia presents a controversial investigative problem

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G DOUGLAS TALBOTT
REGINA FRAYSER

American Medical Research Foundation,
Dayton, Ohio,
and
Department of Medicine,
Indiana University Medical School,
Indianapolis,
Indiana

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Development of Bradycardia during Submersion of the Duck

SOME mammals and birds display structural and functional adaptations which permit them to endure submersion asphyxia for extended periods of time Among the adjustments to submersion of diving animals, those concerned with the cardiovascular system are the most frequently described The most conspicuous and easily observable features displayed during submersion are a sudden onset of apnœa followed by a striking bradycardia

The receptor sites and the quality of the stimuli which bring forth the adjustments observed during submersion are unknown It has been alleged that the actual immersion under water must play a dominant part in the afferent reflex chain, and that naturally diving animals are endowed with special mechanisms for reacting to this stimulus[1]. On the other hand, some authors have advocated that the mechanism mobilized to combat asphyxia is no different in quality in diving animals from what is found in typical non-diving animals[2]

The experiments described here are an attempt to disclose whether water immersion as such is a triggering factor to the submersion bradycardia in the domestic duck

Domestic ducks, *Anas boscas*, were tracheotomized, and catheters for intravascular pressure measurements were introduced in the brachial artery and vein The operative procedures were performed under local anæsthesia. The animals were resting in upright, normal positions during the entire experimental period This precaution was executed carefully to avoid disturbances of the breathing pattern or the vestibular mechanisms Artificial ventilation using various gas mixtures was applied through the tracheal cannula The central arterial and venous pressures were fed into Statham pressure transducers and recorded on a Sanborn four-channel recorder Diving was simulated by immersion of the animal's head in a water container, taking care to retain the upright position of the animal during the immersion period

Fig 1 depicts cardiac cycle-length (C C L) (each point represents the average in a 10-sec period) and arterial

(A P.) and venous (V P) pressures during an experiment involving the following steps The animal was artificially ventilated with air At each inspiration an elevation of the central venous pressure occurred Prior to point *A* is seen the normal resting values of the parameters measured At point *A* the animal was blindfolded by a gentle grip with one hand around the animal's head No undue pressure was exerted and the beak and nares remained uncovered The blindfolding is seen to introduce a moderate change in cardiac cycle-length with minor changes in arterial and venous pressure-levels If anything, the conspicuous sinus arhythmia seen in many birds became particularly prominent The bradycardia amounted to about a 20–30 per cent reduction in frequency. This initial bradycardia could easily be induced by a number of stimuli involving touching of the animal's head or bending its head forward.

At point *B* the animal's head was held slightly forwards, simulating exactly the position of the head during the immersion in water There was no change in cardiac rhythm

At point *C* the animal's head was submerged There was a slight transient increase in heart rate; but the frequency soon levelled off at the value characteristic of the pre-immersion period

At point *D* the artificial respiration was stopped as apparent in the cessation of the sinus arhythmia and the respiratory fluctuations in the venous pressure record From point *D* on, a gradually increasing bradycardia is seen to appear The development of the bradycardia was dependent on a number of factors which are being subjected to further investigation The cardiac slowing became particularly prominent after a period of 40–50 sec of arrested breathing and could amount to a prolongation of the cardiac cycle by a factor of four when the animal displayed muscular efforts involving the thoracic musculature The importance of the muscular activity was amplified as curarization of the animal prevented the conspicuous bradycardia seen otherwise We believe that receptors sensitive to mechanical distortion located within the thoracic cavity or in the respiratory musculature proper are important in the development of the bradycardia during submersion asphyxia No effect of immersion in water was detectable after a slight initial bradycardia had already been established by blindfolding or a number of other unspecific stimuli Our experiments therefore disagree with the idea that immersion in water *per se* plays any major part in the development of bradycardia during submersion of the duck

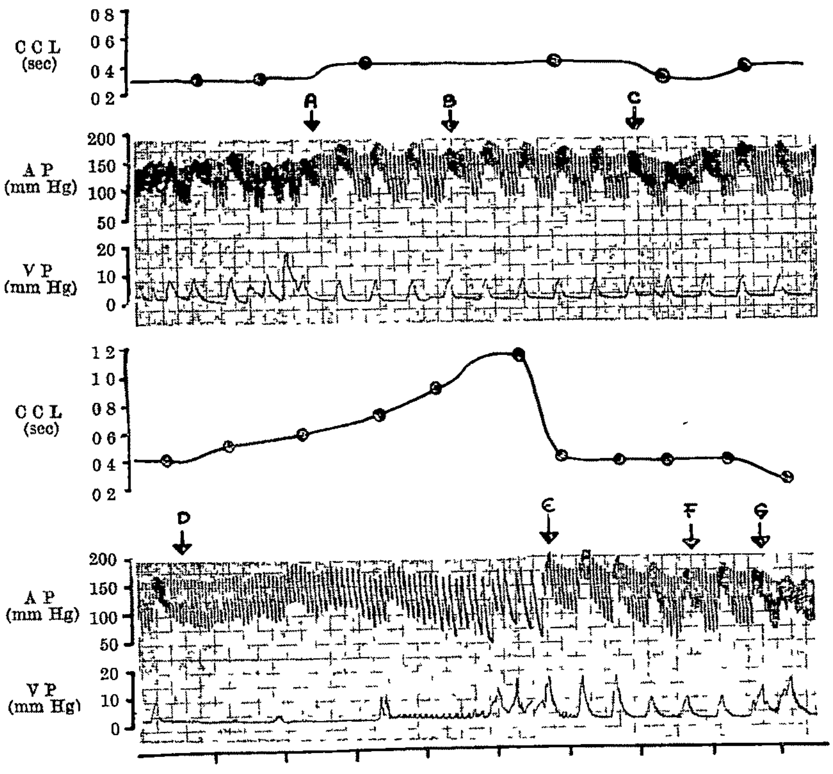

Fig 1

At point *E* the artificial respiration was resumed The heart frequency returned promptly to the values recorded before cessation of the breathing movements At point *F* the animal was taken out of water with no change in cardiac rhythm At point *G* the animal was released from the blindfolding. This act increased the heart frequency further and completed a reversible cycle, giving back the initial frequency that prevailed before onset of blindfolding (point *A*)

O B Reite
J Krog
K Johansen

Institute for Experimental Medical Research,
Ullevaal Hospital, Oslo

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PHARMACOLOGY

Salicylate and Increased Vascular Permeability due to Hyaluronidase

Much work has been carried out on the action of sodium salicylate on the hyaluronic acid hyaluronidase system, and some of it is contradictory In fact, Guerra[1] pointed out that salicylate inhibits the action of hyaluronidase, and on the basis of this, he suggested this inhibition would explain the action of the salicylate in rheumatic diseases These results were completely or partially confirmed by others[2–6]

On the contrary, no inhibition of this enzymatic system by the salicylate could be demonstrated by other workers[7–12] Furthermore, the hyaluronidase was found capable of increasing the atheromatous lesions of rabbits fed hypercholesterolic diet[13–15]

On the basis of the foregoing results, 22 female rabbits were fed a high cholesterol diet (0 2 g/kg a day *per os*) in order to demonstrate a possible inhibiting effect of salicylate on the action of hyaluronidase At the 44th day after the beginning of the treatment, 12 animals were given 15 per cent solution of salicylate intraperitoneally at the dose of 0 5 g/kg a day for 10 days At the 54th day after the beginning of the experiment, 6 of the 10 cholesterol fed animals and 6 out of the 12 cholesterol + salicylate fed animals received twice 1,000 viscosimetric units of testicular lyophilized hyaluronidase diluted into 3 ml of saline solution The other animals were given saline solution only All the animals were killed 30 days later

The entire aortas, previously fixed in formalin and stained with sudan III, were grossly and independently observed by 4 persons following a suitable scheme Afterwards the specimens were cut using the freezer microtome and the sections restained with sudan III

Both macroscopically and microscopically we observed (*a*) more severe atheromatous lesions in the animals treated with hyaluronidase than in those of the other groups, as already described by Cali and Seifter[13–15], (*b*) a slight inhibition of the hyaluronidase by sodium salicylate in the animals treated with cholesterol, hyaluronidase and sodium salicylate, whereas no significant variations were observed in the animals treated with cholesterol and sodium salicylate

Therefore, we believe it may be assumed (*a*) that high doses of sodium salicylate slightly inhibit the hyaluronidase only when the hyaluronic acid–hyaluronidase system is turned towards the hyaluronidase, as in the animals treated with cholesterol and hyaluronidase; it would be advisable to examine the relationship between this effect and the dose of salicylate, (*b*) that it is possible to confirm only in part what Guerra[1] found in his experiments, (*c*) that there is not sufficient evidence to state, as Guerra did, that salicylate is effective in rheumatic diseases because of its antihyaluronidase action It might be considered this is only a part of the therapeutic efficiency of this drug In this respect, on the other hand, it has been recently pointed out[16–19] that other substances of the bradykinin group are important in the pathogenesis of rheumatic diseases and that sodium salicylate completely inhibits the action of these substances

G B Rossi
E Caccavale
L Coscia Porrazzi

Institute of Pathological Anatomy,
University of Naples

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Elevation by Propynylamines of Rat Tissue Norepinephrine-levels and Reserpine Antagonism in Mice

The hydrochlorides of 2-propynylamine, di-2-propynylamine, and tri-2-propynylamine were tested in several systems for monoamine-oxidase inhibition The 2-propynylamines were supplied through the courtesy of Dr Alfred Bader of the Aldrich Chemical Co

Norepinephrine-levels were determined in the pooled brains and hearts of groups of 5 rats each injected intraperitoneally with 10 mg/kg and 40 mg/kg of 2-propynylamine hydrochloride once daily for 4 days The tissues were extracted in duplicate by the procedure of Shore and Olin[1] and norepinephrine determined by the method of Von Euler and Floding[2] using a spectro-photofluorometer The 10 mg/kg dose of 2-propynylamine hydrochloride elevated brain- and heart-levels of norepinephrine to 151 per cent (146–156) and 122 per cent (114–130) of the levels found in control animals The 40 mg/kg dose raised brain and heart norepinephrine-levels to 185 per cent (170–200) and 130 per cent (128–133) of the control values

Table 1 ANTI-PTOTIC AND ANTI-EXTENSOR SEIZURE EFFECTS OF 2-PROPYNYLAMINES IN MICE GIVEN RESERPINE

Compound	Dose (mg/kg, intraperitoneal)	Time before reserpine	Degree of ptosis	mg/kg metrazole required to cause seizures (mean ± *S E*)
None	—	—	0	12 ± 1 0
Reserpine alone	4	—	4	5 8 ± 0 8
+ 2-propynylamine hydrochloride	50	9 days	2 3	11 5 ± 2 2
	50	2 h	0 8	13 8 ± 0 5
	25	2 h	3 4	8 8 ± 3 5
+ Di-2-propynylamine hydrochloride	50	2 h	2 4	12 5 ± 2 8
	25	2 h	3 9	5 3 ± 0 5
+ Tri-2-propynylamine hydrochloride	100	2 h	3 1	11 0 ± 2 0
	50	2 h	3 8	7 8 ± 1 0

The monoamine-oxidase activity of the propynylamines was examined in mice by their ability to reduce the reserpine-induced ptosis and to antagonize the facilitatory effect of reserpine on tonic-extensor seizures[3] Groups of 5 mice each were given the propynylamine compounds by intraperitoneal injection and 2 h or more later were given 4 mg/kg of reserpine, in 5 per cent ascorbic acid solution, by intraperitoneal injection One group of mice without drug treatment and one group given reserpine alone were used in each experiment as controls A 0 5 per cent solution of 'Metrazol' was used to produce tonic-extensor seizures by intravenous infusion Table 1 shows the activities of the 2-propynylamines in both tests At the 50 mg/kg dose-level of 2-propynylamine the anti-reserpine activity, as measured by the 'Metrazol' test, lasted for 9 days Di-2-propynylamine and tri-2-propynylamine both had anti-reserpine activity but were less active than 2-propynylamine The 2-propynylamines alone did not cause any marked retraction of eyelids or produce an anti-convulsant effect on tonic-extensor seizures at the doses used

2-Propynylamine is a potent compound in raising rat tissue norepinephrine-levels, furthermore, 2-propynylamine antagonizes the effects of reserpine in mice

J R McLean
G Chen

Departments of Pharmacology and Chemistry,
Parke, Davis and Co , Research Laboratories,
Ann Arbor, Michigan

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HÆMATOLOGY

Absence of Lysolecithin Formation in C'_3-deficient Rabbit Serum

Recently, Fischer and Haupt have shown[1–3] that the activation of complement in human, guinea pig, rabbit, and pig serum is paralleled by an increase in lytic activity due to an increase of lysolecithin In these experiments complement was activated by the addition of soluble antigen–antibody-complexes or by the addition of heat-aggregated human γ-globulin Complement activity was determined using sensitized sheep erythrocytes, while the lytic activity of lysolecithin was determined in lipid extracts of the same sera by the method of Schubothe and Fok[4] (see also legend to Table 1)

Further evidence for the dependence of lysolecithin formation on the presence of whole complement might be obtained by demonstrating that rabbit sera lacking C'_3 are unable to form lysolecithin

A strain of C'_3-deficient animals (*Freiburg R 3-Stamm*) was described recently and investigated by K and U Rother[5,6] In the hæmolytic system serum of C'_3-deficient animals behaves in the same way as does rabbit serum depleted of C'_3 by treatment with 'Zymosan' Complement activity can be restored by adding any preparation which contains C'_3

Table 1

		Complement activity (50% hæmol units)	Lytic activity of serum extracts (% of total hæmolysis)	Δ lytic activity (%)
Experiment No 1 (rabbit No 1)	Normal rabbit serum	35	14	
	Normal rabbit serum + γ-globulin	0	43 5	c 300
	C'_3-def -rabbit serum	0	16	
	C'_3-def -rabbit serum + γ-globulin	0	16	0
Experiment No 2 (rabbit No 2)	Normal rabbit serum	30	12	
	Normal rabbit serum + γ-globulin	0	23 2	c 100
	C'_3-def -rabbit serum	0	14 6	
	C'_3-def -rabbit serum + γ-globulin	0	14 6	0
Experiment No 3 (rabbit No 3)	Normal rabbit serum	15	24	
	Normal rabbit serum + γ-globulin	0	45 5	c 100
	C'_3-def -rabbit serum	0	24 6	
	C'_3-def -rabbit serum + γ-globulin	0	27 0	c 10
	C'_3-def -serum + *R*1,2,-serum *	8	26 2	
	C'_3-def -serum + *R*1,2-serum + γ-globulin	0	40 0	c 50
	C'_3-def -serum + C'3(pig) †	5	27 8	
	C'_3-def -serum + C'3(pig) + γ-globulin	0	40 6	c 50

* Serum heated at 56° for 25 min

† According to Leon *et al* (ref 8)

The sera of normal and C'_3- deficient animals were incubated simultaneously for 30 min with 1/10 vol of a 5 per cent solution of heat-aggregated (56° C, 30 min) human γ-globulin (Behringwerke, Marburg) Complement activity was determined using standard techniques (ref 7) The lytic activity of serum-extracts (obtained by extracting serum with 20 vol of ethanol/ether 3 1) was estimated in an apparatus originally designed for the determination of erythrocyte fragility (ref 4) The percentage of total hæmolysis obtained under standard conditions is given in the table

The results of three experiments are shown in Table 1

The results show an increase in lysolecithin activity in normal sera after incubation for 30 min at 37° C with aggregated γ-globulin No increase occurred in *R* 3-deficient sera The addition of C'_3 preparations to the deficient serum (experiment No 3) restored some of the complement activity and some of the ability to increase the level of lysolecithin

These results give additional support to the lysolecithin hypothesis[1-3,9] of complement action

I Haupt
H Fischer

Max Planck Institut fur Immunbiologie,
Freiburg-Zahringen

U Rother
K Rother

Medizinische Universitatspoliklinik,
Freiburg im Breisgau

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A Hæmagglutinating Property of Mouse Serum for Human Red Blood Cells

HETEROAGGLUTININS for human red cells have been reported in various species[1,2] Quantitative differences between males and females in a globulin fraction of mouse serum has recently been reported[3] Stimpfling has described a quantitative difference in the anti-sheep and anti-chicken heteroagglutinin content of normal sera from *C*57 *BL*/6 *Sn* mice[4]

An examination of mouse sera from different inbred strains has shown the sera of some strains to agglutinate strongly most samples of human A blood group cells while O and B blood were not agglutinated in the same proportion Only the results of sera from strains *B*10 and *C*3*H* will be reported here

The agglutination technique consisted in mixing, in a slide, a drop of undiluted mouse serum from a pool obtained from adult males or females with a suspension of three-times washed human red blood cells from healthy blood bank donors The reaction occurs in 1 or 2 min at room temperature and can be observed macroscopically

The strength of the agglutination was arbitrarily classified macroscopically as + + + + if all cells were stacked in one clump, + + + when the cells were all agglutinated in several clumps , + + and + when free cells were present, and − when no red cell agglutination was observed Weak and doubtful reactions were read microscopically (× 30)

A test made separately of male and female sera from mice 6–8 months old gave an unexpected sex difference female sera from *B*10 animals strongly agglutinate human A blood group cells while sera from males of this same strain did not agglutinate them to any appreciable extent This difference was not found in male versus female sera of *C*3*H* mice

The agglutination effected by sera from *C*3*H* mice was usually weaker than that obtained with *B*10 sera No difference was observed between human male and female red blood cells The agglutinating property could be made to disappear specifically from *B*10 female sera by absorbing it with three-times washed male tissues (liver plus spleen), from *B*10 or with A blood group red cells

Tissues from male or female *C*3*H* animals did not absorb *B*10 female heteroagglutinins It thus seems that the reaction of female *B*10 sera is specific Two out of thirty-three *B*10 male sera gave a positive though very weak reaction (± or less) with A blood group cells

A sex difference in agglutination was also observed when B and O cells were tested with *B*10 male versus female sera The differences, however, were not so marked as with A cells and the reaction of both male and female

Table 1 HETEROAGGLUTINATION OF HUMAN RED CELLS OF BLOOD GROUPS A, B, AND O BY NORMAL MALE AND FEMALE SERA FROM STRAINS *C*57, *BL*/10 AND *C*3*H*

Blood groups of donors	Mouse serum from strains *C*57 *BL*/10*		*C*3*H*	
	M	F	M	F
A	0/22†	22/22	22/22	21/22
B	9/20	17/20	19/20	17/20
O	4/24	21/24	24/24	23/24

* *B*10 males versus females = $P < 0\ 50$ for group B red blood cells
$P < 0\ 01$ for group O red blood cells

† $\frac{\text{No of samples agglutinated}}{\text{Total No of bloods tested}}$

Table 2 INCREASE IN NORMAL HETEROAGGLUTININS AGAINST HUMAN BLOOD GROUP RED BLOOD CELLS WITH INCREASING AGE OF *B*10 FEMALES

Human A group red blood cells	Hæmagglutination of A red blood cells by *B*10 female sera of ages* Months 1	2	3	4	6
No 5409	±	± +	+ +	+ +	+ + + +
5433	±	± +	+ +	+ + +	+ + + +
5432	±	+	+ +	+ + +	+ + + +
5365	±	+ +	+ +	± + +	+ + + +
5473	±	+ +	+ +	+ +	+ + + +

* The strength of the agglutination was arbitrarily classified macroscopically as + + + + if all cells were stacked in one clump, + + + when the clumps were all agglutinated in several clumps, + + and + when free cells were present and − (negative) when no red cell agglutination was observed

sera with O and B cells was weaker than that observed with A red cells (Table 1)

A study of individual sera from *B*10 females showed a marked age effect on the strength of the agglutinating properties Sera from young females gave weak but consistently positive reactions with human A cells while sera from six months old females gave +++ to ++++ reactions with the same human red blood cells With the exception already mentioned, *B*10 male sera of all ages were negative This increase in agglutinin content with age suggests these agglutinins to be induced by subclinical infections with bacteria carrying antigenic structures related to the ABO system A similar increase in titre of natural heteroagglutinins for red cells and bacteria with age has been described[4,5]

The findings reported here can be interpreted to show a qualitative difference in heteroagglutinating capacity of normal female versus male sera The existence of occasional (2/33) weakly (±) reacting males may be due either to an extreme case of quantitative sex variation or else to technical artefacts The difference between sera from *C3H* and *B*10 strains is striking These strains also differ in that *B*10 females reject male skin grafts while *C3H* females accept them This, the so-called 'Eichwald-Silmser effect', depends on a *Y*-chromosome determined incompatibility between the sexes[6] The possibility remains of a relationship between the male antigen and the human blood group antigen reacting with *B*10 female sera Quantitative differences could be related to an heterotic effect of the *X*-chromosome in double dose in females or to an enhanced antibody production by œstrogen stimulation

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I KIRSHBOM
G HOECKER

Catedra de Biologia,
Instituto Juan Noe',
Universidad de Chile,
Santiago, Chile

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Effects of Surgical Procedures on the Appearance of Slow α_2-Globulin

IT has recently been shown that a variety of conditions mostly related to growth or tissue synthesis evoke a protein in the sera of rats which is not demonstrable in the sera of healthy, non-pregnant, adult rats This material is termed slow α_2-globulin (*SA*$_2$*G*) since, in paper electrophoresis, it exhibits the mobility typical of α_2-globulin, whereas in starch-gel electrophoresis it moves more slowly than both the β-globulin and a slow α_1-globulin[1,2] *SA*$_2$*G* can be demonstrated during prenatal[2–4], postnatal[3–6], and neoplastic[3–8] growth, during pregnancy[3,4,6] and during liver regeneration following partial hepatectomy or carbon tetrachloride poisoning[9,10] While these conditions all involve considerable tissue synthesis, *SA*$_2$*G* also appears in several situations where little or no obvious growth occurs lactation[3,4], tight abdominal bandaging[7], after Caesarean section with retention of a single placenta[6], and in various ill-defined but possibly infectious disease states[6,10]

Since, as already noted by Beaton *et al* [6], the development of new tissue does not appear to be an absolute requirement for the appearance of *SA*$_2$*G*, we undertook to determine the presence or absence of *SA*$_2$*G* after a variety of surgical procedures involving wound healing and, in some but not all cases, compensatory hyperplasia Methods used included unilateral renalectomy and adrenalectomy, *in situ* unilateral ligation of the kidney and adrenal gland, splenectomy, amputation of a forelimb, partial exsanguination, implantation of homologous minced kidney and sham abdominal surgery Only after certain of these treatments did *SA*$_2$*G* appear in the serum.

The animals used, their treatment and the methods of obtaining sera were as previously described[4] Combined unilateral renalectomy and adrenalectomy, splenectomy and combined unilateral ligation of kidney and adrenal without their removal were performed through lateral incisions For the kidney implant operations, a kidney from a donor rat of the same inbred strain was minced with scissors under sterile conditions until the pieces were 2–3 mm on a side They were then implanted into the abdominal cavity through a small ventral incision Surgical sham operations were performed as previously described[10] Limb amputations were performed near the middle of the ulna and radius of the right forearm After a purse-string suture was placed through the skin around the limb a few millimetres proximal to the prospective level of amputation, the skin and muscles were cut, the bones severed with scissors, the purse-string suture tightened and tied In all other operations, the muscles were approximated with silk and the skin closed with wound clips Partial bleedings were performed by severing the rat's tail and collecting 20 ml blood/kg body-weight Bleeding was stopped by a tourniquet and/or cautery These procedures were performed under ether anæsthesia At designated intervals after operation, blood was collected from the tail vessels or by heart puncture Vertical starch-gel electrophoresis was performed by the method of Smithies[11], with minor modifications as previously described[4].

Care must be taken during the slicing of the starch plate not to allow the two newly cut portions to touch each other Should they touch, a small amount of protein may be transferred or 'off-printed' from one to the other Unless the two halves touch exactly in register, a new, artefactual band may be produced This is especially likely in the case of a protein giving a narrow, intense band, for example, slow α_1-globulin The spurious band may be distinguished from a true band by making a cut into the starch parallel to the axis of migration and

Table 1 OCCURRENCE OF SLOW α_2-GLOBULIN AFTER VARIOUS SURGICAL PROCEDURES

Procedure	Presence*	No of animals in each category at indicated No of days after each procedure				
		0	½	2	4	6
Unilateral renalectomy and adrenalectomy	−		0	0	3	2
	+		2	1	6	1
	++		1	4	2	0
Kidney ligation	−		1	0	2	3
	+		2	0	2	0
	++		0	6	0	0
Minced kidney implantation	−			0	1	
	+			4	7	
	++			1	1	
Splenectomy	−		0	0	0	0
	+		0	3	3	2
	++		3	4	3	1
Limb amputation	−			9	6	
	+			1	0	
	++			1†	0	
Partial bleeding	−		6	8	8	
	+		0	1	0	
	++		0	0	0	
Sham surgery	−			22	15	
	+			5†	1	
	++			0	2	
Untreated control rats	−	51				
	+	0				
	++	0				

* − No slow α_2-globulin demonstrable
+ Some slow α_2-globulin demonstrable
++ Much slow α_2-globulin demonstrable
† Signs of wound infection

examining the depth of the bands within the starch: a spurious band will be very shallow, a true band will extend all or most of the way through the starch.

The results obtained are shown in Table 1. In none of the surgical procedures under consideration is there any regeneration of the affected organ in the sense that such regeneration occurs after partial hepatectomy. However, in all but the minced kidney implantation, sham surgery and limb amputation, considerable compensatory hypertrophy or hyperplasia may be expected (see, for example, ref. 12). It is clear from the results obtained that SA_2G is demonstrable in all those conditions involving compensatory growth except the partial exsanguination. The failure of SA_2G to appear after considerable loss of blood might be attributable to: (*a*) failure to remove sufficient blood; (*b*) small amount of wound healing called forth; (*c*) the attributes of a system which produces massive quantities of new tissue at all times, even in the absence of experimental manipulations. In some experiments designed to test the first hypothesis, large quantities of blood were removed once or repeatedly, without, however, evoking a demonstrable production of SA_2G. In the minced kidney implant operation and in some other manipulations at present being used in this laboratory, little wound healing is called forth yet SA_2G becomes readily demonstrable. Hence, the third hypothesis seems most likely at this time. The failure to evoke demonstrable SA_2G by partial bleeding also indicates that loss of blood at surgery is not the effective factor in the other surgical procedures. Compensatory growth of certain organs may, therefore, be one of the stimuli capable of calling forth the demonstrable production of SA_2G.

That conditions other than those leading to some type of growth can bring about the production of demonstrable SA_2G is shown by the frequent or occasional appearance of SA_2G after limb amputation, sham surgery or implantation of minced kidney. In view of the diarrhœa which is often found occurring in the rare cases of spontaneously SA_2G-positive control rats[6,9], and the ever-present possibility of wound infections, it is suggested that inflammatory or infectious disease may be a possible evocating agent. The presence of SA_2G in all rats two days after the implantation of minced kidney tissue and in the majority of rats four days after such implantation may be due to a stimulation, by products of tissue breakdown, analogous to that due to infection. That tissue breakdown can bring about an increase in α_2-globulin (but not necessarily slow α_2-globulin) in man has been known for some time[13]. Finally, it does not appear that mere surgical shock can lead to the production of demonstrable SA_2G since many rats subjected to limb amputation or sham surgery do not exhibit any SA_2G.

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WERNER G. HEIM
J. MICHAEL KERRIGAN
PAULA H. LANE

Department of Biology,
Wayne State University,
Detroit 2, Michigan.

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The Cl^a(Caldwell) Antigen: a New and Rare Human Blood Group Antigen related to the *MNSs* System

Two unrelated families have been found to demonstrate the inheritance of a new and rare red cell antigen, the specificity of which appears to differ from that of all other antigens so far described. It is proposed that the new antigen be named Cl^a(Caldwell). The close genetical linkage of Cl^a to the *MNSs* system has been established. In the two families here reported the coupling of Cl^a is with *Ms* throughout.

The propositus, Mr. Caldwell, had donated blood on six occasions, each time being grouped as O. On testing his seventh donation it was found that his red cells were weakly agglutinated by the anti-B grouping serum in routine use. Caldwell's cells were not polyagglutinable, nor were they agglutinated by any of a battery of high-titre anti-B sera known to be potent in detecting an 'acquired' B antigen[1], nor by a panel of high titre anti-A+B (group O) sera.

Initially we tested 11,000 random donors of all ABO groups in the West of Scotland, but no other Cl(a+) was detected. However, by chance, a second example of the Cl^a antigen was encountered in a cord blood specimen. The cells of Baby Gil. were agglutinated by the routine anti-B grouping serum, but not by an anti-A+B (group O) serum. The infant was in fact group O and Cl(a+). It was established by cross-absorption and elution tests that the Caldwell and Gil. antigens were identical.

Evidence from two generations of the Caldwell family and from four generations of the Gil. family indicates that the Cl^a antigen is the regular expression of the heterozygous state of an autosomal allele. The Gil. Cl^a can by deduction be traced back for 6 generations to an Irish woman born in Ulster about 1830. She must have transmitted her Cl^a to the child of each of her two marriages, so that to-day 27 of her living descendants are known to be Cl(a+). As there is not complete linkage of Cl^a with the *ABO*, *Rh*, *P*, *Kell*, *Duffy* or *Secretor* loci, it can reasonably be taken that the new antigen is not controlled by any of them. However, when the Gil. family data are analysed with respect to *MNSs* and Cl^a it becomes apparent that there are no certain recombinants and 12 certain non-recombinants between *Ms* and Cl^a. That such a finding is due to chance has a probability of 1 in 2^{12}, that is, 1 in 4,000. In addition, the existence of a further 30 almost certain non-recombinants confirms the closeness of this observed linkage. In the Gil. family the absence of a recombinant makes it impossible for us to decide whether Cl^a is part of, or linked to, the *MNSs* system. Cl^a must certainly now be considered as yet another of the rare antigens to be associated with the *MNSs* system in some manner.

Dr. T. E. Cleghorn has confirmed our findings or shown independently that the Cl^a antigen is not serologically identical with the following antigens which are known to belong to the MNSs complex—Hu, He (ref. 2), Mi^a, Vw (ref. 3), Mu(Murrell) (ref. 4), St^a(Stones), Ri^a(Ridley) (ref. 5), Vr (ref. 6), Mt^a (ref. 7) and Mg (ref. 8). The antigen does not appear to be similar to Mg; for example, there are no unexpected dosage effects observable in comparative titrations of Cl(a+) cells against anti-M, -N, -S, or -s sera. However, we have not yet encountered NN Cl(a+) or SS Cl(a+) bloods, and hence have not been able to test them for dosage. Such groupings would have been immediately indicative of recombination in the Gil. family.

Tests (again largely performed by Dr. T. E. Cleghorn) have also excluded identity of the Cl^a antigen with other rare or "private" antigens—C^w, C^x, E^w, V, VS, Bi, Be^a, Bp^a(Bishop) (ref. 9), Bu^a(Boisvert) (ref. 10), By, Donna, Good, Ho, Kp^a, Levay, P^k, Radin-Fleming (ref. 11), Stobo (ref. 12), Sw^a, Tr^a(Traversu) (ref. 9), Wb (ref. 13), Weil, Wr^a, Chr^a, Yahuda (ref. 9), Pritchard (ref. 9), Evans (ref. 14), Hunt (ref. 15), Kennedy (ref. 16) and Go^a (Gonzalez) (ref. 17). Three adult Cl(a+) persons have given normal adult I reactions, while 3 strong anti-Cl^a sera have contained no appreciable anti-i. Cl(a+) cells gave normal positive reactions with anti-Vel, -Ge, -U, -H, -Tj^a, with sera from 'Bombay' and D--/D-- persons, with potato lectin and with human 'D-like' antibody (ref. 18).

Though the Cl^a antigen is, at least in Baby Gil., well developed at birth, there is no evidence of it having been the cause of hæmolytic disease of the new-born in these two families under investigation. The 5 Cl(a−) mothers of Cl(a+) children have no detectable anti-Cl^a in their sera. Seventeen examples of saline-agglutinating anti-Cl^a were recognized in a series of 4,083 random donor sera of all ABO groups, that is, an incidence of 1 in 240 (0·42 per cent). Dr. T. E. Cleghorn found 7 examples of anti-Cl^a in 1,243 random South London donors.

Our anti-Cl^a sera have been apparently of natural origin. All had their optimal temperature of reaction at 4° C or at 12° C, none reacting at 37° C. In keeping with the findings of others[19] regarding the action of enzymes on antigen–antibody reactions within the *MNSs* system, the presence of bromelin, ficin, papain or trypsin was found to inhibit the Cl^a–anti-Cl^a reaction.

Finally, it is interesting to note that at an early stage of the investigation Cl(a+) cells were found positive with a South London anti-B serum, 'Easter', which Dr. I. Dunsford had shown to react with a Sheffield group O donor. Subsequent cross-absorption tests demonstrated that 'Easter' serum contains anti-Cl^a in addition to the antibody against the cells of the Sheffield donor.

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JOHN WALLACE
MARIAN M. IZATT*

West of Scotland
Blood Transfusion Service,
Law Hospital,
Carluke,
Lanarkshire.

* Present address: Genetics Department, The University, Glasgow.

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HISTOCHEMISTRY

Acid-buffered Acetone as a Fixative for Enzyme Cytochemistry

ALTHOUGH acetone has been recommended as a fixative in the histochemical study of enzymes in tissue sections[1,2], there have been but few reports on the use of this fixative in the examination of blood films and related preparations.

In the work recorded here, a variety of hydrolytic enzymes were demonstrated on smears of blood, bone marrow, transudates, exfoliated cells, and tissue culture cells, as well as on organ imprints, using a fixative 60 per cent acetone buffered to pH 4·2 with citrate. Brief fixation (20–30 sec) at room temperature resulted in good preservation of cellular morphology, in most cases of a quality approaching that seen with methanol fixation. The cells showed no tendency to wash off the slide even when fresh and when placed under a vigorous stream of water.

The following enzymes were successfully demonstrated: alkaline and acid phosphatases, esterase (acetyl and chloroacetyl naphthols) using azo dye coupling techniques, peroxidase (benzidine method) and dopa oxidase.

The degree of activity as judged by intensity of dye deposition was greater than or equivalent to that seen with other fixative procedures. Except for acid phosphatase, most preparations exhibited sites of activity and intensity of dye formation similar to that seen on unfixed smears. Acid phosphatase was found primarily in myeloid cells and platelets with weak activity seen in monocytes and lymphocytes, although higher activity was demonstrated in unfixed smears. When 'Naphthol *AS-D*-chloroacetate'[3,4] was used as a substrate, activity was demonstrated in monocytes and platelets, as well as in myeloid cells, and weak activity was sometimes seen in lymphocytes and erythrocytes. On the other hand, Moloney *et al.*[4], and Löffler[5], who used cold 10 per cent formal–methanol fixation[6], found activity only in cells of the myeloid series. When 'Naphthol *AS-D* acetate' or 'Naphthol *AS-OL* acetate' were used as substrates, strongest activity was observed in most monocytes and weaker activity in all other cell types including platelets. Similarly stained

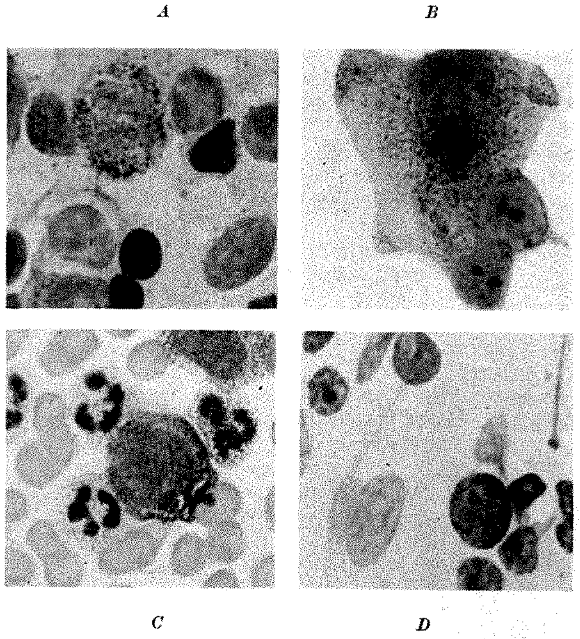

Fig. 1. *A*, imprint of rat spleen stained for chloroacetyl esterase using 'Naphthol *AS-D*-chloroacetate' as substrate, pH 7·1 (× *c.* 1,130); *B*, tissue culture smear, *HEp*-2 (human epidermoid), stained for acetyl esterase using 'Naphthol *AS-OL* acetate' as substrate, pH 7·1 (× *c.* 470); *C*, imprint of rat spleen stained for alkaline phosphatase activity using 'Naphthol *AS-BI* phosphate' as substrate, pH 9·5 (× *c.* 760); *D*, peripheral blood smear, chronic myelogenous leukæmia, stained for chloroacetyl esterase activity using 'Naphthol *AS-D*-chloroacetate' as substrate, pH 7·1 (× *c.* 760)

slides previously fixed in cold 10 per cent formal–methanol showed only very weak staining in some neutrophils and in some monocytes The use of 60 per cent citrate buffered acetone as a fixative for acetyl esterase and cold 10 per cent formal–methanol as a fixative for the enzyme-hydrolysing chloroacetates was helpful in distinguishing immature myeloid elements from immature monocytes, as was pointed out by Moloney[7].

The fixative solution is used and stored at room temperature and is prepared by adding 300 ml of absolute acetone, while stirring, to a mixture of 32 ml of 0 03 M sodium citrate (dihydrate) and 168 ml of 0 03 M citric acid (monohydrate)

Previously, 60 per cent aqueous acetone was suggested as a fixative for succinic dehydrogenase by Quaglino and Hayhoe[8] Optimal cell preservation is not obtained with the aqueous solution and in some cases less activity was observed as compared with that obtained on smears fixed with the acid-buffered acetone mixture

The availability of a fixative capable of simultaneously preserving enzyme activity and cell morphology should prove of value to those utilizing techniques for the cytochemical localization of enzymes in smears.

L S Kaplow*
M. S. Burstone

Diagnostic Research Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland.

*Post-doctoral Fellow of National Heart Institute, U S Public Health Service Present address Department of Pathology, Medical College of Virginia, Richmond, Virginia

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Preparation of Thick Sections for Cytochemistry and Electron Microscopy by a Non-freezing Technique

At present thick $(\sim 50\mu)$ frozen sections of fixed tissues are commonly utilized for cytochemistry and electron microscopy[1-3] despite the fact that freezing and thawing are known to cause disruption of fine structure and loss of enzymatic activity even in fixed tissues[3,4] These disadvantages have been tolerated since use of sections not exceeding 50μ is necessary to ensure even penetration of incubation media[1,2], and no alternative sectioning method has been available

We have recently developed a non-freezing technique for preparing such thick sections that results in greatly improved preservation of cellular structure while retaining higher levels of enzyme activity than the usual frozen section procedures Sections are cut with a McIlwain mechanical tissue chopper (manufactured by Gomshall-Surry), an instrument ordinarily used to prepare somewhat thicker slices of fresh tissue for metabolic investigations[5]

We have found the following technique satisfactory for cutting sections of about 50μ A piece of fixed tissue (about 1 2 × 1 0 × 5–10 mm) is placed in the centre of a pad composed of 6–7 pieces of 5 5 cm filter paper (Whatman No 1) held together with 'Cellophane' tape The tissue is covered with a few drops of liquid 7 per cent agar (or a low melting point paraffin) dripped on to its surface with a warmed dropper pipette (The agar solution is liquefied by heating in a water bath and subsequently stored at 42° C during use) The pad containing the tissue is placed in the refrigerator for several minutes until the agar hardens It is then secured to the circular metal table of the chopper with a few drops of paraffin placed between the bottom piece of filter paper and the metal table The chopper is set to cut at 0 052 mm and a double-edged razor blade (Schick stainless steel) is mounted in the holder The speed control is set at about 200 strokes per minute, and the chopper arm is positioned so that it barely contacts the left edge of the specimen. The instrument is switched on and allowed to remain so until the specimen has been completely traversed by the chopping action of the machine, which passes through the tissue from left to right The sections are then removed from the agar and placed in the desired solution, where they readily separate The entire operation takes less than 10 min

We have utilized the foregoing technique to prepare sections of a number of tissues (for example, pituitary, liver, intestine, kidney, pancreas, skin) fixed in 2–3 per cent glutaraldehyde (buffered at *p*H 7 2 with 0 1 M caccodylate buffer[3]) and stored for variable periods in buffer The sections have afterwards been treated by several histochemical procedures, 'post-fixed' in osmium and embedded in 'Araldite' When examined in the electron microscope these tissues have consistently showed a substantial improvement in general morphology, intensity of histochemical reaction and sharpness of localization of enzyme activity over those prepared by frozen section techniques Improved enzyme survival was demonstrated by the fact that shorter incubation times could be utilized for the non-frozen than for frozen sections to achieve the same intensity of reaction Even more notable is the fact that carefully prepared chopper sections appeared nearly free of the gross defects (tears, gaps, large membrane discontinuities, retraction between cells), which in our experience invariably characterize to some extent the frozen sections

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R E. Smith
M G Farquhar

Department of Pathology,
University of California School of Medicine,
San Francisco 22

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PATHOLOGY

Electrical Charge Density of Spleen Cells sensitized against *L*-strain Fibroblasts

It has previously been shown that homologous and heterologous spleen cells, sensitized against *L*-strain fibroblasts, have a marked cytopathic effect on the fibroblasts *in vitro*[1,2] It was thought useful to examine the electrical surface charge density of these sensitized spleen cells in view of the possible changes which occur at the cell surface during the process of sensitization This communication presents such results obtained by cell electrophoresis

Mice or guinea pigs were sensitized as described by Taylor and Culling[1]· briefly, this involved two intraperitoneal and one intrasplenic injection of *L*-strain fibroblasts over a period of nine weeks Five days after the intrasplenic injection the spleen was removed and weighed Polyvinylpyrrolidone brush smears of the spleen were stained by the acridine orange and Wright's techniques for differential cell counts (lymphocytes, pyroninophilic proplasma and plasma cells and occasional granulocytes) The spleen was then macerated and shaken with glass beads in Medium 199 to obtain a homogeneous cell suspension; sterile conditions were maintained throughout. The spleen cells were then washed four times in Medium 199 and a small aliquot taken for cell electrophoresis. 1 ml of the spleen cell suspension also was added to each of several culture tubes containing a 24-h growth of *L*-strain fibroblasts, viability cell counts on the fibroblasts were performed 24, 48 and 72 h after the addition of the spleen cells to assess the extent of fibroblast damage caused by the sensitized cells. In each experiment identical procedures were performed using normal spleen cells from litter mates.

The cell electrophoresis technique has been described previously[3], the spleen cell suspension was washed in isotonic saline and suspended in phosphate-sucrose electrophoretic buffer (*p*H 7.0, ionic strength 0.083, relative viscosity 1.21), the specific conductivity measurements were made on the cell suspension at the end of an electrophoretic run. Electrophoretic mobilities were converted to surface charge densities using the simplified Gorin equation.

Table 1. EFFECTS OF SPLEEN CELL SENSITIZATION

Exp No	Sensitized or control	Spleen weight (mg)	Viability† counts (× 10³)	Spleen‡ plasma cell series (%)	Cell electrophoresis μ/sec/V/cm	Cell electrophoresis E.S.U. cm⁻²
1*	C	650	140	6	1.40	2.8 × 10³
	S	1,740	63	56	0.94	1.9
2*	C	670	140	5	1.40	2.8
	S	1,020	75	40	1.16	2.3
3*	C	700	155	9	1.30	2.6
	S	1,400	22	54	0.88	1.7
4*	C	740	134	6	1.35	2.7
	S	1,010	51	36	0.97	1.9
5	C	90	241	6	1.21	2.4
	S	270	18	56	1.07	2.1
6	C	100	171	7	1.41	2.8
	S	320	49	40	0.89	1.8
7	C	110	189	8	1.32	2.6
	S	330	29	54	1.03	2.1
8	C	120	170	12	1.41	2.8
	S	200	53	40	1.03	2.1
9	C	90	170	9	1.41	2.8
	S	230	35	33	0.91	1.8
10	C	100	120	7	1.25	2.5
	S	340	12	38	1.01	2.0
11	C	100	120	7	1.25	2.5
	S	320	13	59	0.94	1.9

* Guinea pig (remaining animals *BALB/c* mice)
† Fibroblast culture viability counts at 72 h
‡ Pyroninophilic proplasma and plasma cells as percentage of spleen cell count

The results are summarized in Table 1, where it will be seen that (1) spleens of sensitized animals are approximately two or three times larger than control animals, (2) sensitization causes an increase of plasma and proplasma cells in the spleen, (3) the cytopathic effect of the sensitized spleen cells is reflected by the smaller number of viable fibroblasts after incubation for 72 h with sensitized cells, compared with those incubated with normal cells; (4) the sensitized spleen cell suspensions invariably showed a slower cell electrophoretic mobility than non-sensitized spleen cells ($P > 0.001$), the average loss of surface charge density approximates 10.6×10^3 electron charges/μ^2 cell surface. Evaluation of detailed cellular morphology is difficult in the electrostatic apparatus, thus limiting attempts to correlate cell mobilities with their morphological and functional characteristics. However, despite heterogeneity of the sensitized spleen cell populations, their electrophoretic mobilities were quite uniform.

The fall in surface charge density associated with the emergence of sensitized (plasma and pro-plasma) cells is somewhat surprising in view of the evidence to suggest that proliferating cell populations have a higher surface charge density than their resting counterparts[4]. The fall in surface charge density may be due to (1) an expansion of the cell surface, (2) re-alignment of surface ionic groups, or (3) the presence of γ-globulin on the cell surface; this possibility seems plausible in view of the fact that sensitized cells are involved in antibody synthesis, furthermore, γ-globulin has the slowest electrophoretic mobility of the plasma proteins.

In conclusion, homologous and heterologous spleen cells that have been sensitized against *L*-strain fibroblasts are cytopathic to the fibroblasts in culture. These sensitized spleen cells have a significantly lower surface charge density than normal spleen cells from control animals. This difference may be due to the presence of cytotoxic antibody γ-globulin on the surface of the sensitized cells.

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P. S. VASSAR
H. E. TAYLOR
C. F. A. CULLING

Department of Pathology,
University of British Columbia,
Vancouver

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Inhibition by Urethan (Ethyl Carbamate) of Virus Induction of Leukæmia in *C3H* Mice

URETHAN is now known to be a multipotent carcinogenic agent[1]. Its leukæmogenic action in adult mice is only slight[2], if at all[1,4]. However, it has been shown to act as an 'incomplete' carcinogen in adults in potentiating the leukæmogenic effects of total-body irradiation[3,4], and of œstrogens and polycyclic hydrocarbons[3]. More recently, urethan has been shown to exert a striking leukæmogenic effect when administered to some strains of mice shortly after birth[5-7].

In the work recorded here the effect was examined of urethan introduced during the neonatal period in the low-leukæmic *C3Hf/Lw* strain of mice and in its recently derived, genetically comparable, high-leukæmic sub-line C3Hf*/Lw, in which Moloney leukæmogenic virus (MLV) is transmitted through the mother's milk to successive generations[8,9].

Mice 24–72 h after birth received subcutaneously either a single injection of urethan dissolved in distilled water (4 per cent solution) at a dosage of 1 mg/g body-weight or the same dosage given for 4 consecutive weeks beginning in mice 24–72 h of age. Mortality in the latter group was considerable, but recovery of survivors was rapid.

Table 1 shows the results obtained in a 10-month period of observation. Low-leukæmic *C3H* mice did not develop lymphocytic neoplasms at either dosage-level of urethan. Multiple hepatomas were, however, found at necropsy in both sexes and at 10 months in both groups. Neoplasms of reticular tissues, principally lymphocytic leukæmia, occur spontaneously in the *C3Hf/Lw* strain at a frequency of approximately 10 per cent, but rarely before 12 months of age, and hepatomas occur with a moderate frequency among males, but usually beyond a year of age[10].

A striking inhibition of lymphocytic neoplasms, principally of thymic origin, was found in those high-leukæmic C3Hf*/Lw mice of both sexes given urethan at both dosage-levels. This inhibition is reflected in both frequency and latent period. Untreated controls in these groups 3*b* and 4*b* of Table 1 were either litter mates of the injected mice, housed in the same cages, or were prior or subsequent litters born to the same mothers. In those few C3Hf*/Lw urethan-treated mice which came to necropsy at 10 months of age there was found, as in the low-leukæmic sub-line, a high frequency of multiple hepatomas in both males and females. Lung adenomas were rare.

A group of *BALB/c* mice receiving 1 mg/g urethan at 24–72 h of age remained non-leukæmic and were killed at 10 months of age. No hepatomas were found, but 89 per cent had multiple lung adenomas (25/27 females and 20/24 males).

Although a strikingly enhanced susceptibility to urethan has been recorded in several stocks and inbred strains of mice given the compound parenterally[5-7], or orally[11] as

Table 1. EFFECT OF URETHAN, ADMINISTERED TO NEW-BORN MICE, ON THE FREQUENCY OF LEUKÆMIA AND OTHER NEOPLASMS

Group	Sub-line of mice	Effective No. mice	Dose of urethan	No. and % leukæmic	Age at death in days (range)	No. with hepatomas / No. necropsied	No. with lung adenomas / No. necropsied
(1)	*C3Hf/Lw* (low-leukæmic)	20	1 mg/g	0	—	15/20*	0/20
(2)	,, ,,	12	1 mg/g ×4	0	—	9/10	1/10
(3*a*)	*C3Hf*/Lw* (high-leukæmic)	24	1 mg/g × 4	8† (33%)	225 (150–300)	6/6	0/6
(3*b*)	,, ,,	79	None	66 (84%)	201 (120–270)	—	—
(4*a*)	*C3Hf*/Lw* (high-leukæmic)	18	1 mg/g ×4	1 (5·5%)	300	6/6	2/6
(4*b*)	,, ,,	25	None	13 (52%)	187 (140–234)	1/10	0/10
(5)	*BALB/c*	51	1 mg/g	0	—	0/51	45/51‡

* Multiple hepatomas in urethan-treated mice. Of 24 mice with multiple hepatomas, 15/18 were in females and 9/12 in males; 4–25 separate tumours were found in individual mice and these varied in size from 4 to 10 mm (diam.).

† All the leukæmias encountered in this work were generalized lymphocytic (stem cell) and apparently thymic in origin. No sex differences were observed in frequency or latent period among leukæmics either in those groups showing inhibition by urethan or in the control groups.

‡ 2–6 lung nodules found in individual mice and these varied in size from 1 to 3 mm (diam.).

new-borns or early in life, it is clear from the results presented here that induction of leukæmia (or other neoplasms) is not a general phenomenon but is apparently quite dependent on the genotype of the animal. In another *C3H* sub-line, for example *C3H/Bi*, given urethan parenterally at birth, lymphocytic neoplasms were encountered in 40 per cent of the mice (females only) and a pronounced sex differential was observed in hepatoma induction[12]. Male *C3H* mice of 3 different, but related, substrains have been shown previously to have an increased frequency of hepatomas when given urethan parenterally as adults[13].

C3Hf/Lw mice, though susceptible to MLV and capable of replicating and transmitting virus through successive generations, are insensitive to urethan induction of lymphocytic leukæmia. The inhibitory influence of urethan observed in the high-leukæmic *C3Hf*/Lw* sub-line signifies that the potentiation or promoting effect of urethan[3,4] may indeed also be a limited phenomenon.

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LLOYD W. LAW
ALFREDO PRECERUTTI *

National Cancer Institute,
Bethesda, Maryland.

* Lederle International Fellow from the National Institutes of Health, Buenos Aires.

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Electron Microscope Observations of Tumour Cell Cultures in the Presence of Lysozyme

IN earlier works, one of us[1] reported that lysozyme extracted from chicken egg albumen, added in suitable concentrations to human cell cultures, induced metachromatic granulations in the cell cytoplasm. Little information has so far been obtained on the nature and significance of these granulations, in spite of a number of investigations carried out to investigate their relationships with the host cell, their tinctorial properties and their reactivity to cytochemical tests.

The evidence obtained provided some help for a better outline of further work. Thus, it could be seen that lysozyme granules in cell smears, either dried in the air or fixed by heating, give a metachromatic violet reaction when stained with methylene blue or toluidine blue. Nucleic acid, lipid and polysaccharide reactions were negative. The extracellular origin of these granules was rejected. They were found to occur only in cells under metabolic and reproductive activity, and their presence would not seem to modify normal cell development.

A preliminary electron microscope investigation on lysozyme-treated cells showed the presence, in some cytoplasm vacuoles, of small structureless heaps which probably correspond to the granules observed by the light microscope[2].

Information obtained by a fuller investigation of such processes is reported here, special consideration being given to their relationship with endocytoplasmic structures. Hep 2 strain of epithelial origin, kindly supplied by Dr. A. Fjelde (State Serum Institute, Copenhagen), was used.

Cell colonies were grown on coagulum from chicken plasma and embryonal extract, using 199 or Gey's culture medium to which was added human and calf serum.

Lysozyme chloride was added to the cells being tested, in the proportion of 3 mg/ml. nutrient culture medium. 24–36 h later, after the appearance of lysozyme granules had been proved by the light microscope, colonies were removed, fixed with osmic acid and embedded in metacrylate, following the usual procedures. Sections 400 Å thick were made and examined by electron microscope.

Before describing the changes induced by lysozyme in Hep 2 cells, we should consider the appearance of cytoplasm membranes in untreated cells, since the aforementioned granulations seem to develop on these structures.

In cytoplasms of untreated cells, changes due to collections of multilayered membranes are regularly observed. These show a concentric appearance, are onion- or ring-shaped, or arranged like lamellar coils, grouped at the periphery of a large vacuole in which they are contained (Fig. 1).

Fig. 2 shows the same Hep 2 strain after treatment with lysozyme chloride under the aforementioned technical procedures: the homogeneous small heaps, oval or round-shaped, of different sizes, weakly opaque, contained in the vacuoles or membranous formations, may be identified with lysozyme granules. These granules are closely related to the multilamellar membranous structures from which they seem to originate following a homogenization process induced by lysozyme.

Among the proposed views on the nature and genesis of the granules under discussion, that of a direct action of lysozyme on some constituents of the endocytoplasmic reticulum deserves consideration. This hypothesis would suggest, rather than a simple linkage between proteins of different electric charge, an attack of the lysozyme on cell membranes through an enzymatic mechanism of homogenization of the fibrillar structure, resulting in the formation of granules.

If these experimental observations are confirmed, lysozyme will acquire a significant role in the examination

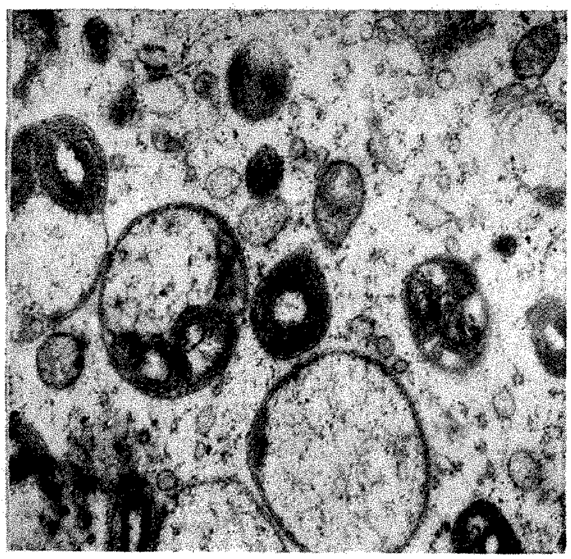

Fig. 1. × 35,500

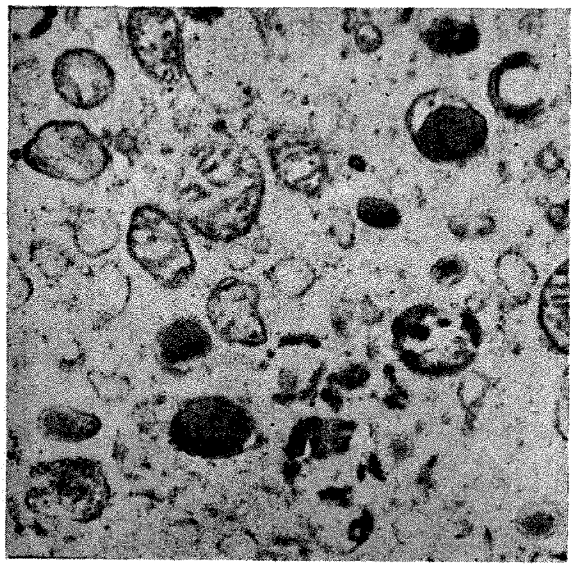

Fig. 2. × 20,500

of the biochemical nature of cytoplasm membranous formations and of interference with the reactive processes of such membranes during viral infections and other pathological conditions of cells.

D. CALLERIO-BABUDIERI
C. CALLERIO

Istituto di Ricerche A. Fleming, SPA,
Milano.

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Effects of *p*-Dimethylaminoazobenzene on the Fine Structure of Rat Liver Cells

INTEREST is at present being shown in the changes which carcinogenic agents induce in the fine structure of liver cells. When substances such as thioacetamide[1], dimethylnitrosamine[2] and ethionine[3] are administered to rats, a constant and early change is a hypertrophy of the smooth elements of the endoplasmic reticulum. In some cells there is also the formation of whorled structures composed of smooth paired membranes. So far as *p*-dimethylaminoazobenzene is concerned, the only investigations reported so far are those of Porter and Bruni[4], and Hübner[5]. These workers described a hypertrophy of the endoplasmic reticulum, but made no mention of whorl formation.

The animals used in the work recorded here were albino male rats of a local strain which had been fed maize containing 1 per cent *p*-dimethylaminoazobenzene. They were killed after 7 months of feeding, by which time several hepatocellular and cholangio-carcinomas had developed. Non-neoplastic portions of liver were fixed in Palade's fixative and embedded in methacrylate. Localization of the changes in relation to the portal tracts was facilitated by examination of thick sections stained by toluidine blue.

It was found that the endoplasmic reticulum had been transformed into a honeycombed mass of vesicles of varying sizes (Fig. 1). They did not contain any electron-dense material and were clearly delimited by smooth cytoplasmic membranes. At random points the membranes were more densely crowded together, and here varying stages of whorl formation were evident (Fig. 2). These structures consisted of circularly arranged paired membranes surrounding an inner zone which contained faintly electron-dense material or poorly outlined membranes. Up to ten pairs of membranes were counted in a single whorl, at the periphery of which the membranes merged with those lining the vesicles. Although most of the mitochondria were normal, some were irregularly shaped, while others were swollen, granular and possessed incon-

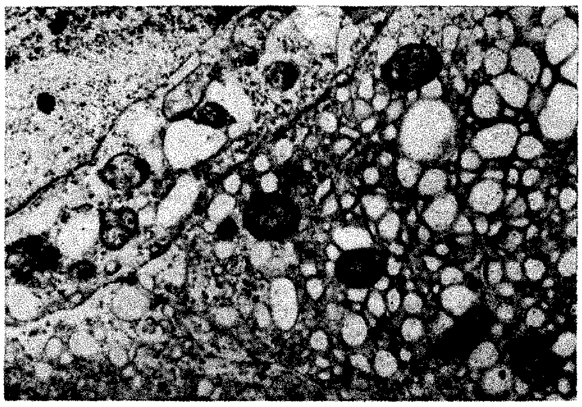

Fig. 1. The endoplasmic reticulum has been converted into a mass of vesicles lined by smooth membranes. Portions of three cells and a nucleus are shown

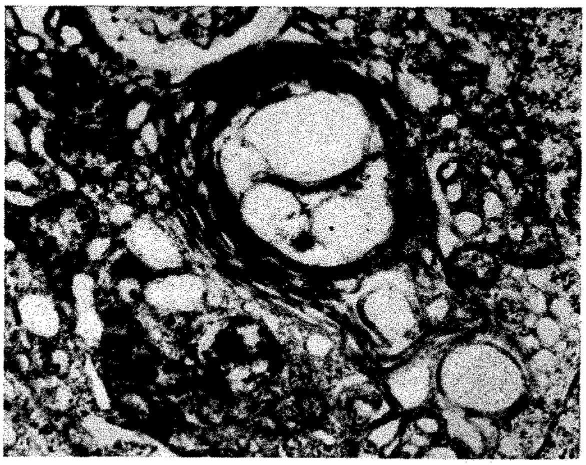

Fig. 2. An area of whorl formation. The paired membranes appear to be continuous with those lining the vesicles. A few irregularly shaped mitochondria are present

spicuous cristæ. Occasional nuclei had a very irregular and crenated outline, but no gross nucleolar abnormalities were noted. Some cells contained many dense bodies and lipid inclusions. Examination of the thick sections revealed that all the cells studied were periportal in situation.

The changes we have observed in the endoplasmic reticulum appear similar to those following the administration of thioacetamide, dimethylnitrosamine and ethionine, but as yet we do not know when they first appear and what proportion of cells exhibit them. It is of interest that four different hepato-carcinogens should produce similar alterations in the fine structure of cells. In attempting to evaluate the significance of the hypertrophy of the endoplasmic reticulum and whorl formation in relation to the mechanisms of carcinogenesis, their possible non-specificity should be borne in mind. In this connexion, laminated aggregates of membranes followed the administration of DDT to rats[6], while whorled structures appeared in mouse liver cells infected with the virus of Rift Valley fever[7]. In the latter case, however, the membranes were studded at regular intervals by dark transverse bands, thus differing from those induced by carcinogenic agents. Hypertrophy of the endoplasmic reticulum without whorl formation has also been described in starvation-refeeding experiments[8] and after feeding DL-β-3-thienylalanine[9].

A. H. TIMME

Department of Pathology,
University of Cape Town Medical School,
Observatory,
South Africa.

L. G. FOWLE

Department of Physics,
University of Cape Town,
Rondebosch,
South Africa.

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Phenacetin-induced Papillary Damage in Experimental Animals

DURING the past ten years many reports have appeared describing the association of chronic interstitial nephritis and renal papillary necrosis with chronic ingestion of phenacetin-containing analgesic tablets[1–5].

Although there is overwhelming circumstantial evidence in favour of such an association, some authors still doubt whether there is a causal relationship between phenacetin ingestion and renal disease[6,7]. In an attempt to clarify the problem large doses of phenacetin have been fed to animals over prolonged periods of time; but these experiments have yielded equivocal results. Thus, Studer *et al.*[8] found little change in the kidneys of rats fed phenacetin and this was later confirmed in rabbits[9] and guinea pigs[10]. Thölen *et al.*[11] described interstitial nephritis in mice, but these animals were prone to spontaneous urinary infection with *Closiella muris*. Similarly, rabbits fed on phenacetin and afterwards injected intravenously with *Escherichia coli* developed interstitial nephritis[12], but this finding has not been confirmed[13]. Angervall *et al.*[14] have shown that phenacetin and *N*-acetyl-*p*-aminophenol fed to rats over a long period of time increased their susceptibility to the development of hæmatogenous interstitial nephritis. Recently, Eisalo and Talanti[15] have described changes of chronic interstitial nephritis in rats given 100 mg of phenacetin daily for one month. The lesions were associated with disappearance of enzyme activity in the renal tubular cells. However, as yet there is no published experimental work describing the occurrence of papillary necrosis in animals fed on phenacetin preparations.

We have administered tablets containing phenacetin alone and others consisting of aspirin, phenacetin and caffeine (A.P.C.) to groups of Wistar rats over fifteen

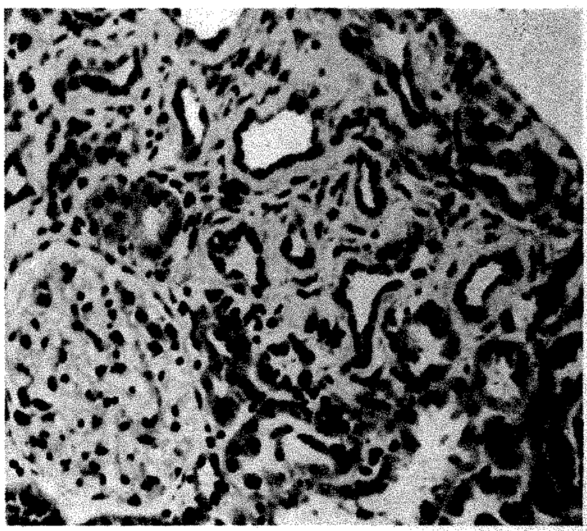

Fig. 1. Section through the renal cortex of a rat fed A.P.C. tablets for a period of seven months. There is interstitial fibrosis, tubular basement membrane thickening and tubular atrophy (hæmatoxylin and eosin, × 600)

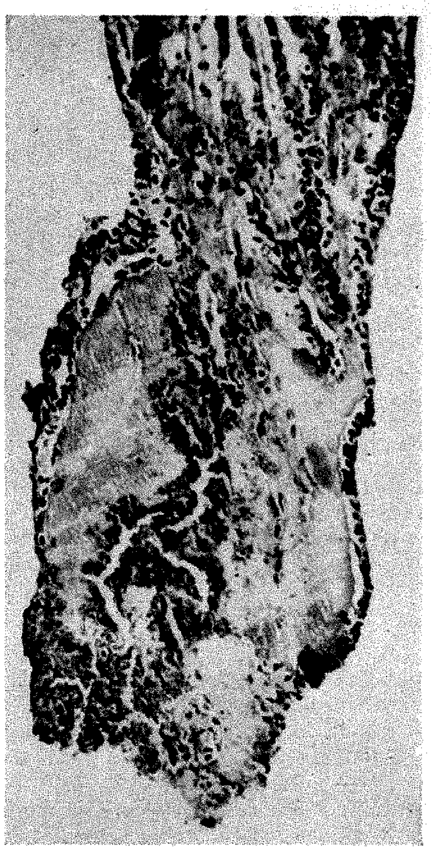

Fig. 2. Section through the renal papilla of a rat fed phenacetin tablets for a period of seven months. The papilla is distended with extensive hæmorrhage (hæmatoxylin and eosin, × 400)

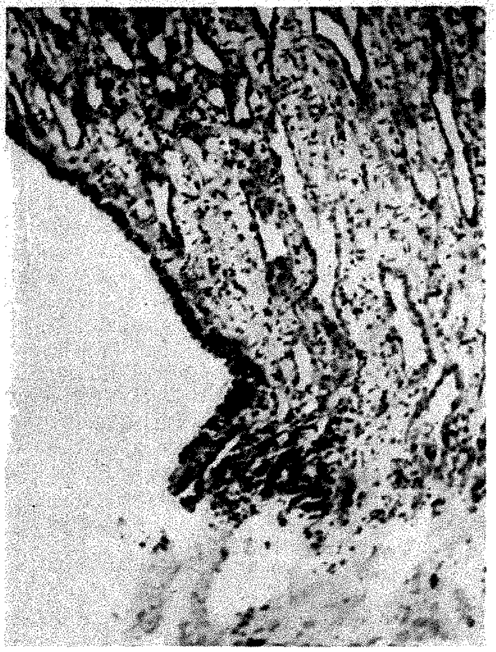

Fig. 3. Section through the renal papilla of a rat fed A.P.C. tablets for a period of eight months showing total papillary necrosis with minimal cellular infiltrate (hæmatoxylin and eosin, × 300)

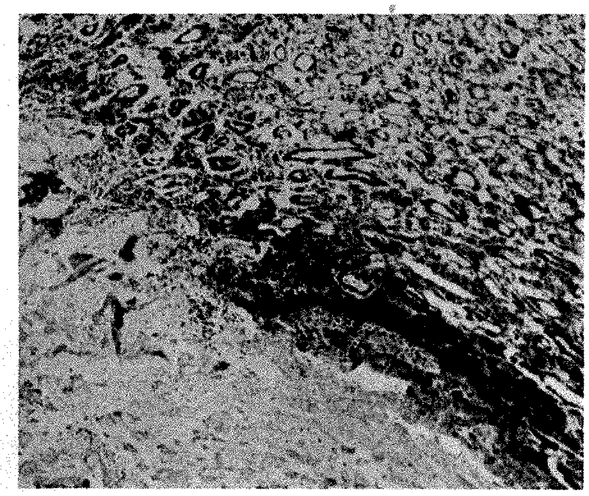

Fig. 4. Section through the renal papilla of a rat fed A.P.C. tablets for a period of 14 months. There is total papillary necrosis with a marked granular and non-granular cell infiltrate (hæmatoxylin and eosin, × 120)

months. The tablets were crushed in a mortar and mixed with fine rolled oats. The total intake of phenacetin by each rat varied from 1,000 to 14,000 mg.

Three types of lesions were observed in the experimental animals:

(*a*) An interstitial fibrosis with thickening of the tubular basement membrane and tubular atrophy occurred in both the phenacetin and A.P.C. groups (Fig. 1). Although this change did occur in control animals, it was quantitatively and qualitatively less marked than in the other two groups.

(*b*) Severe hæmorrhage into the renal papilla occurred in 20 per cent of the rats in the A.P.C. and phenacetin groups (Fig. 2). A small area of hæmorrhage occurred in one control animal.

(*c*) Papillary necrosis was observed in two rats. The first had been fed A.P.C. tablets for 8 months and the renal papilla was totally necrosed. There was minimal cellular infiltrate and bacteria were not present (Fig. 3). The second rat, which had been fed A.P.C. tablets for 14 months, showed papillary necrosis in which there was both a granular and non-granular cell infiltration (Fig. 4). There were scattered foci of Gram-negative bacilli present. No such changes were observed in the control group.

The production of papillary damage in rats fed on phenacetin provides convincing support for causal relationship between prolonged phenacetin abuse and renal damage.

C. ABRAHAMS
A. H. RUBENSTEIN *
N. W. LEVIN †

Departments of Pathology and Medicine,
University of Witwatersrand Medical School,
Johannesburg, South Africa.

* Senior research bursar, Cardio-Pulmonary Unit of the Council for Scientific and Industrial Research.

† Present address: Department of Medicine, University of Illinois College of Medicine, Chicago 12.

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IMMUNOLOGY

Transfer to Neonatal Chickens of the Primary Antibody Response initiated *in vitro*

THERE are numerous reports on the formation of antibody by neonatal animals which had been injected with homologous spleen and lymph node cells mixed *in vitro* with bacterial antigens[1–3]. Transfer to neonatal recipients of a primary response to serum protein antigens under similar conditions has often met with failure or with poor antibody responses[3]. The latter has been attributed either to the unsuitability of the neonatal environment for the cells[4,5] or to the possibility that soluble proteins have a longer induction period, and as a consequence the homograft reaction results in damage of the cells before antibody is demonstrated[6]. To obviate this, Holub and Říha utilized diffusion chambers which protected the transferred adult cells from the rejection mechanism. The present report summarizes further attempts to initiate an antibody response by *in vitro* mixing of chicken spleen cells with either bovine serum albumin (BSA) or with BSA–anti-BSA complexes, and subsequent transfer to neonatal chickens.

Tissue culture Mixture 199 (pH 7·0) containing heparin and penicillin was used throughout this work. The procedure for preparation of spleen homogenates was similar to the method described by Papermaster[8]. Spleens were obtained from adult Austra white hens and homogenized in a Potter tissue grinder. The cells were suspended in the various test solutions and diluted to give concentrations of $1 \times 10^8 - 1 \times 10^9$ cells/ml. The test and control cells were incubated at 37° C for 30 min, and in some experiments the cells were washed 3 times with Mixture 199 after incubation Neonatal cockerels or pullets (12–36 h old) were given a single intraperitoneal injection of 1 0 ml of the cell suspensions, and were bled 6 days later. The sera were heated at 56° C for 30 min, adsorbed with sheep red blood cells, and assayed for antibody by the tanned cell method[9] Sera from the donors had no anti-BSA titres, and the antibody responses of recipient chickens were found to be specific as determined by hæmagglutination inhibition tests

In preliminary experiments, cells which had been incubated with concentrations of BSA higher or lower than 1 0 mg gave poor responses, and no antibody responses were demonstrated in chickens which were injected with either 1 0 or 0 1 mg of BSA without cells In subsequent experiments 1 0 mg of BSA was used as the sensitizing dose, and the results are summarized in Table 1 Various controls were included and are summarized as follows Only 2 of 51 chickens produced hæmagglutinin titres of 1 10 following injection of 1 0 mg BSA (no cells) Cells which had been incubated with BSA followed by disruption by 5 cycles of freezing and thawing failed to form antibody Responses obtained with washed and unwashed sensitized cells were similar Incubation of cells with BSA and chicken anti-BSA antibody complexes (Exp 2 and 4) did not produce titres higher than cells incubated only with antigen Small amounts of antibody appeared in recipients injected with cells which had been incubated with high-titre chicken antibody and washed In Exp 1, high titres were produced by cells which had been incubated with primary chicken anti-BSA antibody containing a high concentration of high molecular weight (19*S*) hæmagglutinin[10], and then followed by incubation with BSA and washing We were unable to repeat the production of these high titres in subsequent experiments, and the results suggest that either a secondary response was elicited, although the donor sera were negative for anti-BSA, or that the antibody was passively transferred

The antibody responses to cells exposed only to antigen were erratic, and this might be related to the sex of the recipients In 3 experiments with cockerels no measurable titres were obtained (1 10 or above), however, there were always some chickens which produced antibody in experiments in which pullets were used Apparently antibody synthesis was initiated *in vitro* rather than as a result of active sensitization of the recipients to antigen transferred with cells Fifty-five chickens (pullets and cockerels) were injected with antigen-exposed cells (washed and unwashed), and 16 chickens or 19 per cent produced titres of 1 10 or greater, whereas only 2 or 4 per cent of 51 chickens injected with BSA produced titres of 1 10 Furthermore, 64 per cent (9/14) of the pullets produced antibody in response to cells plus antigen, and no titres were detected in 13 pullets injected with antigen alone Whether a difference in the degree of maturity between neonatal sexes exists, and, consequently, pullets may represent a more effective host for potentially antibody-producing cells, remains to be clarified

Table 1 ATTEMPTS TO ELICIT A PRIMARY ANTIBODY RESPONSE IN NEONATAL CHICKENS WITH PASSIVELY TRANSFERRED SPLEEN CELLS SENSITIZED *in vitro* WITH BOVINE SERUM ALBUMIN (BSA)

Treatment of spleen cells*	Experiment No 1 (♀)	 2 (♂)	 3 (♀)	 4 (♀)	 5 (♀)	 5 (♂)
Cells + BSA–anti-BSA complexes, incubated, washed 3 times		0 (7)†		0 (1) 10 (1) 80 (2)		
Cells + BSA–anti-BSA complexes, incubated, unwashed		0 (7)		10 (2) 20 (2) 40 (1) 80 (2)		
Cells + BSA–anti-BSA complexes, incubated, washed, disrupted by freeze-thaw					0 (1) 5 (1)	
Cells + anti-BSA, incubated, washed 3 times		0 (7)		5 (1) 10 (1)	5 (2) 20 (1)	
Cells + anti-BSA, incubated, washed 3 times, BSA added, incubated, washed 3 times	640 (1) 10,240 (1) 20,480 (1) 40,960 (1) 320,000 (1)	0 (7)	40 (1) 80 (1) 160 (2) 320 (2) 1,280 (1)	0 (3) 20 (1) 40 (1) 80 (1) 160 (1)	5 (3) 10 (1)	5 (2) 10 (2)
Cells + BSA, incubated, disrupted by freeze-thaw					0 (3)	
Cells + BSA, incubated, unwashed		0 (6)		0 (2) 10 (1) 40 (1) 80 (1)	0 (1) 5 (2) 40 (1)	10 (1) 20 (2)
Cells + BSA, incubated, washed 3 times					10 (2) 20 (2) 80 (1)	0 (34) 5 (4) 10 (1) 20 (2) 40 (1)
1 mg BSA (no cells)	0 (3)	0 (5) 5 (1)			0 (6) 5 (2)	0 (29) 5 (1) 10 (2)

*Neonatal chickens bled 6 days following transfer of $1 \times 10^8 - 1 \times 10^9$ cells

† Reciprocal of hæmagglutinin titre, numbers in parentheses refer to number of chickens

Evidence for the high molecular weight nature of the antibody synthesized in neonatal chickens was obtained by sucrose density gradient ultracentrifugation[10] Hæmagglutinating activity was associated with the 19*S* globulin fractions, thus yielding evidence that a primary response was induced[10,11]

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EUGENE F GOLD *
ALBERT A BENEDICT †

Department of Bacteriology,
University of Kansas,
Lawrence, Kansas

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* Present address Department of Chemistry, University of Indiana, Bloomington, Indiana

† Present address Department of Microbiology, University of Hawaii, Honolulu, Hawaii

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Preparation of *Vipera palestinae* Antineurotoxin using Carboxymethyl-cellulose-bound Neurotoxin as Antigen

THE separation and characterization of two distinct toxic components of *Vipera palestinae* venom, hæmorrhagin and neurotoxin, by the use of electrophoretic and chromatographic methods, have been previously reported[1,2] Numerous attempts made in this laboratory over a number of years to prepare potent viper antineurotoxin by using neurotoxic fractions for immunization have been unsuccessful[3] Superimposed immunization with neurotoxic fraction of rabbits previously immunized with whole venom did raise the antineurotoxin titre but not to a sufficient level[4]

It was observed, during investigations of column chromatography fractionation of *Vipera palestinae* venom, that, while the neurotoxin elutes from a 'DEAE-cellulose' column, it is firmly bound to a carboxymethyl-cellulose column[2] Binding of sea snake toxin to the latter resin was recently reported by Carey and Wright[5] The present report deals with the effect of carboxymethyl cellulose (CMC) on the toxicity and immunogenic activity of the neurotoxic fraction of *Vipera palestinae* venom

The neurotoxin-binding capacity of CMC was tested using CMC in a non-soluble form, prepared according to the method of Peterson and Sober[6] from Whatman ashless standard cellulose powder, mesh 325 (H Reeve Angel and Co , Inc , New York) The CMC preparation was suspended in phosphate buffer 0 005 M, *p*H 6 8 The neurotoxic fraction was an eluate from a 'DEAE-cellulose' chromatography column[2] The amount of neurotoxin which combines with CMC was measured by adding increasing lethal doses of the neurotoxic fraction to constant amounts of CMC Following moderate centrifugation, the supernatant was titrated for toxicity by intraperitoneal inoculation of mice weighing 20 g, and the LD_{50} calculated according to the method of Reed and Muench[7] It was found that 1 g of insoluble CMC binds up to 390 LD_{50} of the neurotoxic fraction

Antisera against the CMC-bound neurotoxin were prepared in rabbits As controls, antisera prepared in rabbits by the use of neurotoxic fraction alone were utilized Since the insoluble form of CMC could not be used for immunization of rabbits, the neurotoxic fraction was bound to CMC in soluble form Toxicity tests of this CMC-neurotoxin complex proved that 300 LD_{50} of added neurotoxin was detoxified by 1 g of soluble CMC

Two immunization experiments were carried out using 3 rabbits each The antigen was mixed in equal parts with Freund's complete adjuvant prior to inoculation Primary immunization was carried out by two intradermal injections into two foot-pads each, within one week, followed after an interval of ten days by a single subcutaneous injection The amounts of CMC-neurotoxin complex used for these two primary inoculations were equivalent to 2 and 17 mouse LD_{50} respectively The rabbits were bled one week following the subcutaneous inoculation Two months later a subcutaneous 'booster' injection was given, equivalent to 37 5 LD_{50} of CMC-bound neurotoxin One week after the booster the animals were re-bled from the heart Pre-, post-primary-, and post-booster immunization sera were tested for their capacity to neutralize the neurotoxic fraction Varying amounts of the respective sera, pooled from 3 rabbits, were mixed with different amounts of LD_{50} The mixtures were inoculated intraperitoneally in mice and the protective effect compared with that of antisera prepared against neurotoxin alone It will be seen in Table 1 that the sera of rabbits immunized by CMC-bound neurotoxin contained an appreciable amount of specific antibody capable of protecting mice against the neurotoxic fraction The titre of antineurotoxin increased significantly following the booster inoculation The antiserum was found to neutralize specifically the neurotoxic component of viper venom and not its hæmorrhagin It did not neutralize whole viper venom, the test animals dying with hæmorrhage

The results obtained in the present work show that large amounts of neurotoxin can be detoxified by binding it to a suitable resin The method used made it possible to strengthen the immunogenic potency of viper neurotoxin to such a degree that minute amounts of resin-bound neurotoxin elicited a high antibody response In view of the low molecular weight shown to be characteristic for several venom neurotoxins[8–10], the increase in immunogenic activity attained by resin binding may be a reflexion of the increase in molecular weight of the antigen[9]

Table 1 NEUTRALIZING POWER OF RABBIT SERA IMMUNIZED WITH CMC-BOUND NEUROTOXIN

Antigen used for immunization	Sera	No of LD_{50} of neurotoxic fraction neutralized/ml serum
CMC-bound neurotoxin	Pre-immunization	0
	Post-primary	9
	Post-booster	30
Neurotoxic fraction alone	Pre-immunization	0
	Following numerous primary and booster inoculations	6

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CHAYA MOROZ
NATAN GOLDBLUM
ANDRE DE VRIES

Rogoff Medical Research Institute,
Department of Experimental Biology,
Tel Aviv University and the Labor Sickfund,
Beilinson Hospital, Petah Tikva,
and Department of Preventive Medicine,
Hebrew University—Hadassah Medical School,
Jerusalem

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Formation of Antibody in Tissue Culture : Effect of Certain Mucopolysaccharides and Hexosamine

RECENT investigations have given insight on the mechanism of antibody synthesis *in vitro*[1–3] Fishman[4,5] has further developed *in vitro* systems in which a primary induction of antibody was achieved The reports of Alfred and Pumper[6] and of Ozzello[7] have described the use of certain mucopolysaccharides and other non-protein substances for growth of primary isolations and established tissue culture cell lines As a continuation of these investigations, the present communication aimed at testing the ability of two mucopolysaccharides and *N*-acetyl-*d*-glucosamine to promote the 'growth' and antibody formation by tissue cultures of chicken spleen fragments It is obvious that an *in vitro* system that would permit a clearly defined examination of the antibody induction process and the sequence of biochemical events which occur during the differentiation of cells into antibody-forming clones is a major problem

Adult chickens (2–3 months old) were given a single intravenous injection of 1 0 ml (5×10^8 organisms of *Salmonella paratyphi B* vaccine) After 24–28 h, the spleen was removed, cut into fragments (3 mm³, 15–20 mg each), and placed on stainless steel wire mesh strips (4 cm long, 1 5 cm wide) Three fragments were placed on each strip, each overlaid with a drop of 1 per cent agar[8], and allowed to stand at room temperature for 15–20 min The agar-embedded fragments were then cultured in 17-mm roller tubes in 2 0 ml of each of the following media (*a*) synthetic medium (*M*-199) alone, (*b*) *M*-199 + 50 per cent horse serum , (*c*) *M*-199 supplemented with either chondroitin-sulphate, hyaluronic acid, or *d*-glucosamine at varying concentrations The cultures were kept for 9 days during which period the growth fluids were collected, replaced with fresh medium, and the level of antibody was measured The antibody assay procedure was a measure of the agglutinating capacity of the growth fluid by 0 02 ml (1×10^7 organisms) of the original vaccine according to Corvazier and Cochrane[9]

An average of the results of 10–12 experiments is shown in Table 1 Medium-199 (minus purines, pyrimidines, and nucleotides) was nutritionally insufficient for antibody formation by cultured spleen tissue from sensitized chickens However, when this basal medium was supplemented by serum (group 2), chondroitin-sulphate (group

Table 1 COMPARATIVE STIMULATORY ACTIVITY OF MUCOPOLYSACCHARIDES, *N*-ACETYL *d*-GLUCOSAMINE AND SERUM ON THE ANTIBODY-FORMING CAPACITY OF CULTURED SPLEEN TISSUE

Culture group	Test material in *M*-199	Concentration of test material	Reciprocal of agglutination titre Days 0	 1	 3	 6	 9
1	*M*-199 alone	—	0	0	24	0	0
2	Serum	50 per cent v/v	32	128	1,024	4,096	256
3	Chon-SO$_4$	1 0 mg/ml	0	4	64	128	4
4	Hyaluronic acid	1 0 ,,	0	16	64	64	0
5	*d*-Glucosamine	2 0 ,,	0	4	128	256	32
6	,,	1 0 ,,	0	4	128	512	32
7	,,	0 5 ,,	0	4	4	16	4
8	,,	0 25 ,	0	0	4	16	0
9*	Serum	50 per cent v/v	32	32	32	32	32

* Control Spleen fragments from non-injected chickens

3) hyaluronic acid (group 4), or *d*-glucosamine (group 5), each of these mixtures permitted the biosynthetic processes of antibody formation It was found that there was a limiting concentration of each of these supplements below which antibody formation decreased or disappeared The chemically defined mixture of *M*-199 + *d*-glucosamine was of interest since it appeared to have a stimulatory activity higher than that of chondroitin-sulphate or hyaluronic acid and possibly analogous to the activity of the serum component

It was further demonstrated that there was a lag period in antibody formation in a nutritionally optimal medium and antibody synthesis reached a peak in 6 days and continued in some cases for longer periods Fig 1 shows a comparison of the antibody-forming capacity of tissue cultivated in various media as a function of incubation time Control cultures, containing spleen tissue from non-injected chickens, grown in each of the media, showed the absence of antibody formation These controls also demonstrated that the low agglutinating antibody titre to *Salmonella paratyphi B* normally found in the serum of chickens (1 8) before injection of antigen was not detectable in the cultured spleen fragments or saline soluble extracts of homogenates of the spleen tissue prior to cultivation

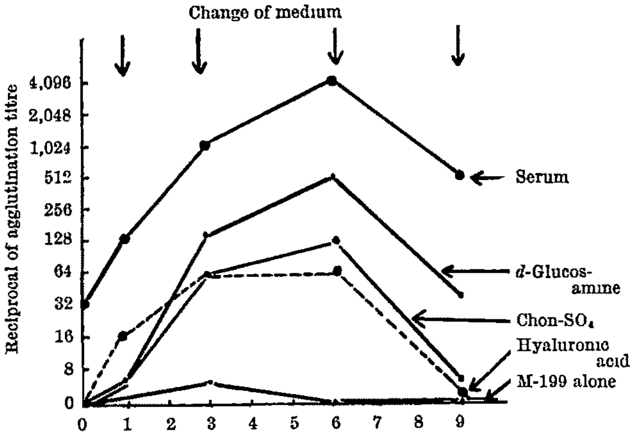

Fig 1 Response of cultured spleen fragments to serum (50 per cent), mucopolysaccharides (1 mg/ml), and D-glucosamine (1 mg/ml), each dissolved in *M*-199

It was desirable to know if the antibody material which was produced in the synthetic mixture of *M*-199 + glucosamine could be separated from low molecular components of the growth medium and recovered in a reactive form (agglutinating ability) This was accomplished by applying a sample of freshly collected culture fluid to a 'Sephadex *G*-50' column which had been equilibrated with 0 005 M sodium phosphate buffer at *p*H 6 8, and washing the column with the same buffer At least 75 per cent of the agglutinating activity was eluted as a sharp peak in the initial column volume This manipulation afforded a simple way for obtaining a concentrated antibody-containing solution directly from the culture tube

These preliminary results indicate that the requirements for antibody formation *in vitro* need not be a complex protein-containing medium but that simplified solutions can establish the proper physiological conditions for this process The use of chemically defined glucosamine medium gives rise to a relatively clean environment in which one might study the nature of antibody precursors and of the finally synthesized antibody protein The fact that chondroitin-sulphate and hyaluronic acid promote antibody-forming activity in this system suggests that the hexosamine residue of these materials might be the active site However, the viscosity of the culture medium as affected by these mucopolysaccharides cannot be disregarded as a possible essential physiological condition It should be noted that the agar-overlay which was used to hold the spleen fragment on the wire mesh may have served another purpose in aiding in the maintenance of a certain degree of organ tissue organization Such organization may be necessary for the successful continuation of antibody formation which was induced *in vivo* Present experiments are directed toward the elucidation of the relationship between the changes in cell types of these spleen fragments at different time intervals of culture and the possible role of the nuclear RNA fraction of the cell types associated with the sequence of antibody synthesis

L J ALFRED*
P CORVAZIER
P GRABAR

Service de Chimie Microbienne,
Pasteur Institute, Paris

---

* Post-doctoral Fellow (9717-*C*2), National Cancer Institute, National Institutes of Health, Bethesda, Maryland Present address Section of Cell Biology, Weizmann Institute of Science, Rehovoth, Israel

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ANATOMY

Influence of Trypan Blue on Resorption of Rat Embryos

TERATOGENIC agents have been found to cause an increase in fœtal mortality as well as developmental malformations In a series of 206 embryos between 10½ and 14½ days of gestation from Wistar rats injected with 1 ml of a 1 per cent solution of trypan blue at 8½ days of pregnancy, 45 embryos, or 21 8 per cent, were found to be undergoing resorption These resorption products were examined and eight of them were serially sectioned at 5μ for histological investigation A number of sites of complete resorption were observed, but they were not counted, so that the total number of resorptions was considerably in excess of the 45 products examined Even so, this proportion of resorptions (21 8 per cent) is considerably greater than the 6 0 per cent of resorptions recorded by Beck *et al* [1] for a control group of Wistar rats, although much lower than the total resorption rate (56 2 per cent) recorded for rats injected with 1 per cent trypan blue in the same experiment

On gross examination, the resorption products were seen to be considerably shrunken while retaining a recognizable shape in the early stages Later resorption products gradually lost their embryonic shape and merged into the surrounding extra-embryonic tissues These embryos were more brittle than usual and the tissues flaked easily In advanced cases the amniotic secretion had formed a white gel, although at earlier stages there was amniotic fluid present and a white deposit on the surface of the embryos particularly in the depressions between the limb buds and the body wall The resorption products and their chorionic vesicles were invariably smaller than other embryos of the same litter Litter mates which were not resorbing showed a variation in size, some being retarded in general development with no other apparent deviation from the normal, while larger embryos displayed an abnormal heart structure

Microscopically the resorption products showed little evidence of the normal architecture of the embryo and displayed an irregular, cellular mass comprising macrophages, nucleated red blood cells and nuclear debris Many of the red cell nuclei had lost their surrounding cytoplasm and most of the nuclei were pycnotic. There were also areas of structureless coagulum

It has been suggested recently[2] that fœtal death is a secondary effect of the malformations produced by trypan blue The findings in the investigation reported here do not support this view Invariably the embryos which showed even the slightest degree of resorption were smaller than their litter mates but in the absence of resorption the smallest embryos did not display the greatest degree of malformation and usually showed no evidence of abnormal development apart from the retardation in growth

The extent to which the mammalian embryo relies for survival in the uterus on the development of its own tissues is very small indeed The main requirement is for adequate umbilical vessels and conducting vessels within the embryo, and these are not generally described as being affected by teratogenic agents, while abnormalities of the heart and great vessels are commonly found[3-5]. Indeed development of the embryo may continue following decapitation[6,7]

The resorption of rat embryos does not necessarily require the presence of a teratogenic agent such as trypan blue It is a non-specific phenomenon It has been the experience of the staff of the animal department of the Otago Medical School that complete resorption of whole litters may occur following rough or inordinate handling of the pregnant dam or without any obvious cause It is also known to occur in crowded conditions Such resorption may occur quite late in the pregnancy at a stage where differentiation (as opposed to growth) could be expected to be virtually completed

Trypan blue increases the resorption rate in pregnant rats by a mechanism which appears to affect all the embryonic tissues and the amniotic fluid, but this effect does not seem to be dependent on, or even specifically related to, developmental malformations other than that of growth retardation

The malformations in this series of rats will be reported at a later date

W N ADAMS SMITH*

Anatomy Department,
University of Otago Medical School

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* Present address Department of Human Anatomy, University of Oxford

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RADIOBIOLOGY

Production of Hydrogen Atoms in Teeth by X-irradiation

THE purpose of this communication is to report several electron magnetic resonance observations in human teeth *in vitro* The sample was a small section of the upper left incisor tooth of a 7-year-old female child The sample volume was 1–2 mm^3 Electron magnetic resonance spectra were obtained at the K-band (24 Kmc) with a high sensitivity electron magnetic resonance spectrometer using 100-kc/s field modulation and a cylindrical cavity operating in the TE_{011} mode The sensitivity of this spectrometer is approximately 10^{12} $(\delta H)^2$ spins, where δH is the resonance line-width in gauss

An initial search in the unirradiated material at room temperature disclosed no paramagnetic resonances in the region 3,300–10,200 gauss Since the $g=2$ value of magnetic field for the operating frequency was 8,500 gauss, this corresponds to a scan of g value from 5 2 to 1 7. respectively This means that there is no single paramagnetic species in the specimen which exceed the concentration 10^{12} $(\delta H)^2$ and the g value of which lies in this region

X-irradiation was afterwards performed in air at room temperature for 10 min with a tungsten target tube operating at 50 kV and 50 m amp and with the sample placed 4 cm from the target Immediately following irradiation the intense electron magnetic resonance spectrum shown in Fig 1*a* was observed at room temperature Fig 1*b* is the spectrum in the same sample 2 5 days following irradiation During this interval the specimen was maintained in air at room temperature Fig 1*c* is the integral of the central portion of Fig 1*b* and represents the absorption curve The spectra exhibit three salient features

(1) A broad line centred at $g=2$ 003 and approximately 20 gauss wide between the inflexion points A comparison of Figs 1*a* and *b* shows that this line has decreased by about a factor of 6 during the intervening time

(2) A 'Pake' shape[1] line the absorption curve maximum of which lies at $g=2$ 0004 This line is easily recognized in Fig 1*c* and has a full width of 20 gauss The signal intensity is essentially undiminished during the 2 5-days interval

(3) A weak doublet centred at $g=2$ 0029 ± 0 0003, corrected for second-order effects, and a splitting of 498 ± 2 gauss The width of each component is 5 gauss and the signal diminished by about a factor of 2 over the 2 5-days interval The spectrum was observed at 4 2° K with no change in shape The splitting of the doublet increased slightly to 502 ± 2 gauss However, the electron relaxation time at 4 2° K is longer than 0 1 sec

If the spin Hamiltonian of a paramagnetic species contains terms involving tensor quantities, for example, hyperfine and quadrupole tensors, the species will exhibit broad electron magnetic resonance lines of complex shapes[2] in a polycrystalline or amorphous material The lines near $g=2$ are of this type Identification of spectra 1 and 2 is therefore highly problematical Spectrum 2 probably arises from a defect having an anisotropic g tensor and containing only nuclei with zero spin Considering the chemical composition of teeth, this unpaired electron may be associated only with the calcium and/or oxygen nuclei Similarly, identification of spectrum 1 is not attempted at this time

Several conclusive arguments can be made for the identification of the doublet in spectrum 3 as hydrogen atoms A doublet electron magnetic resonance spectrum indicates that the unpaired electron in this defect interacts with only one nucleus with nuclear spin equal to ½ Wide-line nuclear magnetic resonance spectra on this sample show that large concentrations of both hydrogen-1 and phosphorus-31 are present No fluorine-19 line was observed indicating only a trace amount is possible These are the only spin ½ nuclei which are likely to be present in more than trace concentrations The observed splitting of 500 ± 2 gauss and the observed g-value of 2 0029 ± 0 0003 are in excellent agreement with the free atom values[3] of 508 gauss and 2 0023 Deviations of this

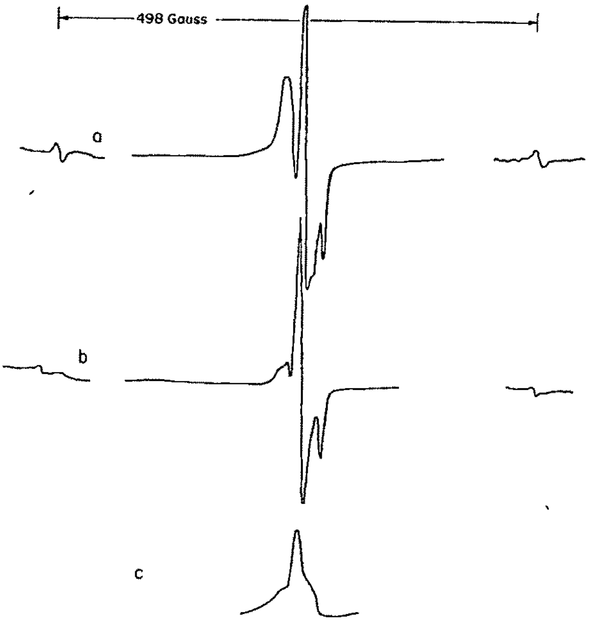

Fig 1 Electron magnetic resonance spectrum in X-irradiated tooth at room temperature *a*, First-derivative of the absorption immediately after irradiation, *b*, first-derivative of the absorption 2 5 days after irradiation, *c*, absorption curve corresponding to the central portion of *b* obtained by electronic integration Spectrometer gain was reduced by a factor of 10 in the central portions of *a* and *b*

magnitude and even greater have often been measured for trapped hydrogen atoms in a solid matrix[4]

The small width and symmetric shape for the doublet lines indicate that both the *g*-value and electron-nuclear hyperfine interaction are isotropic This fact also agrees with previous observations of hydrogen atoms in solids Conceivable molecular defects containing one hydrogen atom would have considerable anisotropy in both *g*-value and hyperfine coupling and would also show a much smaller doublet separation

Any phosphorus-containing defect in which the phosphorus nucleus is located at a point of less than tetrahedral symmetry would likewise possess *g* and hyperfine anisotropy[5] Phosphorus located at a defect of tetrahedral symmetry, as in phosphorus-doped silicon, would have isotropic hyperfine coupling of only 20–30 gauss[6] Atomic phosphorus likewise has a hyperfine splitting of 20 gauss[7]

Production of hydrogen atoms by radiation damage at low temperatures in several materials is well established The unusual aspect of this work is that hydrogen atoms have been produced and are relatively stable at room temperature in teeth The number of hydrogen atoms produced in the 1 mm^3 sample with 10-min irradiation is about 10^{15} atoms For the stronger unidentified species about 10^{18} defects are produced

We thank Dr J. J Lambe of this Laboratory for his aid in this work

TERRY COLE
ARNOLD H SILVER

Scientific Laboratory,
Ford Motor Co ,
Dearborn,
Michigan

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A Fast-neutron Source for Radiotherapy

IT would be a pity if recent correspondence[1,2] were to give the impression that 14-MeV neutrons are known to be valueless for radiotherapy on account of their low linear energy transfer To my knowledge no measurements of the oxygen enhancement ratio of mammalian cells irradiated by 14-MeV neutrons have yet been published. An estimate of the value of this irradiation in treating tumours containing anoxic foci is therefore a matter of speculation.

In considering the effective linear energy transfer of 14-MeV neutrons, account must be taken of all the ways in which energy can be deposited in tissue At low energies nearly all the energy is deposited by recoil protons (94 per cent at 0 3 MeV[3]) As the neutron energy rises the proportion of energy deposited by recoil protons becomes less, owing to the falling cross-section for this process, and is only 70 per cent at 14 MeV[4] Randolph[4] made calculations at this energy allowing for the other interactions which can occur, and found a significant contribution from α-particles (mainly from the reaction $^{16}O(n,\alpha)^{13}C$) These α-particles presumably have a high relative biological effectiveness and so are biologically more effective than recoil protons An estimate of the radiobiological effect based on the linear energy transfer of recoil protons only is therefore likely to be a poor approximation

It is not at present possible to make a reliable estimate of the biological effects of these α-particles However, there is a general picture that, as the linear energy transfer rises, the relative biological effectiveness of mammalian cells rises to a maximum around 50 keV/μ and then falls again[5], while oxygen enhancement ratios fall steadily[6] The important parameter for therapy is the 'gain factor' defined by Alper[7] A gain factor of about 1 7 has been found for cyclotron-produced neutrons having a mean energy of 6 MeV[8] The gain factor for 14-MeV neutrons can only be estimated roughly, as the necessary radiobiological evidence is lacking It is likely to be less than 1 7, and a calculation assuming plausible values for the radiobiological properties of the components of 14-MeV neutron radiation (recoil protons and nuclei and α-particles) suggests it may be in the region of 1 5, which would still be of therapeutic value

Fowler[2] points out that the optimum neutron energy is likely to be a compromise between low energies giving high linear energy transfer but poor penetration, and high energies giving lower linear energy transfer but better penetration This implies that the optimum depends on the depth of the tumour treated It is quite possible that 14 MeV may turn out to be a suitable neutron energy for treating deep-seated lesions On the other hand, the difficulties of collimating the beam may be greater than seem to be imagined by Lomer and Greene[9]

While the value of a source of 14-MeV neutrons for therapy remains in doubt, the construction of a more powerful source than is at present available should be welcomed as an aid to radiobiological research

D. K. BEWLEY

Medical Research Council Cyclotron Unit,
Hammersmith Hospital,
London, W 12

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BIOLOGY

Effect of Lymphocyte Depletion by Thoracic Duct Fistula and Administration of Antilymphocytic Serum on the Survival of Skin Homografts in Rats

SINCE lymphocytes appear to play a crucial part in the homograft reaction it seemed of interest to examine the behaviour of homografts in recipients subjected to chronic lymphocyte depletion by (*a*) drainage of lymph through a thoracic duct fistula, (*b*) repeated administration of a hetero-specific antilymphocytic serum, (*c*) a combination of these procedures

The use of a thoracic duct fistula is based on the observation of Gowans, McGregor, Cowen and Ford[1] that rats challenged with tetanus toxoid or sheep erythrocytes after such a fistula has been maintained for 5 days fail to produce antibodies

The effect of a hetero-specific (rabbit-anti-rat) antilymphocytic serum on the behaviour of homografts of skin and endocrine tissues was investigated some years ago by Woodruff, Woodruff and Forman (cited by Woodruff[2]), but it was not found possible to maintain a lymphocytopenia for more than about 10 days, and no significant effect on homograft survival was observed, the serum used in the experiments recorded here has, however, proved to be much more effective

Albino rats of an inbred strain were used as skin graft donors, and hooded rats of a second inbred strain as recipients Fitted split skin grafts (2 cm × 2 cm) were used as described by Woodruff and Simpson[3] Thoracic duct cannulation was performed as described by Bollman, Cain and Grindlay[4] and Gowans[5] In rats undergoing lymph depletion the fistula was maintained for 5 days, during which time the total output was 1–2 × 10^9 lymphocytes

Antilymphocytic serum was prepared initially by giving random bred rabbits three intraperitoneal injections of 2 × 10^8 hooded rat thoracic duct lymphocytes at weekly intervals and bleeding 10 days after the last injection Immunized rabbits received a 'booster' intraperitoneal injection of 100–200 × 10^6 rat lymphocytes 10 days before subsequent bleeding The serum was used without prior absorption against rat erythrocytes It was assayed by measuring its capacity to produce a lymphocytopenia in normal hooded rats, and was accepted as satisfactory only if 2 ml injected intraperitoneally to a 200-g rat resulted in a fall in the absolute lymphocyte count of the peripheral blood to less than 2,000/mm^3 in 4 h

The treatment used and the period of homograft survival (assessed macroscopically) in the various experimental groups are set out in Table 1

It will be seen that lymph drainage and administration of antilymphocytic serum both resulted in prolonged homograft survival, the maximum effect was obtained when treatment was begun before grafting and continued thereafter

These results will be reported more fully elsewhere with an appraisal of their biological significance and their possible application in the surgery of replacement

M F A WOODRUFF
N A ANDERSON

Department of Surgical Science,
University of Edinburgh, and
Medical Research Council Research Group on Clinical and
Experimental Problems of Transplantation

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Control of Egg Size in Chickens through Dietary Amino-acid Balance

THERE are conflicting reports regarding the influence of the level of protein and of specific amino-acids in the diet of the hen on egg size Results from an experiment in this laboratory suggested that the balance, rather than absolute levels, of amino-acids determines the response to different amounts of dietary protein and to amino-acid supplementation of the laying diet There was also evidence that egg size may be modified quickly by altering dietary amino-acid balance The following experiment was designed to test the validity of these observations

Forty-eight White Leghorn laying pullets, 8 months of age, were placed in individual laying cages The percentage composition of the basal diet (1) fed was ground wheat 69 09, ground yellow corn 10 0, soy-bean oil meal (44 per cent protein) 7 5, distillers' dried solubles 2 0, iodized salt 0 35, feeding oil (2,250 U S P units vitamin A, 300 I C U vitamin D_3/g) 0 5, vitamin B_{12} premix (20 mg/lb) 0 06, stabilized animal fat 5 0, bonemeal 1 75, limestone 3 75, manganese sulphate 0 015. The other diets consisted of the basal diet supplemented with amino-acids as follows (2) 0 25 per cent L-lysine monohydrochloride plus 0 25 per cent DL-methionine (98 per cent pure), (3) 0 255 per cent glycine, (4) 0 25 per cent L-lysine monohydrochloride plus 0 25 per cent DL-methionine plus 0 255 per cent glycine. The basal diet contained 14 per cent protein

Table 1

Treatment*	Effect of lymphocyte count	No of animals	Survival of skin grafts (days)	
			Individual values	Mean
Nil	Nil	12	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 9	8
Lymph drainage from day −5 to day 0	Fell during drainage period to about half initial level, began to rise 1 week later but still below normal by time all grafts destroyed	10	11, 11, 11, 12, 12, 13, 14, 15, 15, 15	13
Antilymphocytic serum 1 ml /day i p on days +1 to +14 inclusive	Fell during period of treatment to about quarter initial level, then slowly increased	10	11, 11, 12, 14, 14, 14, 14, 16, 18†, 41	16
Antilymphocytic serum 2 ml /day i p on days −7 to −1 inclusive, and 1 ml /day i p on days +1 to +14 inclusive	Fell during pre-grafting treatment to about one-fifth initial level, then gradually increased for 13 days after which it fell again	10	18, 18, 22, 27, 29, 30, 30, 32, 32, 46	28
Normal rabbit serum 2 ml /day i p on days −7 to −1 inclusive, and 1 ml /day i p on days +1 to +14 inclusive	Increased slightly throughout period of treatment	10	8, 8, 8, 8, 8, 8, 8, 8, 8, 8	8
Lymph drainage from day −5 to day 0 Antilymphocytic serum 1 ml /day i p on days +1 to +14 inclusive	Fell during period of treatment to about one-tenth original level, then gradually rose and was almost normal 3 weeks after grafting	10	17, 18†, 22†, 26†, 33, 38, 42, 45, 54, 60	35

* The day of skin grafting is reckoned as day 0 † Animal died with healthy graft

Table 1 EGG PRODUCTION AND ANALYTICAL DATA

Diet	Amino-acid supplement	Group	Period	Production (%)	Av egg wt (g)
1	None	*A*	1	77.4	56.4
		B	2	62.9	56.4
		A	3	62.2	56.6
		B	4	62.5	57.4
2	Methionine + lysine	*B*	1	76.5	57.2
		A	2	64.3	57.6
		B	3	67.4	58.4
		A	4	65.1	58.3
3	Glycine	*C*	1	76.8	55.0
4	Glycine, methionine-lysine	*C*	2	61.3	56.0
3	Glycine	*C*	3	58.5	55.7

The pullets were divided into 3 groups of 16 birds each, distributed in individual laying cages so as to effect uniformity of environmental factors The three groups of birds were subjected to the dietary régimes indicated in Table 1 and Fig 1 Groups *A* and *B* alternated between diets (1) and (2) Group *C* alternated between diets (3) and (4)

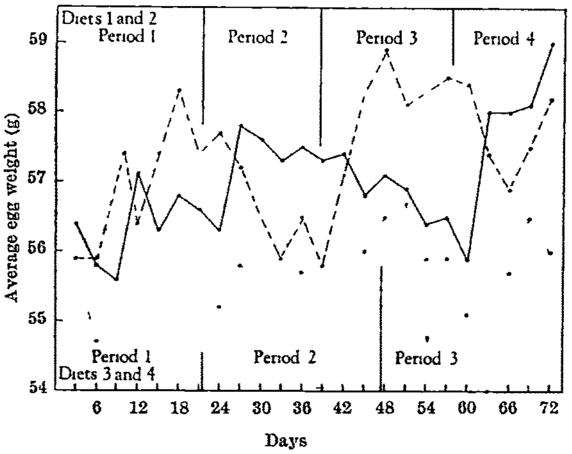

Fig 1 Average weights of eggs laid by birds fed diets of different amino-acid balance —, Group *A*, – – –, group *B*, , group *C*

Individual daily record was kept of the weights of eggs laid by the birds within each group The experiment was of 72 days' duration The weights of the component parts (shell + shell membranes, albumen and yolk) of all eggs laid on 20 days during periods 3 and 4 were determined The moisture content of the albumen from several days' production of eggs was also determined during the last period

The average weights of the eggs laid are shown in a graph by 3-day periods in Fig 1, and a summary of the results is given in Table 1 The rank of the diets in their effect on egg size was similar throughout the experiment· diet (2), diet (1), and diet (3) or (4)

Since egg size was depressed when glycine was added to the basal diet and as supplementation with lysine and methionine increased egg size, it may be concluded that amino-acid balance and not protein-level was the factor limiting egg size Supplementation with lysine and methionine of the diet already supplemented with glycine, that is, substitution of diet (4) for diet (3), did not increase egg size to the weight of the eggs laid by birds receiving diet (2), which was supplemented with lysine and methionine only Evidently, in the case of diet (4), although the absolute levels of lysine and methionine were similar to those in diet (2), the levels of these amino-acids relative to the over-all amino-acid composition of the diet were not optimum for maximum egg size.

When lysine and methionine were either added to or withdrawn from the basal diet, size of the eggs laid was affected on the fourth day following modification of the diet Alternation of diets (1) and (2) between groups *A* and *B* produced alternation in the relative size of eggs laid by the birds in the two groups

Table 2 COMPOSITION OF EGGS LAID BY BIRDS FED DIFFERENT DIETS

	No of eggs analysed	Av wt of eggs (g)	% of total weight shell	yolk	albumen	No of eggs analysed	% solids in albumen
Group *A*							
diet 1	121	56.5	9.11	28.04	62.83		
2	70	57.9	8.88	27.91	63.12	49	12.3
Group *B*							
diet 1	69	57.4	9.12	28.26	62.62	45	12.2
2	139	58.2	8.87	27.65	63.48		
Group *C*							
diet 3	146	55.5	9.02	28.96	62.03	35	11.9

The percentage composition of the eggs analysed is shown in Table 2 The percentage of albumen was greatest in the eggs laid by birds fed diet (2), followed in order by eggs laid by birds fed diets (1) and (3) The percentage of solids in the albumen was significantly ($P < 0.01$) lower in the eggs laid by the birds fed diet (3)

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B E MARCH
J. BIELY

Department of Poultry Science,
University of British Columbia,
Vancouver, 8, B C.

Infra-red Spectra of Embryonic Chick Feathers

THE demonstration by Bell and Thatachari[1] of sequential changes in the X-ray diffraction pattern of embryonic chick feathers from 'immature' to 'mature' β-keratin has suggested that the nature of this metamorphosis might be further revealed by infra-red spectra of the developing feather. The infra-red spectrum of embryonic feather has not been described, to our knowledge

Whole or homogenized washed feathers from the mid-dorsal region of white Leghorn chick embryos from the twelfth to nineteenth days of incubation were examined in

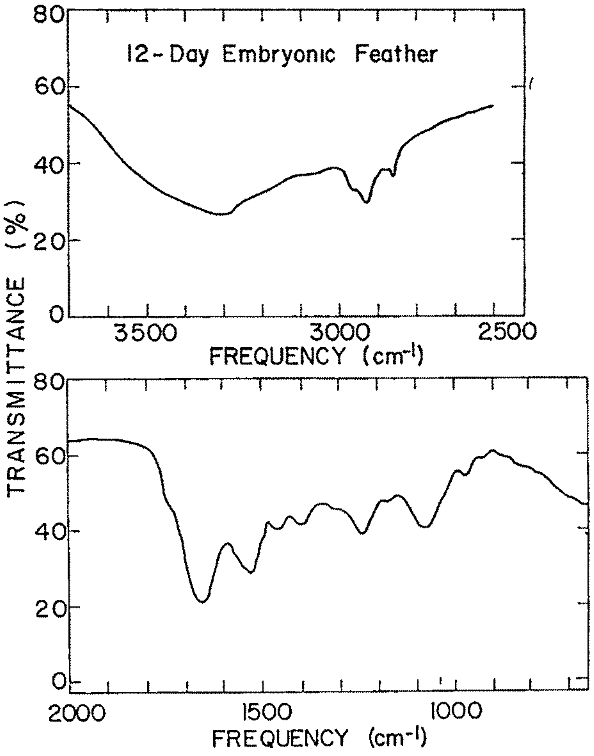

Fig 1 Infra-red spectrum of 12-day feather

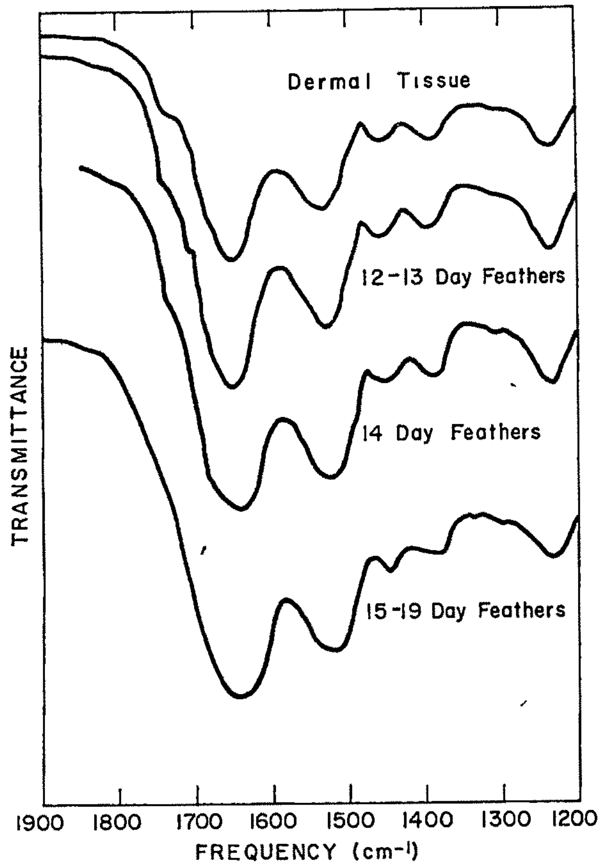

Fig 2 Loss of carbonyl-ester band during embryogenesis

a Perkin-Elmer double-beam infra-red spectrophotometer, model 421. Samples were usually prepared as potassium bromide pellets, but whole feathers pressed between sodium chloride plates and suspended in 'Fluorolube' were also examined to eliminate the possibility of artefacts caused by grinding and pressing Dermis, as subcutaneous tissue, and ether-extracted feathers from 13-day embryos were similarly studied

A representative spectrum of 12-day feather from 4,000 cm^{-1} to 700 cm^{-1} and spectra in the 1,800–1,200 cm^{-1} range for all specimens examined are shown in Figs 1 and 2, respectively In general, the patterns are similar both to each other and to those reported for adult swan and eagle quill[2,3] and are characteristic of class *B* proteins[3] The significant change seen during embryogenesis is that the pronounced shoulder at 1,730 cm^{-1} on the high-frequency side of the amide I band observed in 12- and 13-day specimens is decreased at 14 days and gone thereafter This shoulder, which lies in the region of ester-carbonyl absorption, is quite prominent in dermis, much diminished in ether-extracted 13-day feathers and not present in native collagen[4]

These results suggest that the structural changes detected by X-ray diffraction during embryogenesis of the feather are of a sort not readily detected by infra-red spectroscopy and that the changes are explicable by differences in molecular orientation rather than by appearance of new chemical groupings Disappearance of the band at 1,730 cm^{-1} is probably a manifestation of retraction of the mesodermally derived feather core but could also be consequent in part on loss of lipid from other cells during development

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RONALD A MALT
KARL A HARTMAN, jun

Department of Biology and
Spectroscopy Laboratory, Massachusetts
Institute of Technology, Cambridge, and
Departments of Surgery, Massachusetts
General Hospital and Harvard Medical
School, Boston

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Reversed Diurnal Vertical Migration of Planktonic Crustacea in Inland Waters of Low Hydrogen Ion Concentration

EXPERIMENTAL investigations[1,2] have shown that pH has an influence on the vertical migration of planktonic crustaceans In particular it has been demonstrated that tactic responses at a pH of 9 0 may be in the reverse direction to those at a pH of 7 0 However, little attention has been directed to the fact that such reversed responses may occur under natural conditions of high pH

A reversed diurnal vertical migration was recently reported[3] for 2 species of planktonic crustacea in a New Zealand lake with a high pH No causal relationship between the high pH and the unusual pattern of migration was suggested in this report However, it was afterwards discovered that of the very few previous reports of such migratory behaviour in inland waters[4-6], one[6] was concerned with an African lake which showed a striking similarity to the New Zealand one with respect to pH and the relative proportions of the major inorganic ions (Table 1) The main chemical difference is that Lake Rudolf is 5–6 times more concentrated and has a greater proportion of sulphate than Lake Aroarotamahine. A further similarity is that both lakes are relatively opaque with a limit of visibility between 2 and 3 m Worthington and Ricardo[6] stated "The most striking fact is that the migration (of *Diaptomus banforanus*) appears to be the reverse of the normal . .", and mentioned the possibility that high pH might account for the aberrant behaviour The significance of their observation appears to have been somewhat overlooked

Table 1 CHEMICAL COMPARISON OF REVERSED MIGRATION LAKES

	Lake Rudolf Rift Valley Central Africa[9]	Lake Aroarotamahine Mayor Island New Zealand (Feb 1963)
pH	9 5	9 0
Alkalinity	0 020 N	0 0035 N
Na^+	770 mg/l	125 mg/l.
K^+	23	4 0
Ca^{++}	5 0	0 9
Mg^{++}	4 0	1 2
$SO_4^=$	56	4
Cl^-	429	76
$CO_3^=$	181*	10
HCO_3^-	958*	193
Total CO_2	830	147
Reversed migrants	*Diaptomus banforanus*[6]	*Boeckella propinqua* (C VI ♂♂) *Daphnia carinata*[3]

* Calculated from pH and stated value [9] for CO_2

The chemical results shown in Table 1 for Lake Aroarotamahine were obtained from the analysis of a surface sample. The values stated for pH, $CO_3^=$ and HCO_3^- were obtained from field measurements made immediately after taking the sample. The pH of this lake is, of course, subject to considerable seasonal and vertical variation During summer when thermal stratification is well developed the zooplankton is limited to the epilimnion where the pH is not less than 9 0 During winter the lake is in a state of complete circulation and the pH is approximately 8·2 throughout. *Daphnia carinata* showed a reversed migration under both these conditions, but in the case of *Boeckella propinqua* males there was no evidence of reversed migration during the period of complete circulation.

Lake Rudolf and Lake Aroarotamahine are both associated with alkaline rhyolites in the form of either comendites or pantellerites[7,8] which are rich in sodium and potassium but very poor in calcium and magnesium. As a result of the chemical weathering of these rocks relatively large amounts of extremely soluble sodium carbonate have found their way into these lakes, so that they have come to contain a sodium carbonatochloride water in which alkaline earth and sulphate ions are present in only very small proportions.

It is interesting to note that the very low calcium content of the lakes mentioned here is not a limiting factor with respect to zooplankton productivity. On the contrary, there is evidence that both lakes are highly productive. In a series of 4 lakes, Worthington and Ricardo[6] found the zooplankton numerically richest in Lake Rudolf. Likewise Bayly[3] found far greater zooplankton densities in Lake Aroarotamahine than in 9 other lakes examined.

I. A. E. Bayly

Department of Zoology,
University of Queensland,
Brisbane.

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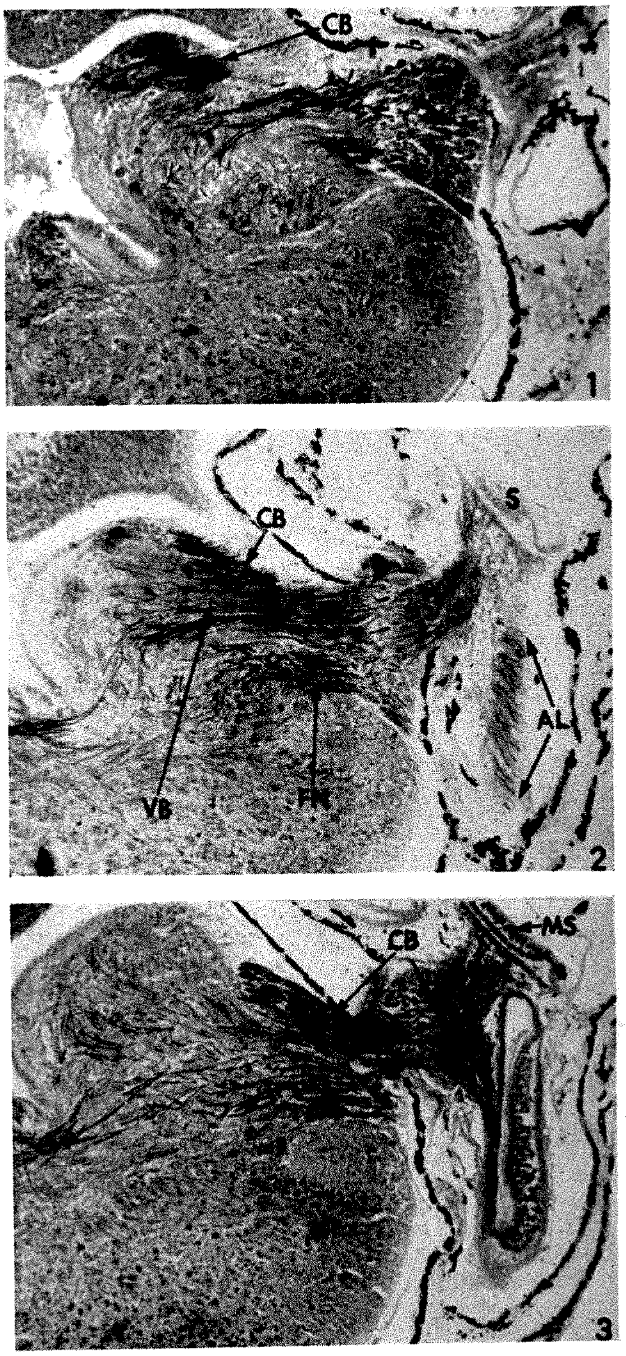
Posterior Division of the Eighth Cranial Nerve in *Lacerta vivipara*

Little information is available in the literature on the innervation of sensory areas in the inferior, or saccular, portion of the inner ear of the lizard. In order adequately to show nervous structure, histological techniques have been used in the past that preclude dealing with the intact animal; usually the brain must be removed, thereby disrupting anatomical relationships. A notable exception is the work of Beccari[1] on embryonic material of *Lacerta*; his primary interest, however, was in finding homologies in the central nervous system between reptiles and mammals, and he gives little consideration to the central terminations of fibres from specific sensory areas. Weston[2,3] has speculated on central terminations of sensory fibres in *Sphenodon* and has discussed structure of vestibular and auditory areas in brains of a number of reptiles.

Serial sections through the inner ears of two de Castro-stained specimens of *Lacerta vivipara* were available for examination, and de Castro-stained specimens of *Anniella pulchra* and *Anguis fragilis* were available for comparison, along with Bodian-stained serial sections of the inner ears in *Gongylus ocellatus*, *Calotes versicolor* and *Sceloporus undulatus*.

Nerve fibres from the macula of the utricle and cristæ of the anterior and lateral ampullæ project to the central nervous system through the anterior acoustic foramen and terminate rostral to the central projections of the remaining sensory areas (Fig. 4).

Of interest here is the qualitative difference in the posterior division of the VIIIth cranial nerve between fibres which project to the dorsal, acoustic area of the brain stem, and those that project ventrally to the vestibular areas of the brain stem.



Figs. 1, 2 and 3. Cross-sections through the inner ear in *Lacerta vivipara*, anterior to posterior, to show the course of the cochlear bundle and relationships between the sensory areas of the membranous labyrinth and the central nervous system. (For key to abbreviations see legend to Fig. 4)

Fibres which originate in the anterior, lagenar, portion of the cochlear duct (Fig. 2) and from the basilar papilla course posteriorly in the otic capsule to emerge from the capsule (via the posterior acoustic foramen) and, as a distinct, dark-staining bundle, cross caudal to fibres that arise from the macula of the saccule (Fig. 3). The bundle courses dorsally and rostrally within the central nervous system to terminate on small cells in the acoustic area of the brain stem (Fig. 1). For convenience, this group of fibres is termed the cochlear bundle.

The fibres projecting from the macula of the saccule, joined by those from the papilla neglecta of the utricle and crista of the posterior ampulla, terminate ventral to the termination of the cochlear bundle. The cells in the vestibular area with which these fibres synapse are larger

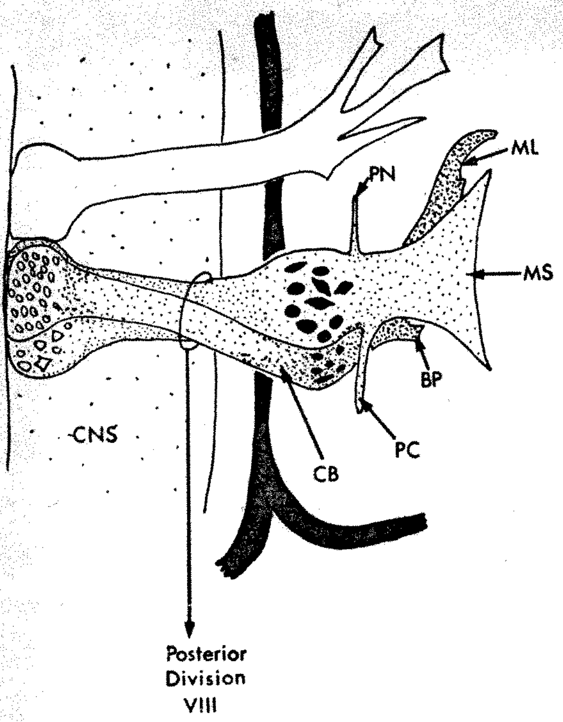


Fig. 4. Schematic representation of the course and relationships of the fibres of the posterior division of the VIIIth cranial nerve in *Lacerta vivipara*. The anterior division of VIII, with ramifications from the utricle, and anterior and lateral ampullæ, is shown in white.

AL, anterior, lagenar, part of the cochlear duct; *BP*, basilar papilla; *CB*, cochlear bundle; *CNS*, central nervous system; *FN*, facial nerve; *ML*, macula of the lagena; *MS*, macula of the saccule; *PC*, posterior crista; *PN*, papilla neglecta; *S*, saccule; *VB*, vestibular part of the posterior division of VIII

than those associated with the cochlear bundle (Figs. 1 and 2).

These relationships are shown schematically in Fig. 4. In *Lacerta*, then, the central terminations of fibres from the macula of the lagena are the same as those from the basilar papilla, and different from those of the macula of the saccule. A reasonable conclusion from the structural evidence in *Lacerta* is that the macula of the lagena has some auditory function, while the macula of the saccule is concerned mainly with equilibration.

This is not the case in forms in which sensory areas in the cochlear duct are reduced relative to the macula of the saccule, such as in *Anniella* and *Anguis*. In the latter specimens, there is no secondary tympanic membrane, and it is probable that these lizards do not hear as well as free-living forms, such as *Lacerta*, in which a well-defined secondary tympanic membrane is present. Other evidence (which will be published elsewhere) indicates that the inner ear in *Lacerta* is more highly evolved than that in either *Anniella* or *Anguis*, and it is possible that auditory function of the macula of the lagena is a phylogenetically advanced feature.

DAVID W. HAMILTON*

Anatomy School,
University of Cambridge.

* Present address: Department of Anatomy, Harvard Medical School, Boston 15, Mass.

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Action of Piperazine on the Neuromuscular System of *Ascaris lumbricoides*

PIPERAZINE is the drug most commonly used for the treatment of ascariasis, in preference to other anthelmintics, because it produces a flaccid paralysis of the parasites[1] rather than hyperactivity. Though no direct evidence was available, it was suggested that such paralysis might be due to a curare-like effect, since piperazine prevents the stimulating action of acetylcholine on *Ascaris* muscle[2].

The electrical activity of the somatic muscle cells of *Ascaris lumbricoides*, var. *suum*, has recently been examined in this laboratory[3] and we have now taken advantage of the techniques and preparations used in this work to investigate the action of piperazine on the neuromuscular system of the worm.

This system is represented in Fig. 1. Each somatic muscle cell is connected to one of the two nerve cords by a slender process, or muscle 'arm', which tapers down and divides into a number of branches or 'fingers'. The fingers from adjacent arms interlace, forming a closely woven network which, shaped as a thin ribbon, runs parallel to the nerve cord. Although little is known about the fine structure of this network, experimental evidence[3] has shown that it behaves as a functional syncytium, the individual muscle cells being electrically interconnected at this level.

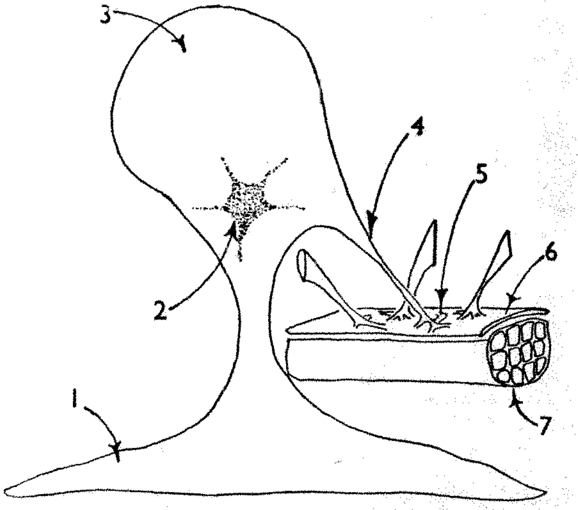

Fig. 1. Highly diagrammatic representation of the neuromuscular system of *Ascaris lumbricoides*. Only one complete muscle cell has been drawn, and the arms of three others; see text. It should be pointed out that the 'arm' of the muscle cell forms a right angle with the 'spindle', the latter being parallel to both the nerve cord and the longitudinal axis of the worm
1, Spindle; 2, nucleus; 3, nuclear bag or 'belly'; 4, arm; 5, fingers; 6, syncytium; 7, nerve cord

The complex formed by the nerve cord and associated syncytium has many of the properties of vertebrate smooth muscle, including that of autorhythmicity or automatic production of spike potentials. Microelectrode recording from the muscle cell 'bellies' shows a resting potential (average value about 30 mV) interrupted by rhythmic spike potentials, see Fig. 2*A*, which originated at the syncytium and are conducted along the arms and bellies to the spindles—the only part of the muscle cell which contains contractile material.

When piperazine citrate, as a solution adjusted to *p*H 7, is added to the saline bathing the *Ascaris* preparations (30 per cent sea-water) the amplitude of the spontaneous spike potentials is seen to decrease until the cells become electrically silent (Fig. 2). This effect is accompanied by an increase in the resting potential. In one experiment, for example, an average resting potential of 28·3 mV was increased to 41·0 mV on the addition of piperazine to the bathing solution to a final concentration of 10^{-4} (w/v). Increments of up to 15 mV have been observed when recording continuously from a belly.

In many cells the increase in the resting potential is concomitant with a considerable reduction in the frequency of the spike potentials, though in others the spikes disappear before any marked changes in their frequency can be detected. Often, the resting potential is seen to go on increasing after the spike activity is blocked.

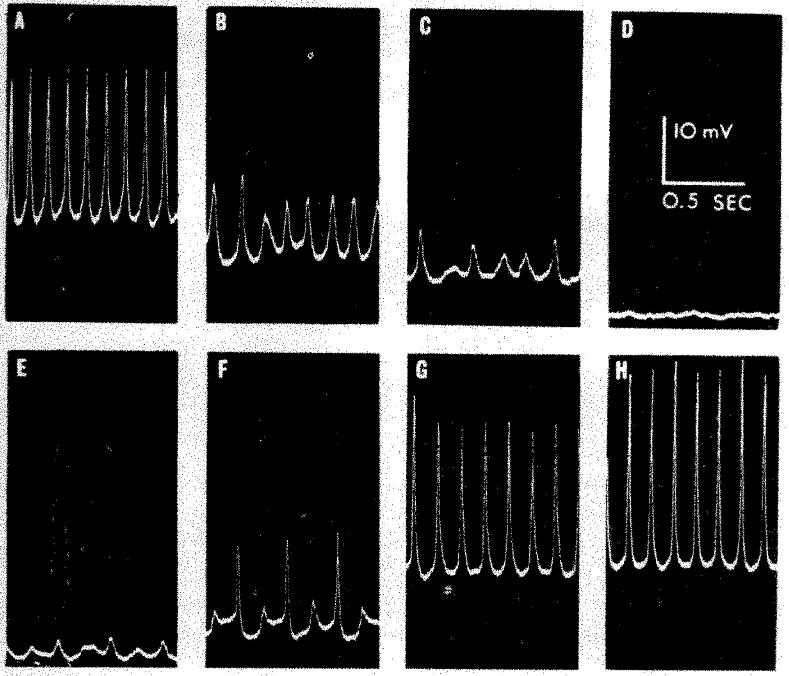

Fig. 2. Intracellular recordings illustrating the action of piperazine on the resting potential and spontaneous spikes of a somatic muscle cell of *Ascaris lumbricoides*. The microelectrode was inserted into the cell 'belly'. Between records *A* and *B* piperazine citrate was added to the bathing solution to a final concentration of 10^{-4} (w/v). Record *D* was taken about 5 min after record *B*. Between records *D* and *E* the piperazine was washed out of the chamber. The records are arranged in each row, so that the relative position of the traces shows actual changes in membrane potential which was, approximately, 29 mV in *A* and *H*, and 41 mV in *D* and *E*

Control experiments, in which equivalent amounts of sodium citrate were added to the bathing solution, showed that the effects described here are not due to the citrate anion, nor to a lowered concentration of calcium ions. As can be seen in Fig. 2, all the effects of piperazine are easily reversed by washing the preparation with fresh saline.

The action of piperazine on *Ascaris* muscle appears to be similar to that exerted by inhibitory transmitters and pharmacological analogues on the transmembrane potential of smooth and cardiac muscle cells. This resemblance is particularly interesting since it has been shown that the nerve cord of *Ascaris* does contain a system of muscle inhibitory fibres. Goodwin and Vaughan Williams[4] have recently reported that high-frequency electrical stimulation of the nerve ring situated in the pharyngeal region of the worm results in an inhibition of both the spontaneous contractions of the body and those elicited by local stimulation of motor nerves.

Our experiments have shown that inhibitory fibres can also be stimulated in fragments of *Ascaris* which do not include the nerve ring by electric shocks applied to the ventral nerve cord. Marked hyperpolarizations of the muscle cell membrane, accompanied by a block of spike activity, can be observed following single stimuli while recording from the belly (Fig. 3). Repetitive stimulation of the inhibitory fibres increases the resting potential of the bellies to a steady level from 40 to 45 mV.

Piperazine does not interfere with the function of the inhibitory fibres. On the contrary, small concentrations of this compound increase the effectiveness of inhibitory stimulation, and small inhibitory potentials can still be recorded from hyperpolarized cells in paralysed preparations.

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J. DEL CASTILLO
T. A. MORALES
V. SANCHEZ

Department of Pharmacology,
School of Medicine,
San Juan 22,
Puerto Rico.

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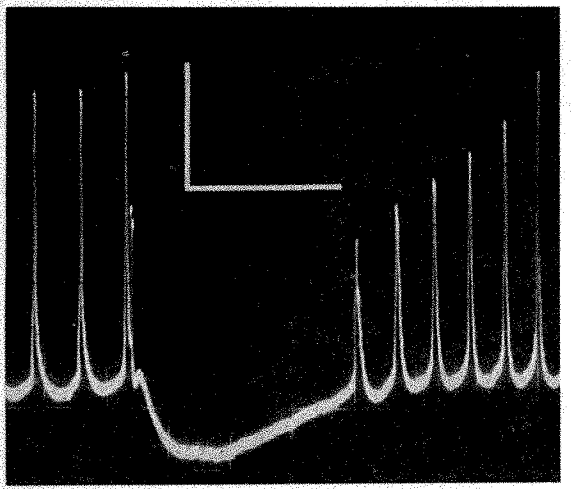

Fig. 3. Effects of the electrical stimulation of inhibitory nerve fibres in the nerve cord of *Ascaris lumbricoides*. Two pulses were applied to the nerve cord at the points marked by the artefacts. They resulted in a hyperpolarization of the muscle membrane and the blockade of the spontaneous spike potentials. Records were taken with a microelectrode inserted into the cell belly. Calibrations: vertical, 10 mV; horizontal, 0·25 sec

Gaseous-ion-induced Stimulation of Cytochrome *c* Biosynthesis

IN earlier papers we described the growth acceleration observed when seedlings of *Avena sativa*, *Hordeum vulgaris* and *Lactuca sativa* were exposed to air ions[1-3]. (+) or (−) Ions generated in pure air produced statistically significant increases in stem-length, integral elongation and wet and dry weight. The degree of growth stimulation roughly paralleled ionic density and was accompanied by a proportional increase in flow of current to ground. Analysis of ion-treated plants showed no deviation from normal composition. This communication presents evidence that gaseous ions stimulate the biosynthesis of cytochrome *c*.

Germinated seeds of *Hordeum vulgaris* (Dayton Winter and California Mariout) with roots 1–2 cm long but without coleoptiles were grown in an iron-free chemically defined nutrient solution. The details of the plant culture system and radioisotopic generators used are described elsewhere[4]. The essential points are: units of 36 seedlings each were exposed to (+), (−) or (0) air ions in clear plastic chambers under greenhouse conditions. The greenhouse was supplied with air purified by passage through an electrostatic precipitator and an activated carbon filter. The minimum temperature was 25° C, but maximum temperature varied with the season. The volume of solution was

Table 1. EFFECT OF UNIPOLAR IONIZED AIR ON DEVELOPMENT OF IRON CHLOROSIS IN SEEDLINGS OF *Hordeum vulgaris* GROWN IN CHEMICALLY DEFINED IRON-FREE NUTRIENT SOLUTION. THERE WERE 36 PLANTS IN EACH TEST GROUP AND THE ANALYTICAL DATA OF 3 SEPARATE EXPERIMENTS PERFORMED DURING THE SUMMER HAVE BEEN AVERAGED

Air ion charge	No. of days exposed to air ions	Appearance of leaves	Cytochrome *c* fraction absorbance at 550 mμ	μg Protein/ml. of cytochrome *c* fraction	Fe in p.p.m. in cytochrome *c* fraction	Total chlorophyll mg/g fresh weight
			× 10^{-3}		× 10^{-3}	× 10^{-2}
(−)	7 days in all 3 experiments	Green No chlorosis	50·2 ± 0·9*	32·1 ± 0·2*	9·6 ± 0·7*	8·0 ± 1·7*
(+)			47·2 ± 1·3	30·2 ± 0·1	8·0 ± 0·8	8·2 ± 1·9
(0)			31·4 ± 0·8	21·4 ± 1·8	3·4 ± 1·3	10·3 ± 2·6
(−)	12, 14 and 20 days, iron added after seedlings harvested	Severe chlorosis	123·7 ± 1·5	74·2 ± 0·4	19·4 ± 1·5	22·0 ± 1·2
(+)			102·3 ± 1·4	70·6 ± 1·4	20·1 ± 1·8	24·2 ± 1·3
(0)			87·4 ± 2·5	54·2 ± 0·6	16·2 ± 2·5	45·4 ± 3·7
(−)	19, 21 and 27 days	Green Recovered from chlorosis	189·8 ± 3·2	123·4 ± 6·6	46·4 ± 6·8	96·3 ± 0·6
(+)			188·7 ± 2·4	120·8 ± 6·4	36·2 ± 2·7	95·4 ± 7·1
(0)			90·5 ± 6·6	63·7 ± 6·4	16·6 ± 8·2	94·2 ± 2·5

* Standard error $e = \frac{S}{\sqrt{N}}$ $N = 3$ S = Standard deviation = $\sqrt{\frac{\Sigma d^2}{N-1}}$

kept constant by addition of distilled water. Ion levels were regularly monitored and were maintained at > 3·1–3·8 × 10^5 (+) or (−) ions/cm²/sec at the tops of the seedlings. Planters were rotated in position to avoid effects due to variations in incident light. At intervals after starting exposure of the seedlings to ions, samples were removed for analytical studies. Their content of cytochrome *c* and chlorophyll was determined and the cytochrome *c* fractions were analysed for protein and for iron content[5–8].

Seedlings cultivated under these conditions in a normal atmosphere developed iron chlorosis. In the presence of (+) or (−) ionized air chlorosis developed earlier and the production of cytochrome *c* was accelerated (Table 1). Thus at 7 days, although the leaves were normal in appearance, the ion-treated plants had only 80 per cent as much chlorophyll as the untreated plants, but 160 per cent as much cytochrome *c*. From the twelfth to the twentieth day all the leaves showed pronounced chlorotic changes; the [chlorophyll] in the ion-treated plants was 50 per cent and the [cytochrome *c*] 120 per cent of the corresponding values obtained in untreated plants. At this time iron was added to the nutrient solution and the plants recovered rapidly from chlorosis. By the nineteenth–twenty-seventh day the leaves in all three groups appeared normal and there was good agreement in [chlorophyll] values. Nevertheless the plants exposed to (+) or (−) ions contained nearly 210 per cent as much cytochrome *c*/g as the plants grown in a normal atmosphere. Substantially the same results were obtained in two additional experiments performed during the autumn and winter months. While the development of severe chlorosis was slower recovery after adding iron was just as fast.

A separate experiment was performed growing the seedlings in a sand substrate with an iron-containing chemically defined medium (Table 2). Here no chlorosis developed in any of the plants, and the only gross effect was the characteristic ion-induced acceleration of growth.

Table 2. EFFECT OF UNIPOLAR IONIZED ATMOSPHERE ON CYTOCHROME *c* AND CHLOROPHYLL CONTENT OF *Hordeum vulgaris* SEEDLINGS GROWN IN SAND CULTURE WITH CHEMICALLY DEFINED NUTRIENT SOLUTION CONTAINING 2·5 P.P.M. IRON. A TOTAL OF 20 PLANTS WAS USED IN EACH EXPERIMENTAL GROUP. EXPOSURE PERIOD 20 DAYS

Air ion charge	Stem-length (mm)	Cytochrome *c* fraction absorbance at 550 mμ	μg Protein/ml. of cytochrome *c* fraction	Fe in p.p.m. × 10^{-2} in cytochrome *c* fraction	Total chlorophyll mg/g fresh weight
		× 10^{-3}		× 10^{-2}	
(−)	115 ± 6·5	85 ± 2·0	17·2 ± 0·7	6·0 ± 1·2	1·22 ± 0·16
(+)	110 ± 5·8	75 ± 1·8	16·5 ± 1·2	5·8 ± 2·0	1·25 ± 0·12
(0)	72 ± 8·3	40 ± 3·0	8·7 ± 1·4	2·1 ± 2·5	1·11 ± 0·20
	$N = 20$	$N = 3$			

Standard error $e - \frac{S}{\sqrt{N}}$ S standard deviation $\sqrt{\frac{\Sigma d^2}{N-1}}$

Cytochrome *c* of the ion-treated plants was double that of the controls.

The cytochrome *c* fractions prepared from the plants contained small amounts of other extractable substances. Accordingly, the percentage change in [cytochrome *c*] for a given sample displays only approximate agreement among the three criteria used; namely, absorbance at 550 mμ, [cytochrome *c* protein] and [cytochrome *c* iron].

The simplest explanation of the experimental results described here appears to be that when a seedling grows in an iron-free environment air ions cause most of the limited store of iron present in the seed to be diverted to the synthesis of cytochrome *c*. Accordingly not enough iron is available to support the normal biosynthesis of chlorophyll and chlorosis develops rapidly. If iron is present in normal amounts in the substrate, exposure to ionized air results in typical ion-induced increase in growth rate associated with an increased production of cytochrome *c* but without hampering chlorophyll production. The supply of iron evidently is sufficient to provide for both biosynthetic processes and chlorosis is avoided. The increased [cytochrome *c*] may represent an important metabolic factor in making possible the accelerated growth rate regularly observed in ionized air.

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A. P. KRUEGER
S. KOTAKA
P. C. ANDRIESE

Department of Bacteriology,
University of California,
and the
Naval Biological Laboratory
of the
School of Public Health,
University of California,
Berkeley,
California.

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MICROBIOLOGY

Nitrogen Fixation in Cell-free Extracts of *Azotobacter vinelandii* prepared by Lysis with Phage A_{22}

WITHIN the past few years nitrogen fixation has been achieved in cell-free extracts of micro-organisms including *Clostridium pasteurianum*, *Azotobacter vinelandii*, *Bacillus polymyxa*, and in a variety of blue-green algae[1-5]. We now report that cell-free lysates of *A. vinelandii*, prepared by adding A_{22} phage[6] to cultures of the bacteria during early exponential growth, incorporate nitrogen gas.

A. vinelandii OP was grown in 4-l. aerated cultures in Burk's nitrogen-free medium at 30° for 12 h. At this stage when cell counts were about 2×10^7 cells/ml., A_{22} phage was added (phage to cell input 3:1) and incubation continued for another 6 h. Phage counts of the incubated cultures, made on agar plates layered with *Azotobacter* cells in a nitrogen-free medium, showed more than a 100-fold increase in phage plaque-forming units per ml. The lysed material was collected at 44,000*g* in a continuous-flow Servall centrifuge at 4°, and the residue was washed three times with 0·05 M phosphate (*p*H 7·5). The residue suspended in the dilute phosphate buffer was recentrifuged at the various *g* values shown in Table 1. Less than 10^3 cells/ml. were found by plate counts of the suspended residue. Before exposing to the nitrogen-15 gas mixture the pellets collected at various *g* values were suspended in 0·05 ml. phosphate buffer *p*H 7·5. The effects of adding medium from 6-h cultures, from which the bacteria had been removed by centrifuging at 40,000*g* for 1 h (absence of whole cells checked by plating experiments) to supernatant fractions and to the suspended pellets were investigated. In some experiments the cell-free medium was boiled for 15 min, cooled and then added to the test fractions. Samples containing between 0·2 and 1·5 mg protein were exposed for 2 h in Warburg flasks to a gas mixture containing (atmospheres) 0·2 nitrogen, 0·2 oxygen and 0·8 helium. The nitrogen gas contained 33·4 atomic per cent nitrogen-15 excess. The extracts were then digested in sulphuric acid containing a copper-selenate catalyst. Total nitrogen was determined by the micro-Kjeldahl procedure and nitrogen-15 by the method of Simms and Cocking[7] using a mass spectrometer.

Table 1. NITROGEN FIXATION IN EXTRACTS OF *Azotobacter vinelandii* PREPARED BY LYSING CELLS WITH PHAGE A_{22}. ATOMIC PER CENT NITROGEN-15 EXCESS

	Sample	Exp. I	Exp. II
	Whole cells (12 h growth)	0·56	0·68
	Cell-lysates* centrifuged successively at the following *g* values:		
1	5,000*g* for 30 min (pellet)	0·007	0·025
2	(1) plus *M*†	0·009	0·030
3	5,000–14,000*g* for 30 min (pellet)	0·018	0·026
4	(3) plus *M*†	0·490	0·254
5	14,000–27,000*g* for 30 min (supernatant fluid)	0·028	0·032
6	(5) plus *M*†	0·380	0·540
7	27,000–44,000*g* for 60 min (residue)	0·029	0·034
8	(7) plus *M*†	0·240	0·340
9	44,000*g* for 60 min (supernatant fluid)	0·016	0·023
10	(9) plus *M*†	0·104	0·132

* Whole cells (12 h growth) treated with A_{22} phage and incubated for a further 6 h to effect lysis. Lysate collected at 44,000*g* suspended in 0·05 *M* phosphate (*p*H 7·5) and centrifuged successively at the various *g* values.

† *M*, medium from 6 h cultures of *Azotobacter* centrifuged at 40,000*g* for 1 h to remove whole cells. An equal volume of the medium added to the fractions.

The results in Table 1 show that there is little fixation in the cell-debris left after centrifuging the suspended residue at 5,000*g*; but the fractions collected at 14,000*g* or above have an appreciable enrichment of the isotope. The addition of the cell-free culture medium to the various fractions stimulated nitrogen fixation as reported earlier[3,5]. The main activity was found in the particulate fractions collected between 14,000*g* and 44,000*g* although there was an appreciable enrichment even in the resultant supernatant fraction. When the latter was centrifuged at 144,000*g* for 1 h the activity was found in the pellet. The stimulatory effect of the medium on nitrogen fixation was not destroyed by boiling.

It is of interest that electron paramagnetic resonance spectra of the active particulate fractions showed pronounced signals at gauss 1·94, presumably non-hæm iron, and at gauss 1·97, probably Mo^{5+}, as well as one for a flavin semiquinone. These results are similar to those reported for active nitrogen-fixing fractions prepared from *Azotobacter* by other methods[8].

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M. W. NIMECK*
P. W. WILSON

Department of Bacteriology,
University of Wisconsin,
Madison.

D. J. D. NICHOLAS

Department of Chemical Microbiology,
Long Ashton Research Station,
University of Bristol.

* Present address: Microbial Development Division, Squibb Institute for Medical Research, New Brunswick, New Jersey.

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Significance of Deoxyribonuclease Production by *Staphylococci*

METHODS of differentiating pathogenic species of *Staphylococci* (*Staphylococcus aureus*) from commensal strains (*Staph. albus*) are of prime importance to the medical bacteriologist. The method generally used to effect this separation is one of the many modifications of the coagulase test, and it is usually considered that new tests adopted for this purpose should correlate highly with the coagulase index. Among the alternatives that have been proposed to the coagulase test are colonial pigmentation, phenolphthalein phosphatase activity and the possession of a fibrinolysin.

None of these has found favour. Chromogenesis has been considered unreliable, partly because of the subjective difficulties involved, but the introduction of glycerol monoacetate agar by Willis and Turner[1] has done much to eliminate this problem. Further, this new medium permits a subdivision of epidemiological importance within the pathogenic group. Barber and Kuper[2] suggested that phosphatase activity paralleled coagulase production, but there is some disagreement about the value of this test because of the large number of coagulase-negative strains that produce a phosphatase. It was noted by Christie and Wilson[3] that the fibrinolytic activity of human strains of *Staphylococci* generally correlated with the coagulase test and with pathogenicity, but in our experience there are many important exceptions to this generalization.

More recently, Weckman and Catlin[4], using a viscometric technique, showed that high deoxyribonuclease production was a characteristic of coagulase-positive

Staphylococci, and suggested that this property might be of use in their identification. Other workers[5-7], using the plate method of Jeffries *et al.*[8], have produced some evidence in support of this view.

In work recorded here we have investigated the relation between staphylocoagulase production and a number of other physiological activities. Colonial pigmentation was determined on glycerol monoacetate agar[1], and phosphatase activity on a slight modification of the medium used by Barber and Kuper[2]. A modified heated horse blood agar and a new fibrin agar medium were devised to detect proteolysis and fibrinolysis. Deoxyribonuclease activity was studied on an agar medium containing a final concentration of 0·2 per cent of the sodium salt of thymus gland deoxyribonucleic acid (B.D.H.). This was not autoclaved before its incorporation into the sterile basal medium. Full details of the preparation and use of these media will be described elsewhere.

Table 1 summarizes the results of tests on 227 coagulase-negative *Staphylococci* and 1,467 coagulase-positive strains. Results of tests for the phosphatase activity of 333 of these strains are shown in Table 2.

Table 1. REACTIONS OF 1,694 STRAINS OF *Staphylococci* OF HUMAN ORIGIN ON VARIOUS MEDIA

Staphylococci	Deoxyribonuclease activity		Fibrinolytic activity		Pigment on monoacetate agar		Proteolysis on heated blood agar	
	+	−	+	−	+	−	+	−
Coagulase-positive	1,467	0	971	496	1,467	0	826	641
Coagulase-negative	5 (weak)	222	1 (weak)	226	1	226	8 (weak)	219
Total	1,694		1,694		1,694		1,694	

Table 2. PHOSPHATASE ACTIVITY OF 333 STRAINS OF *Staphylococci* OF HUMAN ORIGIN

Staphylococci	Phosphatase activity on phenolphthalein phosphate agar		Total
	Phosphatase positive	Phosphatase negative	
Coagulase-positive	106	0	106
Coagulase-negative	190	37	227
Total	296	37	333

Apart from phosphatase production, coagulase-negative *Staphylococci* showed little activity against any of the substrates tested. Thus, only 5 of the 227 strains produced detectable amounts of deoxyribonuclease and even these caused only a weak reaction. Similarly, only one strain showed any fibrinolytic activity and only 8 were even weakly proteolytic. All but one strain failed to produce pigment, the colonies being porcelain white on glycerol monoacetate agar.

In contrast, the coagulase-positive organisms were, in general, highly active against the substrates utilized. All strains tested split phenolphthalein phosphate, all produced deoxyribonuclease and all formed pigmented colonies on glycerol monoacetate agar. Fibrinolysis was displayed by 66·3 per cent while 55·2 per cent were proteolytic on the modified heated blood agar medium. Within the coagulase-positive group, however, there is a well-defined and commonly encountered sub-group of organisms which are non-fibrinolytic, non-proteolytic and non-phage-typable, and in these respects resemble the saprophytic species. These organisms form a yellow, rather than an orange, pigment on glycerol monoacetate agar, and despite their similarities to *Staph. albus* are often of more serious epidemiological concern than are other varieties of *Staph. aureus* (see Jacobs *et al.*[9], Willis *et al.*[10]).

These results show that phenolphthalein phosphatase production, fibrinolysis and proteolysis are of little determinative value in separating the pathogenic from the saprophytic species. Deoxyribonuclease is produced by all coagulase-positive *Staphylococci* but by only a few strains of the coagulase-negative group. Thus the test for deoxyribonuclease production, which is simple to perform, has a useful place in the diagnostic laboratory for the recognition of potential pathogens. The parallel observed between the apparently dissimilar coagulase and deoxyribonuclease activities warrants further investigation.

Although no definite statement can be made about the role of deoxyribonuclease production in staphylococcal pathogenicity, it is of interest that all strains regarded as potentially pathogenic produce this enzyme in additon to staphylocoagulase. The ability of a staphylococcus to destroy deoxyribonucleic acid would seem to be more closely linked to a mechanism of pathogenesis than its capacity to coagulate blood plasma, having regard to the wide distribution of deoxyribonucleic acid in the host body, and its fundamental importance in the living cell. It is well known that some strains of *Staph. albus* cause infections in man despite their inability to form coagulase. It may well be that these are representatives of a group that forms detectable deoxyribonuclease.

S. I. JACOBS
A. T. WILLIS
GILLIAN M. GOODBURN

School of Medicine,
Leeds 2.

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Vibrio cholerae and Colicines

COLICINES originally demonstrated in *E. coli* have since been found in many other Gram-negative bacilli present in the intestine, *Ps. pyocyanea* and some Gram-positive bacilli[1-4]. Such antibacterial substances are now called bacteriocines[5]. It has also been confirmed that bacteria of different genera may produce a similar colicine[6] and that the range of antibacterial activity of colicines exhibits interesting specificities[3,4]. The possible role of bacteriophages, colicines and other factors producing disintegration of vibrios with subsequent liberation of toxin has been postulated to explain the pathogenesis of cholera[7] which prompted this investigation into the colicinogenicity and colicine-susceptibility of 60 strains of *V. cholerae* (45 Inaba and 15 Ogawa) and 15 strains of El Tor vibrios. Sixteen standard colicinogenic strains producing different types of colicines and 4 colicine-sensitive indicator strains were kindly presented by Prof. P. Fredericq of the University of Liège.

Twenty-nine strains of *V. cholerae* and 4 strains of El Tor vibrios were tested for production of colicine against other members of the species by the technique of Shannon[8]. This technique was at first standardized with known colicinogenic indicator strains in sheep blood agar plates. For the remaining 31 strains of *V. cholerae* and 11 strains of El Tor vibrios, the stab-technique of Fredericq[3] was used, as he found the technique of perpendicular streaks of Shannon was suitable only for the very actively colicinogenic strains. Each strain was tested against other members of the species and also against the known indicator strains, namely, *E. coli*, φ of Gratia, B, C and K-12.

Standard colicinogenic strains of various Gram-negative bacilli producing 16 different types of colicines, namely, colicines *A–K*, *V*, S_1–S_4 were also tested for their antibacterial effect on these vibrios by the same technique of Fredericq.

None of these 60 strains of *V. cholerae* and 15 strains of El Tor vibrios was found to produce bacteriocine-like antibiotic substances against other members of the same species or against the known colicine-sensitive strains of *E. coli*. All the vibrios were also found not to be susceptible to the known colicines produced by the common intestinal organisms using the experimental methods eminently suitable for demonstration of colicinogenicity and colicine-susceptibility of *E. coli* and other Gram-negative bacilli.

Bhaskaran[9], working on recombination of *V. cholerae*, found strains with fertility factor (P^+ strains) producing plaque-like clearings when tested on strains without fertility factor (P^- strains) under his experimental conditions which was not due to phage. The possibility of the clearing being due to bacteriocine was suggested by Prof. Lederberg. Farkas-Himsley and Seyfried[10] found lysogenic streptomycin-sensitive strains of *V. cholerae* under conditions of reduced redox potential undergoing lysis and liberating a substance lethal to their streptomycin-resistant mutants when tested aerobically. This inhibiting substance has been called vibriocin by them, as they excluded the possibility of its being a phage.

In spite of these observations, there seems to be little justification for postulating the possible role of colicines in the pathogenesis of cholera in the light of the findings reported here. Moreover, the hypothesis of the relation between production of colicines and pathogenicity does not find much support from the extensive investigations of Halbert[11] on the relation of antagonistic coliform organisms to *Shigella* infections.

D. Barua

Department of Bacteriology,
School of Tropical Medicine,
Calcutta, 12.

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CYTOLOGY

Preservation of the Ultrastructure of Plant Cells using Glutaric Acid Dialdehyde as a Fixative

Recently, Sabatini *et al.*[1] reported the use of glutaric acid dialdehyde (glutaraldehyde) and other aldehydes as fixatives for electron microscopy. Excellent preservation of the submicroscopic structure of pancreas and muscle cells was obtained when the tissues were fixed in 6·5 per cent glutaraldehyde. It was stressed that the value of this fixative lies in its application to ultrastructural cytochemistry, since it permits stabilization of the structural components of the cell without inactivating many of the enzymes.

Carasso and Favard[2] discuss in detail the chemical mode of action of formaldehyde as a fixative. Addition-reactions between formaldehyde and proteins and nucleic acids involve sulphydryl and amino-groups. The formation of methylene bridges between adjacent molecules can take place and aids in the stabilization of the structure. The reactions between the other aldehydes and the structural components of the cell are probably similar to those observed with formaldehyde. Preservation of ultrastructure may well depend on the reactivity of specific groups in proteins and nucleic acids with the aldehyde and on the ability of the aldehyde to form stabilizing cross-linkages. Since glutaraldehyde is a dialdehyde it could form two types of cross-linking bridges, involving either one or both of the aldehyde groups. Similarity between the fixation mechanism of formaldehyde and glutaraldehyde is suggested by the inability of glutaraldehyde to fix lipids. Accordingly, the absence of all cellular membranes is the most striking feature of the specimens fixed with glutaraldehyde.

Root-tips from etiolated seedlings of *Zea mays* were excised and fixed at 4° C for various lengths of time (0·5–12 h) in an aqueous solution of 4 per cent glutaraldehyde buffered at *p*H 7·8 in 0·028 M sodium veronal and sodium acetate. In some of the experiments a post-fixation in similarly buffered 1 per cent osmium tetroxide was carried out for 30 min at 4° C. The tissues were embedded in 'Vestopal *W*' and thin sections were stained for 1 h with half-saturated uranyl acetate.

A low-magnification electron micrograph of a section through the apical meristem of a maize root fixed in glutaraldehyde is shown in Fig. 1*a*. Three structural components can be recognized in the prominent nucleus: the nucleolus, the chromatin and the interchromatic substance. All three are granular in structure, and at higher magnification fine filaments can be observed to connect the granules. The nuclear envelope is absent, but its former location is marked by the chromatin with which it was closely associated. Small vacuoles are present, but the tonoplast is absent. Electron-dense bodies which are 0·3–0·4μ in diameter are identified as proplastids (Fig. 1*a*). They have undergone shrinkage during the process of fixation, dehydration and embedding. The surrounding void which they formerly occupied does not contain ribosomes. Thin fibrils extend perpendicularly through this region connecting the proplastids and the surrounding cytoplasmic structures. Although the proplastids contract and appear denser, ribosome-like particles can be recognized within them. Sometimes these particles occur in rows suggesting their former association with membranous structures.

A mitochondrion and a portion of the surrounding cytoplasm are shown in Fig. 1*b*. The mitochondria in the cells of the apical meristem can be identified by the electron-transparent areas in their centre. The existence of similar electron-transparent areas has been reported previously in plant mitochondria fixed by other methods[3,4]. The cristæ are poorly preserved; their presence is indicated by amorphous material, which has been tentatively identified as the proteinaceous components of the membranes. In addition to the structures which are interpreted to be cristæ, the mitochondria also contain filaments and ribosome-like particles. It should be noted that, unlike the proplastids, no shrinkage of the mitochondria has occurred. This may well be related to a difference of the matrices of both organelles. In the cytoplasm ribosomes can be recognized. They occur in small clusters or in rows, which suggest the former existence of ergastoplasm. A unique feature is the denser appearance of the ribosomes which are in the vicinity of the vacuoles. This may be due to a greater inherent density, possibly resulting from a contraction, or to an increased uptake of uranyl acetate. Whether this reflects a functional difference is not known.

The membranous components of the cell organelles can be retained if the tissue is post-fixed for 30 min in buffered osmium tetroxide. As a result of this double fixation the aspect of the membranes and other structural components is similar to that obtained with a normal osmium tetroxide fixation. Such a post-fixation was used in cytochemical work after a specific treatment for enzyme localization or the extraction of structural components with enzymes. A treatment consisting of an initial fixation in glutaraldehyde for 1 h or longer, followed by an incubation period in a pH 7 buffer at 37° C for 2 h and a post-fixation in osmium tetroxide, did not result in any visible damage to the cell structure. Some damage was observed if the initial fixation was only 0·5 h.

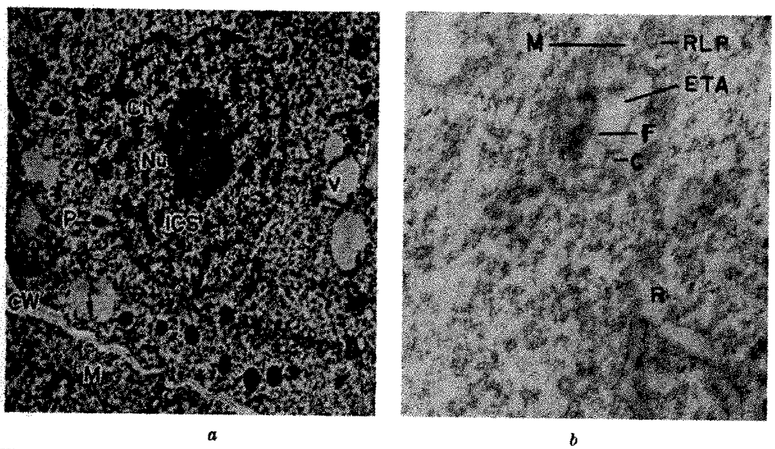

Fig. 1. Portion of a cell from the apical meristem of the root of ***Zea mays*** fixed for 2 h in 4 per cent glutaraldehyde buffered at pH 7·8. Thin sections were stained with half-saturated uranyl acetate for 1 h. *a*, Nucleolus (*Nu*); chromatin (*Ch*); interchromatic substance (*ICS*); vacuole (*V*); proplastids (*P*); mitochondrion (*M*); cell wall (*CW*). (× *c.* 6,600); *b*, ribosomes (*R*); mitochondrion (*M*) with electron-transparent area (*ETA*), crista (*C*), filaments (*F*) and ribosome-like particles (*RLP*). (× *c.* 30,000)

Cytochemical experiments revealed that the enzymes acid phosphatase, alkaline phosphatase and esterase are still active after a 90-min fixation in glutaraldehyde and can be localized in the tissue. The extraction of ribonucleic acid and deoxyribonucleic acid can be achieved with the corresponding nucleases after a fixation of 2 h. The Feulgen reaction can be applied to glutaraldehyde-fixed tissues embedded in 'Vestopal'. The interference of the fixative resulting in a light red background of the tissue does not affect the interpretation of the results. The removal of chromatin is complete in fixed specimens following a 3-h incubation period with deoxyribonuclease. Extraction of ribonucleic acid can also be achieved by treating the thin sections with 0·3 N potassium hydroxide at 37° C. After 3 h the ribosomes are completely removed and the electron density of the nucleolus is greatly decreased.

These preliminary results indicate that the ultrastructure of plant cells can be successfully preserved when glutaraldehyde is used as a fixative. Stabilization of the ultrastructure of the cell does not inactivate certain of the enzymes or prevent the extraction of some structural components. The interpretation of the cytochemical data is aided by a post-fixation with osmium tetroxide because it stabilizes the membranous structures and allows the structural components of the cell to be more readily recognized. It is believed that glutaric acid dialdehyde will be of considerable value for both cytochemical and structural studies of plant cells.

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Maarten J. Chrispeels*
A. E. Vatter

Department of Pathology,
University of Colorado Medical Center,
Denver 20, Colorado.

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*Present address: Department of Agronomy, University of Illinois, Urbana.

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Mitosis in Metamyelocytes

The metamyelocyte of bone marrow is usually classified with the more mature forms of granulocyte[1] and is considered to be in the maturation fraction[2,3] rather than in the proliferative group of bone marrow cells. There are numerous reports that the metamyelocyte is unable to divide, and that mitosis is confined to less-differentiated forms of granulocyte[2,4-8].

Under certain experimental conditions, we have found evidence of labelling in metamyelocytes of guinea pig bone marrow exposed to tritiated thymidine for only short periods of time (not exceeding 1 h). The tritiated thymidine S.A. 360 mc./mM (Schwarz Biochemical Inc., New York) was injected either intravenously or into the peritoneum. During 30 min–1 h after the injection the animal was anæsthetized with ether and samples of femoral marrow were removed and washed in homologous serum. Bone marrow smears were prepared, air-dried and fixed in methyl alcohol. The films were covered with Kodak '*A.R.*10' stripping film and after three weeks' exposure they were developed. The smears were stained through the film with a 0·1 per cent solution of new methylene blue at pH 5·5.

Tritiated thymidine is incorporated into newly formed DNA (ref. 9), and since synthesis of DNA precedes mitosis our observation of this process in metamyelocytes suggests that these cells do in fact have a proliferative capacity. We have identified metamyelocytes labelled with tritiated thymidine in the bone marrow (Fig. 1 *a*, *b*, *c*, *d*) of guinea pigs three days after the onset of an acute anæmia due to loss of blood. In each animal approximately 12 ml. of blood was withdrawn and this caused the red cell count to fall from about 5,000,000 per c.mm. to approximately 2,500,000. Labelled metamyelocytes have also been seen in the bone marrow of young guinea pigs recovering from sub-lethal whole-body γ-irradiation[10]. Apart from the experimental conditions, labelling has been seen in metamyelocytes of normal bone marrow.

Cronkite *et al.*[11] used tritiated thymidine to study the proliferation of hæmopoietic cells and found that labelling in metamyelocytes did not appear until three hours after the injection. They concluded that the metamyelocyte is a non-dividing cell. In our investigations of anæmic and irradiated guinea pigs the labelling of metamyelocytes occurred within 1 h of injecting the label. This interval[12] excludes the possibility that the metamyelocytes are labelled because they are the daughter cells of labelled progenitors. The possibility is also excluded by the absence of labelled mitotic figures in the smears (Fig. 1*e*).

The presence of mitotic figures in metamyelocytes would provide additional evidence that these cells can in fact divide. These figures would have to be observed at a very early stage of mitosis when the typical shape of the metamyelocyte nucleus, as seen in the non-dividing cell, is still present. Figures of this type are rarely seen since the duration of this phase appears to be very short. In the later stages of mitosis when the shape of the metamyelocyte is lost, it would be virtually impossible to distinguish between a myelocyte or a metamyelocyte in mitosis. A metamyelocyte nucleus in an early stage of division is illustrated in Fig. 1*f* and its shape may be compared with that of the resting nucleus shown in Figs. 1 *a*, *b*, *c*, *d* and *g*.

How may the present findings be interpreted? It could be suggested that the label is taken up by cells which are myelocytes at the time of uptake but that they differentiate into metamyelocytes in the short interval

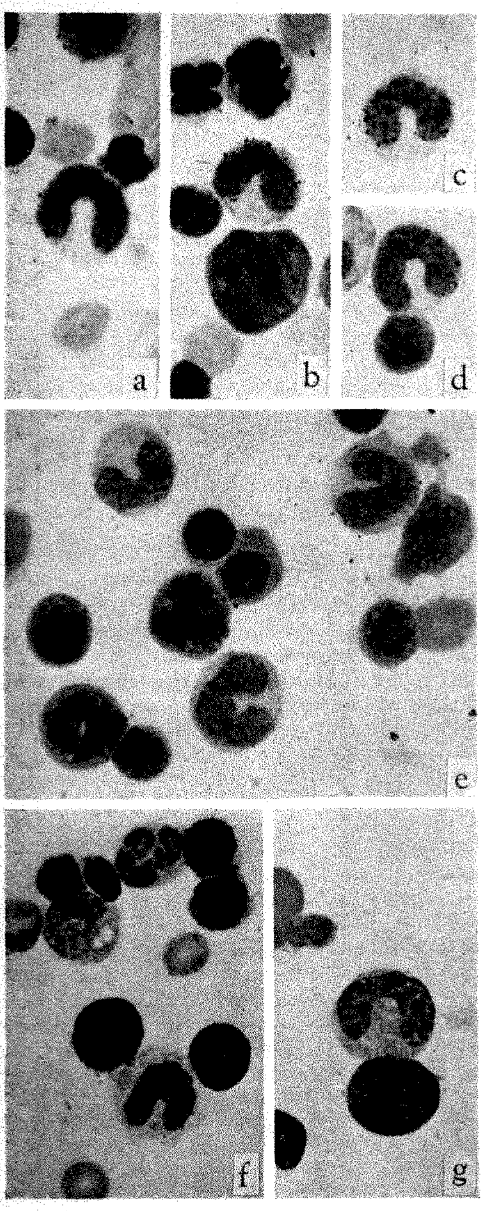

Fig. 1. *a, b, c, d*, Metamyelocytes in smears of guinea pig bone marrow labelled with tritiated thymidine; *e*, absence of labelling in mitotic figures; *f*, a metamyelocyte nucleus in an early stage of division; *g*, metamyelocyte nucleus in resting phase. *a, b, c, d, e*, Cells stained new methylene blue; *f, g*, cells stained MacNeal's tetrachrome. (× *c*. 1,166)

between injection of the label and the preparation of marrow smears. In the experiments recorded here, assuming that there is an immediate uptake of thymidine, the maximum time available for this differentiation would be no more than 1 h. This transformation appears to be improbable from the studies of Patt and Maloney[13], who found that approximately 11 h are required for the transition of a myelocyte into a metamyelocyte in the dog.

It might also be suggested that the evidence of proliferation in metamyelocytes has chiefly been seen under conditions which call for an increase in the production of cells. In these circumstances, if there is a common progenitor for both granulocytes and red cells, there might be competition for a basic stem cell. In the anæmic animals there would be a considerable demand for stem cells to differentiate into the erythroid series, and this might curtail the number of stem cells available for granulopoiesis. Under these conditions metamyelocytes might be stimulated to divide to help maintain the granulocyte population. However, proliferation appears to be a property of normal metamyelocytes since we have seen synthesis of DNA in metamyelocytes of normal bone marrow.

It seems reasonable to postulate that mitosis might still occur in some metamyelocytes since these cells are intermediate in differentiation between the proliferative myelocytes and the non-proliferative stab granulocyte. Moreover, in stage of differentiation the metamyelocyte appears to be comparable with the polychromatic erythroblast, and it is generally agreed that this cell can divide. We do not claim that all metamyelocytes are able to divide, and, at the present, we have no information on the proportion which do so.

P. F. Harris
J. H. Kugler

Department of Anatomy,
University of Sheffield.

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GENETICS

Deoxyribonucleic Acid and the Ancestry of Wheat

Rees[1] has suggested that data relating to the DNA content of nuclei may provide a further clue regarding the parental composition of allopolyploid plants like *durum* and bread wheats. From Rees's results[1] it is clear that *Aegilops speltoides* is more likely to be the donor of the *B* genome of tetraploid wheat than the other members of the *Sitopsis* section of *Aegilops*. With the view of understanding: (*a*) the relationship between somatic chromosome length and DNA content; (*b*) the extent of variation in DNA content of different varieties and species of *Triticum* and *Aegilops*, we estimated the DNA content of mitotic prophase nuclei using a microspectrophotometer constructed on the two wave-length principle of Ornstein[2] and Patau[3]. We preferred prophase nuclei to interphase nuclei since inter-slide differences were less in the former. When prophase nuclei were used in measurements, we did not find chromatin diminution to the extent inferred earlier[4]. Examination of chromosome-length and DNA content indicated a definite relationship between the two[4,5]. The DNA values of *Triticum* and *Aegilops* species (Table 1), however, did not lend themselves to interpretations such as those which Rees[1] could draw.

While Rees[1] found the DNA content of *A. speltoides* to be significantly lower than that of *A. bicornis* and *A. longissima*, our results reveal no appreciable difference. Among tetraploid species Rees[1] used only *T. durum*, and if the differences in DNA values recorded by us between the more primitive *T. dicoccoides* and *T. dicoccum* on one hand and the free-threshing *T. durum* on the other are confirmed, the types of extrapolation as carried out by Rees[1] may become difficult since the parental species

Table 1. MEAN DNA CONTENT (IN ARBITRARY UNITS) OF 4c NUCLEI IN SPECIES OF *Triticum* AND *Aegilops*

Species	2*n*	No. of measurements	Mean DNA content in arbitrary units ± *S.E.*	Coefficient of variance
T. monococcum strain 1	14	115	28·06 ± 0·36	13·8
T. monococcum strain 2	14	51	26·89 ± 0·54	14·3
T. aegilopoides	14	73	27·73 ± 0·47	14·5
T. dicoccoides	28	76	53·18 ± 0·84	13·8
T. dicoccum	28	84	54·39 ± 0·82	13·8
T. durum	28	100	59·36 ± 0·85	14·3
T. aestivum var. Chinese Spring	42	108	75·39 ± 1·03	14·2
T. aestivum var. *C*-591	42	102	71·15 ± 0·60	8·5
Ae. speltoides var. *ligustica* strain 1	14	25	29·46 ± 0·89	15·1
Ae. speltoides var. *ligustica* strain 2	14	90	29·43 ± 0·48	15·5
Ae. bicornis	14	71	30·16 ± 0·59	16·5
Ae. longissima	14	64	29·85 ± 0·62	16·6
Ae. squarrosa	14	54	25·09 ± 0·45	13·2

involved would have to vary in the different tetraploids. The two varieties of *T. monococcum* and *T. aestivum* examined also showed a difference. The coefficient of variance values are rather high in our investigations, as well as those of Rees[1], and a higher order of precision in measurement will enable the use of the mean values with greater confidence. While the DNA content of nuclei would undoubtedly provide additional information of great value in tracing the phylogeny of hybrids and polyploids, an extensive survey of the extent of variability in DNA content occurring within a group of related species would have to precede the use of DNA values for such a purpose. The presence of heterochromatic segments may also influence DNA values[6,7], although in *Triticum* this may not be a complicating factor[4,5].

M. D. UPADHYA
M. S. SWAMINATHAN

Division of Botany,
Indian Agricultural Research Institute,
New Delhi, 12.

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Effects of *n*-Butyl Methanesulphonate and Related Mutagens on Barley

MANY types of alkylating mutagens had been applied to barley before the mutagenicity of sulphonates was observed. Ethyl methanesulphonate (EMS) was the first tested and is now widely used in mutation research. The related compounds methyl methanesulphonate (MMS) (refs. 1, 3, 4), *n*-propyl methanesulphonate (*n*PMS) (refs. 1–3), isopropyl methanesulphonate (isoPMS) (ref. 3) and others also have been shown to be mutagenic on barley.

This communication presents preliminary results of the effects of alkyl sulphonates not previously reported. These agents are *n*-butyl methanesulphonate (*n*BMS) and isobutyl methanesulphonate (isoBMS). Results of treatments with MMS, *n*PMS, and isoPMS are included and compared with those obtained recently with EMS, in order to evaluate the influence of the different alkylating groups on these compounds. The biological responses induced by these chemical mutagens are compared with those obtained by X-radiation.

The sulphonates used, except MMS which was acquired from Eastman Organic Chemicals, were synthesized and tested for purity in the laboratory of the Department of Agricultural Chemistry at this University.

Seeds of hull-less Himalaya barley were stored in desiccators over saturated salt solutions for 2–4 weeks to obtain a moisture content of 14 per cent. The chemicals were applied by immersion of the barley seeds in aqueous solutions buffered at *p*H 7 with 0·2 M *tris* (2-amino-2-(hydroxymethyl)-1,3-propanediol) at 20° C for times according to treatment design. The treatment solutions of isoPMS only were changed every 50 min because this compound hydrolysed much faster than the others. After the chemical treatment the seeds were soaked in distilled water for 5 h at 20° C to reduce delayed action of the mutagens.

The biological responses of barley seeds to the various agents were measured by the percentage of seedling growth of treated versus control plants, the rate of survival (plants with germinating M_2 seedlings relative to the number of M_1 seeds planted), and the fertility, estimated from the average number of M_2 seedlings per M_1 spike. Chromosome aberrations were scored in 200 mitotic cells of shoot-tips of germinating M_1 seeds and 100 microsporocytes of field-grown M_1 plants from each treatment. The frequency of mutations was estimated from the number of chlorophyll-deficient mutations in M_2 seedling progenies.

The results (Table 1) show that all the sulphonates examined induce chlorophyll-deficient mutations. In all cases, the mutations were induced by treatments that produced low rates of damage to seedling growth and plant survival and to chromosome structure. The new chemical mutagen, *n*BMS, appears to be about as efficient as the related ethylating compound EMS, particularly since higher doses than those investigated can be applied (Froese-Gertzen, unpublished results).

Data for isoBMS were not listed in Table 1 since a treatment with 0·02 M concentration for 32 h at 20° C was not sufficient to induce either noticeable damage to seedling growth and survival rate or an appreciable number of genetic changes. However, subsequent investigations of the chemical reactivity of this compound revealed that the hydrolysis rate of isoBMS is only 1/6 that of *n*BMS. It is expected that a treatment adjusted to the slow reactivity will induce an increased mutation rate.

Treatments of barley seeds with *n*PMS were reported earlier by Ehrenberg, who obtained chlorophyll-deficient mutations in 26 per cent (ref. 1) and 12 per cent (ref. 2) of the spikes analysed. The conditions for these treatments were not described. In the experiments with *n*PMS reported by Heslot and Ferrary[3] a higher concentration than 0·02 M was used for a soaking time comparable to 30 h at 20° C, inducing mutations in 8·3 per cent of the spikes. The

Table 1. EFFECTS OF ALKYLATING SULPHONATES ON BARLEY

Agent	Treatment Concentration	Treatment Time	% Seedling growth	% Survived M_1 plants	M_2 Seedling per M_1 spike	Chromosome aberrations in mitosis % Bridges	Chromosome aberrations in mitosis % Fragments	Chromosome aberrations in meiosis % Transloc.	Mutations per 100 M_1 Plants	Mutations per 100 M_1 Spikes	Mutations per 100 M_2 Plants
*n*BMS	0·021	26	91	80	20	2·5	12	2·0	112	28	3·8
*n*PMS	0·02	30	90	78	28	1·0	15	×	51	13	1·2
MMS	0·0023	12	75	65	29	0	42	1·0	21	5	0·5
isoPMS	0·0063	10	93	76	12	1·0	15	2·5	64	20	3·4
EMS	0·02	20	89	77	12	2·0	14	×	103	35	5·0
X-rays	30 kr.		89	77	20	52	395	×	29	8·0	1·2
Water		30	100	79	32	×	×	×	0	0	0

Treatment at 20° C in aqueous solutions buffered at *p*H 7 with 0·2 M *tris*, followed by soaking in distilled water for 5 h at 20° C
Based on four replications with 200 seeds each.

mutation frequency of 13 per cent per M_1 spikes obtained in our studies does not appear to represent the optimum mutagenic efficiency of this compound since the treatments applied had little effect on seedling growth and survival

The mutation frequency of 5 per cent per M_1 spikes reported here for MMS is low in comparison with the results of Minocha and Arnason[5] (32 5 per cent), Ehrenberg[1] (16 per cent), and Heslot and Ferrary[3] (7 8 per cent) However, it seems possible that higher doses may increase the mutation rate to a level comparable with that reported by other investigators The concentration used by Minocha and Arnason was ten times higher than that in the experiments of Heslot and Ferrary[3] and the work recorded here However, it is difficult to understand that a treatment time for 2 h at 20° C (used by Minocha and Arnason[5]) was sufficient for diffusion of the mutagen solution into the seeds and for the induction of 32 5 per cent mutations, unless most of the mutations were caused by delayed action of the mutagen MMS as well as other methylating sulphonates used in recent investigations on barley appear to be more toxic, that is, methylating compounds cause more physiological damage and death at similar doses than other sulphonates, so that 1/10 of the concentration had to be used (Froese-Gertzen, unpublished results)

The exploration of isoPMS as a mutagen for barley was initiated by determining its chemical reactivity The first order hydrolysis rate constant (6 4 × 10^{-3}/min at 20° C) was similar to that of diethyl sulphate (3 45 × 10^{-3}/min at 20° C) (ref 4), and similar treatment conditions were developed Accordingly, the 0 0063 M isoPMS solution was renewed every 50 min after 30 per cent of hydrolysis had occurred Thus, these treatments cannot be compared with those of Heslot and Ferrary in which 0 055 M solutions were applied without renewal for 24 h at 24° C and in which 10 per cent mutated M_1 spikes were obtained The higher mutation rate of 20 per cent observed for isoPMS in our experiments is probably due to the fact that a higher concentration of mutagenically active agent was available when the solution penetrated into the germinal tissue.

EMS has repeatedly been reported to be a 'good mutagen' on barley Some recent results obtained with EMS are cited here for comparison with the other sulphonates This comparison shows that *n*BMS, *n*PMS and isoPMS especially merit further investigation

Data obtained from barley seeds (14 per cent moisture content) exposed to 30 kr of X-rays at room temperature show that the induction of mutation in 8 per cent of the spikes is associated with chromosome aberrations of 53 bridges and 309 fragments per 100 cells This level of injury to chromosome structure, as well as to seedling growth and plant survival in relation to the amount of induced mutation, demonstrates the lower mutagenic efficiency of radiation compared with that of most chemical mutagens investigated here

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EDITH E FROESE-GERTZEN
R A NILAN
C F KONZAK
R R LEGAULT

Departments of Agronomy and Chemistry,
Washington State University,
Pullman.

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VIROLOGY

Effect of Ether on Rabies Virus

Andrewes and Horstmann[1] grouped rabies virus as ether resistant, based on reports of other investigators[2,3] During a recent investigation in this laboratory, strains of arctic rabies virus lost 95–97 per cent of their infectivity following exposure to 20 per cent ether at 4° C for 16 h This observed reduction in infectivity and the variations in reported methods for determining the effect of ether on rabies virus led to investigations comparing the action of ether on different strains of rabies virus

Five strains of rabies virus were tested 2 arctic strains isolated from foxes from Greenland, a bat rabies virus isolated from a Mexican freetail bat, a street virus obtained from a naturally infected cat, and fixed virus (CVS–25) A 20 per cent infected brain suspension prepared with 10 per cent egg-yolk diluent was mixed with anæsthetic ethyl ether (20 per cent by volume) and incubated at 4° C for 16 h Ether was removed by evaporation in an open Petri dish Control specimens without ether were tested simultaneously Serial ten-fold dilutions were prepared, and 0 03 ml of each dilution was inoculated intracerebrally into five 3-week-old mice

Table 1 EFFECT OF 20 PER CENT ETHER ON DIFFERENT STRAINS OF RABIES VIRUS AT 4° C FOR 16 H

Virus strain	Titre (MLD_{50})		Reduction in infectivity	
	Control	Treated	Logs	(%)
Arctic 209	3 16	1 49	1 67	97 9
Arctic 219	4 49	3 15	1 34	95 4
Street rabies	4 49	2 5	1 99	99 0
Bat rabies	4 0	2 83	1 17	93 2
Fixed (CVS)	6 3	4 0	2 3	99 5

Table 2 EFFECT OF VARIOUS CONCENTRATIONS OF ETHER ON ARCTIC RABIES VIRUS AT DIFFERENT TIME-INTERVALS AND TEMPERATURES

Concentration of ether (%)	MLD_{50} titre of virus	
	37° C for 2 h	4° C for 16 h
5 0	3 49	3 49
10 0	1 49	2 88
20 0	<1 0	2 63
Control	3 67	3 63

Results are shown in Table 1 Loss in infectivity varied from 93 2 to 99 0 per cent with the field strains Reduction in fixed virus infectivity was shown to be slightly greater (99 5 per cent)

In one experiment the effect of anhydrous ether on fixed virus was compared with that of anæsthetic ether, with no apparent difference

The effects of various concentrations of ether at different time-intervals and temperatures were examined with one arctic strain The results are given in Table 2 The quantitative reduction in virus infectivity was shown to range from 0 18 logs (12 6 per cent) with 5 per cent ether to greater than 2 67 logs (99 8 per cent) with 20 per cent ether at 37° C for 2 h

Andrewes[4] has also recently demonstrated fixed rabies virus to be ether sensitive

The property of ether sensitivity supports the recent suggestion that rabies virus may be related to the myxovirus group[5,6]

ROBERT A CRANDELL
WILLIAM D HILLIS

U.S Air Force Epidemiological Laboratory,
Aerospace Medical Division,
Lackland Air Force Base, Texas

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Cultivation of Horsesickness Virus in Tissue Culture

Nieschulz[1] and Alexander[2] independently showed that horsesickness virus multiplies in the brains of Swiss albino mice. Alexander[3] reported later the successful cultivation of mouse-adapted horsesickness virus in the brains of guinea pigs, rats and other rodents. He also succeeded in cultivating a mouse-adapted strain of virus in embryonated hen's eggs, in which multiplication of the virus occurred in the brain of the embryo without producing any specific mortality[4]. McIntosh[5] propagated viscerotropic horsesickness virus strains in 8-day-old embryonated hen's eggs at an optimum temperature of 32° C. Embryo mortality occurred regularly during the initial passages.

This communication describes the successful propagation of horsesickness virus in tissue culture. The third mouse brain passage of a type 3 virus strain (designated as 13/63), with a mouse LD_{50} titre of $10^{-6·0}$, was selected. After removal of the nutrient medium, roller tubes containing monolayers of chicken embryo fibroblasts were each seeded with 0·2 ml. of a 1 per cent suspension of the mouse brain antigen in Hanks's balanced salt solution. The inoculated tubes were rolled at 37° C for 30 min and the monolayers were then washed. Finally, each tube received 1·0 ml. of medium consisting of Hanks's balanced salt solution containing 0·5 per cent lactalbumen hydrolysate, 10 per cent bovine amniotic fluid and 2 per cent bovine serum. The tubes were kept in roller drums at 37° C and were examined daily under low magnification. No cytopathic effects were observed.

At daily intervals the supernatant fluid plus macerated cells of two tubes, selected at random, were titrated in day-old mice by intracerebral inoculation of decimal dilutions. The virus titres were calculated by the method of Reed and Muench[6] and expressed as the negative logarithm of the dilution end-points. Table 1 represents the virus titres of the first three serial passages in chicken embryo fibroblasts.

Table 1

T C passage	Mouse LD_{50} of tissue culture material on days after seeding							
	1	2	3	4	5	6	7	8
1st	4·0	4·0	3·5	3·7	3·0	2·9	2·5	4·0*
2nd	—	—	1·0	1·0	1·5*			
3rd	0	4·0	4·0	4·0	4·0	5·0*		

* Material used for passage

The specificity of the mortality in mice was controlled by a complement fixation test[7] on the brains of mice sick after inoculation with material from the third and fifth tissue culture passages.

With the sixth passage the growth curve of the virus in tissue culture was determined. For this purpose a monolayer of chicken embryo fibroblasts in a 100-ml. medicine flat bottle was used. The culture was seeded as described before using 1·0 ml. of the fifth tissue culture passage of virus (titre $10^{-4·0}$ mouse LD_{50} per 0·03 ml.) as seed material. After adsorption, the monolayer was washed, 10 ml. of medium was added and the bottle incubated at 37° C. Aliquots of 1·0 ml. of supernatant fluid were removed daily from the bottle and titrated in day-old mice. One ml. of fresh medium was used to replace the sample removed.

In a parallel experiment a number of roller tubes were also seeded with virus as before and four tubes were collected daily. The supernatant fluids from each group of four tubes were collected, pooled and centrifuged to remove cellular material. The monolayers were washed three times with Hanks's balanced salt solution and then macerated in a volume of medium corresponding to the volume of supernatant fluid removed. Each sample of supernatant fluid and cells were titrated separately in mice. The results are summarized in Table 2.

Table 2

Culture	Mouse LD_{50} of T C material on days after seeding					
	1	2	3	4	5	6
Bottle-100 ml. flat	0	2·5	3·8	4·2	3·7	3·9
Tubes (*a*) Cells	0	2·5	3·8	4·8	4·1	4·5
(*b*) Supernatant fluid	0	1·2	3·7	4·0	3·8	4·2

Although no specific cytopathic changes were noticed during six serial passages of the virus, it is quite evident from Tables 1 and 2 that monolayers of chicken embryo fibroblasts supported the growth of horsesickness virus under the conditions described. An appreciable amount of virus appeared to be cell bound.

By using the foregoing method, a type 4 horsesickness virus strain (designated as 32/62) in its second mouse brain passage has also been adapted to multiply in chicken embryo tissue culture, and work is now in progress to adapt strains of the remaining seven types of horsesickness virus and to determine optimal conditions of growth with special reference to cell type, *p*H and temperature of incubation.

I thank Dr. K. E. Weiss, head of the Virology Section of this Institute, for advice.

B. J. Erasmus

Section of Virology,
Veterinary Research Institute,
Onderstepoort,
South Africa

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STATISTICS

Generalized Inverse of a Singular Matrix

In a recent communication[1] a technique for obtaining a generalized inverse of a singular matrix $X = \begin{bmatrix} A & B \\ C & D \end{bmatrix}$, where the square sub-matrices A and D are non-singular, has been given. The g-inverse is derived in two steps, the first involving the calculation of an approximation, and the second its correction.

Dr. M. Zelen (private communication) has pointed out that in the expressions for the sub-matrices, corresponding to those of X, which correct the approximate g-inverse, two sub-matrices $(BD^{-1}C-A)$ and $(CA^{-1}B-D)$, which are both singular, are involved. That they are singular can be proved as follows.

Let (γ, δ) be a row vector such that the constituent vectors γ and δ are non-zero, and their lengths are equal to the number of rows in A and D respectively. Then since X is singular

$$(\gamma, \delta) \begin{bmatrix} A & B \\ C & D \end{bmatrix} = 0 \qquad (1)$$

where 0 is a null vector of the same length as (γ, δ). Equation (1) yields the conditions $\gamma(BD^{-1}C-A) = 0 = \delta(CA^{-1}B-D)$, and since γ and δ are non-zero vectors, $(BD^{-1}C-A)$ and $(CA^{-1}B-D)$ must be singular.

However, despite this circumstance, the approximate g-inverse can still be corrected by using in the expressions for the correction matrix (already given by me) the approximate g-inverses of these singular sub-matrices.

J. C. Koop

Department of Experimental Statistics,
University of North Carolina,
Raleigh

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 18

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S W 7), at 5 p.m.—Symposium on "Denudation in Limestone Regions"

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London, W C 1), at 5 p m —Dr M H Richmond "Biological Activity of Structural Analogues" (Further lecture on November 25)*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Mr G B Townsend "The Colour Performance of the Secam Colour Television System"

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Mr F P W Winteringham "Insect Cross-resistance Phenomena Their Practical and Fundamental Implications".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 6 p m.—Mr Hulme Chadwick "One Man's Colour" (First of three Cantor Lectures on "Colour in Modern Life")

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W C 2), at 6 30 p m —Mr M. T. Hills "Electronic Telephone Exchanges"

Tuesday, November 19

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (joint meeting with the INDUSTRIAL WATER AND EFFLUENTS GROUP, at 14 Belgrave Square, London, S W 1), at 10 30 a m —Meeting on "Agricultural Effluents"

UNIVERSITY OF LONDON (at Imperial College, London, S W 7), at 1 30 p m —Mr D E Broadbent "The Input—How We See and Hear" (Third of five lectures on "The Basis of Experimental Psychology")*

UNIVERSITY OF LONDON (at the Royal Institution, Albemarle Street, London, W 1), at 5 p m —Prof Jacques Monod (Institut Pasteur, Paris) "Controlling Elements and Regulatory Circuits in Cellular Metabolism and Growth" *

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr Harold Scrutton and Mr B J O'C Donelan "Some Features of the Civil Engineering Work in Cammell Laird's Shipyard".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Prof F C Williams, F R S , Dr J F Eastham and Mr L S Piggott "Analysis and Design of Pole Change Motors using Phase-Mixing Techniques", Mr C St J Lamb "Commutatorless Alternating Voltage-Fed Variable-Speed Motor"

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "A New Approach to Teaching Reliability" opened by Mr R C Winton

UNIVERSITY OF LONDON (at Imperial College, London, S W 7), at 5 30 p m —Prof A R Hall "The Historical Relations of Science and Technology" (Inaugural Lecture)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr R P Michael "Neurological Mechanisms and the Control of Sexual Behaviour" (Tenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Is Machine Development Outstripping Man's Capabilities ?"

PLASTICS INSTITUTE, LONDON SECTION—ENGINEERING SUB-GROUP (at the Wellcome Building, Euston Road, London, N W 1), at 6 30 p m —Mr. J R Baines and Mr P M George "Developments in Extrusion Techniques for Unplasticized P V C "

Tuesday, November 19—Wednesday, November 20

INSTITUTION OF GAS ENGINEERS (at Church House, Westminster, London, S W 1), at 9 30 a m daily—29th Autumn Research Meeting

Wednesday, November 20

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 2 p m —Dr P Walker "Biochemical Procedures in the Differential Diagnosis of Metabolic Bone Disease" *

PLASTICS INSTITUTE (at Brunel College, Woodlands Avenue, Acton, London, W 3), at 2 30 p m —Dr C L Child "Some Factors Affecting Products Design" (Students' Lecture)

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 2 30 p m —Mr D I Lawson "Research into Fire Protection"

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W 1), at 5 p m —Dr M H P Bott "Gravity Measurements on the North-East Part of the Irish Sea", Mr C S Hutchison "A Gabbro-Granodiorite Association in Singapore Island" (to be read by Prof J Sutton).

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 15 p m —Dr D J Newell "Statistical Aspects of the Demand for Maternity Beds"

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W 1), at 5 30 p m —Mr M J Van der Zijden and Mr S R Hills "Control of Gasoline Quality in Relation to Inlet System Deposits"

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the SCIENCE AND GENERAL DIVISION, at Savoy Place, London, W C 2), at 5 30 p m —Dr D N Truscott "Computers in Control of Processes—the Coming Revolution in Industry".

SOCIETY OF APOTHECARIES OF LONDON, FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY (at Apothecaries' Hall, Blackfriars Lane, London, E C 4), at 5 30 p m —Prof Alan Kekwick "The Soho Cholera Epidemic, 1854".

BRITISH INSTITUTION OF RADIO ENGINEERS, EDUCATION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Mr R A T Howell, Group Captain J H Stevens, Mr W K Newson and Mr H Arthur "Systematic Selection Procedures for Technical Courses".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Prof J Z Young, F R S 'Measuring and Computation by Brains and Machines" (Thomas Hawksley Lecture)

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the SOUTH-WEST ESSEX TECHNICAL COLLEGE CHEMICAL SOCIETY, at the South-West Essex Technical College, Forest Road, Walthamstow), at 7 p m —Dr P N Campbell "The Biosynthesis of Proteins"

Thursday, November 21

REINFORCED CONCRETE ASSOCIATION (joint meeting with the SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP, at the Institution of Structural Engineers, 11 Upper Belgrave Street, London, S W.1), at 2 30 p m —Meeting on "Lightweight Aggregates"

ASLIB (at 3 Belgrave Square, London, S W 1), at 5 p m —Mr S B Page "The Production of Library Publications"

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Dr C W Nutt and Dr K Bromley "Conditions for the Flotation of Beryl", Mr R. Greenberg "Structure-Gold Relationships as Revealed by some Witwatersrand Mine Survey Plans"

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W 1), at 5 p m —Dr Léon Croizat "Problems of Panbiogeography"

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Annual General Meeting Dr M L Cartwright "From Non-linear Oscillations to Topological Dynamics" (Presidential Address)

INSTITUTION OF CIVIL ENGINEERS, TRAFFIC ENGINEERING GROUP (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Informal Discussion on "Integration of Motorways in Urban Areas" introduced by Dr T E H Williams.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m.—Dr M Shepherd "Epidemiology and Mental Disorder" (Eleventh of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W 1), at 6 p m —Scientific Papers

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S E 1), at 6 p m —Informal Discussion on "Pneumatic v Electronic Controls"

OCCAM SOCIETY (at the Northampton College of Advanced Technology, St John Street, London, E C 1), at 6 15 p m —Discussion on "The 5000 Year Leap (Technology, Social Science and the Underdeveloped Countries)" opened by Dr E T Sherwood

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 30 p m —Mr P Irving "Modern Trends in Two-stroke Petrol Engine Design"

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 7 30 p m —Laboratory Meeting

Thursday, November 21—Saturday, November 23

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W 1), at 10 30 a m daily—Discussion Meeting on "The Physical and Chemical Basis of Muscular Contraction" organized by Prof A F Huxley, F R S , and Dr H E Huxley, F R S

Friday, November 22

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINE GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 7 p m —Mr L S Snell "The Design and Evolution of an Aircraft Rocket Engine".

Friday, November 22—Saturday, November 23

BRITISH SOCIETY FOR IMMUNOLOGY (at the Wellcome Building, 183 Euston Road, London, N W 1), at 10 a m daily—Autumn Meeting Prof. Astrid Fagraeus (Sweden) "Mixed Hæmadsorption—a New Antibody Tracing Technique and some of its Applications" (Prausnitz-Giles Lecture)

Saturday, November 23

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Dr Bengt Danielsson "The Islands of the South Seas in the Modern World".*

Monday, November 25

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C 2), at 2 30 p m and 5 30 p m —Colloquium on "Recent Advances in D C Amplifiers"

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 4 p m —Prof A W Woodruff "Recent Advances in Tropical Medicine" *

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Is Load Shedding Necessary and How Can Its Effects be Minimized ?" opened by Mr A E Hawkins

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Dr E Schatzman (Paris) "Nuclear Reactions at the Surface of Stars and Stellar Evolution" *

BRITISH MEDICAL ASSOCIATION (at the National Hospital for Nervous Diseases, Queen Square, London, W C 1), at 6 p m —Dr Macdonald Critchley, F R C P "The Problem of Visual Agnosia" (Twelfth Victor Horsley Memorial Lecture) *

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 6 p m —Dr. T Vickerstaff "Scientific Aspects" (Second of three Cantor Lectures on "Colour in Modern Life")

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

GRADE B LECTURER (with an honours degree) IN MATHEMATICS IN THE DEPARTMENT OF MATHEMATICS AND PHYSICS—The Director of Education, The Polytechnic, 309 Regent Street, London, W 1 (November 22)

LECTURER (medical or non-medical graduate) IN ANATOMY—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W C 1 (November 23)

LECTURER (Grade II) IN PSYCHOLOGY—The Registrar, The University, Bristol (November 25)

LECTURER or ASSISTANT LECTURER AT THE TIDAL INSTITUTE AND OBSERVATORY—The Registrar, The University, Liverpool, quoting Ref CV/422 (November 25)

JUNIOR LECTURER, LECTURER or SENIOR LECTURER IN PHYSICS at Massey University College of Manawatu, Palmerston North, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (New Zealand and London, November 29)

BIOCHEMIST (with an honours degree in biochemistry or equivalent) IN THE DEPARTMENT OF STEROID BIOCHEMISTRY, to study the biosynthesis and metabolism of androgenic steroids—The Secretary of University Court, The University, Glasgow (November 30)

LECTURER (graduate in science with qualifications in botany or agricultural botany, and preferably with training in crop or plant physiology) IN BOTANY—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C 2 (November 30)

SENIOR DEMONSTRATORS (2) (with a science degree with physics as a major subject, preferably with honours, and some teaching experience) IN PHYSICS at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, November 30)

CHAIR OF ZOOLOGY at the University of Malaya—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Kuala Lumpur, December 7)

LECTURER IN ORGANIC CHEMISTRY at QUEEN'S COLLEGE, Dundee—The Secretary, University of St Andrews, Queen's College, Dundee (December 7)

LECTURER (with special interest in one or more of the following fields soils, conservation or geomorphology) IN GEOGRAPHY—The Secretary, University College, Gower Street, London, W C 1 (December 7)

ASSISTANT KEEPER (Grade I or Grade II) IN THE DEPARTMENT OF ZOOLOGY—The Secretary, National Museum of Wales, Cardiff (December 10)

CHAIR OF BIOLOGY (with special reference to ecology)—The Secretary, University of Lancaster, Bailrigg House, Lancaster (December 14)

SENIOR LECTURER IN PHYSICAL CHEMISTRY at the University of Western Australia—The Registrar, University of Western Australia, Nedlands, Western Australia, or The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 14)

CHAIR OF PHYSIOLOGY at St Mary's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W C 1 (December 17)

LECTURER IN GEOCHRONOLOGY AND ISOTOPE GEOLOGY IN THE DEPARTMENT OF GEOLOGY—The Registrar The University, Leeds 2 (January 1)

MASTER TO TEACH BOTANY to Scholarship level (any other science subject, particularly chemistry, would be welcome)—The Headmaster, Blundell's School, Tiverton, Devon

POST-DOCTORAL RESEARCH ASSISTANT, for work in the field of colour centres in the alkali halides—Dr P W M Jacobs, Department of Chemistry, Imperial College, London, S W 7

RESEARCH STUDENT (with a good honours degree) IN THE DEPARTMENT OF HUMAN BIOLOGY AND ANATOMY, for developing methods of fixation and embedding for electron microscopy—Prof R Barer, The University, Sheffield

RESPIRATORY PHYSIOLOGIST, for work mainly in the wide-open field of high-pressure physiology—The Superintendent, Royal Naval Physiological Laboratory, Alverstoke, Hants

TECHNICIAN or STUDENT TECHNICIAN FOR THE BIOLOGY DEPARTMENT—The Secretary, Royal Free Hospital Medical School, 8 Hunter Street, London, W C 1

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Institute of Physics and The Physical Society Problems Facing University Physics Department (A Report prepared by a Committee appointed by the Council of The Institute of Physics and The Physical Society) Pp 26 (London The Institute of Physics and the Physical Society, 1963) [49

Meteorological Office Scientific Paper No 18 Airflow Around a Model of the Rock of Gibraltar By J Briggs Pp 20 (M O 744) (London H M Stationery Office, 1963) 3*s* net [49

Publications of the Royal Observatory, Edinburgh Vol 3, No 4 The Effect of Rotation on Balmer Line Intensities of B-Type Stars By B N G. Guthrie Pp 83–107 (Edinburgh and London H M Stationery Office, 1963) 6*s* net [49

Bulletin of the British Museum (Natural History) Entomology Vol. 14, No 2 The Acridoidea (Orthoptera) of Madagascar 3 Pyrgomorphidae By V M Dirsh Pp 49–103 16*s* Geology Vol 8, No 6 Certain Palaeoniscoid Fishes and the Evolution of the Snout in Actinopterygians By Dr Brian George Gardiner Pp 255–325+plates 1 and 2 20*s* Vol 9, No 1 The Ordovician Trilobite Faunas of South Shropshire, IV By Dr William Thornton Pp. 1–18+plates 1 and 2. 9*s* (London British Museum (Natural History, 1963) [49

Department of Scientific and Industrial Research Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1962, with Report of the Geological Survey Board Pp iii+91 (London H M Stationery Office, 1963) 5*s* 6*d* net. [109

The Society for Analytical Chemistry Report of the Analytical Methods Committee, 1961/62 Pp 31 (London The Society for Analytical Chemistry, 1963) [109

Ministry of Housing and Local Government Sixth Progress Report of the Standing Technical Committee on Synthetic Detergents Pp 12 (London H M Stationery Office, 1963) [109

Other Countries

United States Department of the Interior Geological Survey Professional Paper 297-C Exploration for Beryllium at the Helen Beryl, Elkhorn and Tin Mountain Pegmatites, Custer County, South Dakota By Mortimer H Staatz, Lincoln R Page, James J Norton, and V R Wilmarth Pp iv+129–198+plates 13–20 Professional Paper 341-C Geology and Ore Deposits of the Itabira District, Minas Gerais, Brazil By John van N Dorr and Aluizio Licinio de Miranda Barbosa Pp v+110+8 plates Professional Paper 369 Geology of San Nicolas Island, California By J G Vedder and Robert M Norris Pp vi+65+plates 1–5 (Washington, D C Government Printing Office, 1963) [109

United States Department of the Interior Geological Survey Geophysical Abstracts, No 198, July 1963 By James W Clarke, Dorothy B Vitaliano, Virginia S Neuschel, and others Pp iii+573–664 35 cents Geophysical Abstracts, No 199, August 1963 By James W Clarke, Dorothy B Vitaliano, Virginia S Neuschel, and others Pp iii+665–750 35 cents (Washington, D C Government Printing Office, 1963) [109

United States Department of the Interior Geological Survey Water-Supply Paper 1613-B Availability of Fresh Ground Water, Montauk Point Area, Suffolk County, Long Island, New York By Nathaniel M Perlmutter and Frank A DeLuca Pp iv+39+plates 1 and 2 Water-Supply Paper 1615-D Principles of Siphons With Respect to the Artificial-Recharge Studies in the Grand Prairie Region, Arkansas By R T Sniegocki and J E Reed Pp iii+19 Water-Supply Paper 1619-BB Ground-Water Exploration and Test-Pumping in the Halma-Lake Bronson Area, Kittson County, Minnesota. By George R Schiner Pp iv+38+plates 1 and 2 Water-Supply Paper 1619-CC Ground Water in the Raft River Basin, Idaho, with special reference to Irrigation Use, 1956–60 By M J Mundorff and H G Sisco Pp iii+23+plate 1 Water-Supply Paper 1669-1 Water Regimen of the Inner Valley of the San Pedro River Near Mammoth, Arizona, (a Pilot Study) By Harry G Page Pp iii+22+plates 1 and 2 Water-Supply Paper 1669-M Specific-Yield and Particle-Size Relations of the Quaternary Alluvium, Humboldt River Valley, Nevada By Philip Cohen Pp iv+24 (Washington, D C Government Printing Office, 1963) [189

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Sandia Corporation Fallout Report Beta–Gamma Radioactivity in Air at Sandia Laboratory, July thru December 1962 By R E Womelsduff Pp 14 (Albuquerque, New Mexico Sandia Corporation, 1963 Available from the Office of Technical Services, Department of Commerce, Washington, 25, D C) 50 cents [189

Bulletin of the American Museum of Natural History Vol 125, Article 3 The Miocene Faunas from the Wounded Knee Area of Western South Dakota By James Reid Macdonald Pp 139–238 (New York American Museum of Natural History, 1963) 3 dollars [189

Forest Research Institute, Dehra Dun Indian Forest Leaflet No 165 *Rauwolfia serpentina* Further Studies (with 3-year-old plants) on Methods of Propagation and their Effect on Root Production and Alkaloidal Content of Roots By R L Badhwar, R M Beri, G V Karira, S Ramaswami and H Sethi Pp 15 (Delhi Manager of Publications, 1962) Rs 1 35 nP, 3*s* 2*d* [189

The Resources Agency of California Department of Fish and Game. Fish Bulletin 122 The Kelp Bass (*Paralabrax clathratus*) and Its Fishery, 1947–1958 By Parke H Young Pp 67 Fish Bulletin 123 The California Oyster Industry By E M Barrett Pp 103 (Sacramento State of California, Department of Fish and Game, 1963) [189

Conseil Permanent International pour l'Exploration de la Mer, Service Hydrographique, Charlottenlung-Slot, Danemark ICES Oceanographic Data Lists, 1958, No 1 Pp xxiii+259 (Copenhague Andr Fred Høst et Fils, 1963) 15 D kr [189

Conseil National de la Politique Scientifique Rapport Annuel 1962. Pp 171 (Bruxelles Conseil National de la Politique Scientifique, 1963) [189

Engineering Digest, Vol 1, No 1, (January–March 1963) (For Departmental circulation only) Pp xviii+1–64 (Andhra Pradesh Public Works, Electricity, Highways and Public Health Department, 1963) [189

Indian Forest Records, (New Series) Mycology Vol 2, No 3 Fungal Diseases of Sal (*Shorea robusta* Gaertn & Fils) Part IV *Fomes caryophylli* (Rac) Bres, a Destructive Heart-Rot of Sal By Dr K Bagchee Pp iv+25–59+20 plates (Delhi Manager of Publications, 1961) Rs 10 00, 16*s* [189

Geological Survey Department, Suva, Fiji Bulletin 8 The Geology of the Tavua Area, Viti Levu, (Viti Levu Sheet 1) By P Ibbotson Pp iv+25 5*s* Economic Investigation No 2 Waimivesi Zinc Property, Tailevu—Diamond Drilling, 1961–62 By P Rodda Pp iv+12 (Suva, Fiji Geological Survey Department, 1962 and 1963) [189

Comptes Rendus des Travaux du Laboratoire Carlsberg Vol 33, No 4 Investigations on Mitochondria in Homogenates of the Amoeba *Chaos chaos* L By Nils Andresen and Charles W Mushett Pp 265–287 4 kr Vol 33, No 5 Normal Versus Synchronized Division in *Tetrahymena pyriformis*—a Study with Antimetabolites By G G Holz, Jr, L Rasmussen and E Zeuthen Pp 289–300 3 kr Vol 33, No 6 A Freeze-Drying Procedure for Protozoa By K K Hjelm and K Max Møller Pp 301–317 3 kr (Copenhague Danish Science Press, Ltd, 1963) [189

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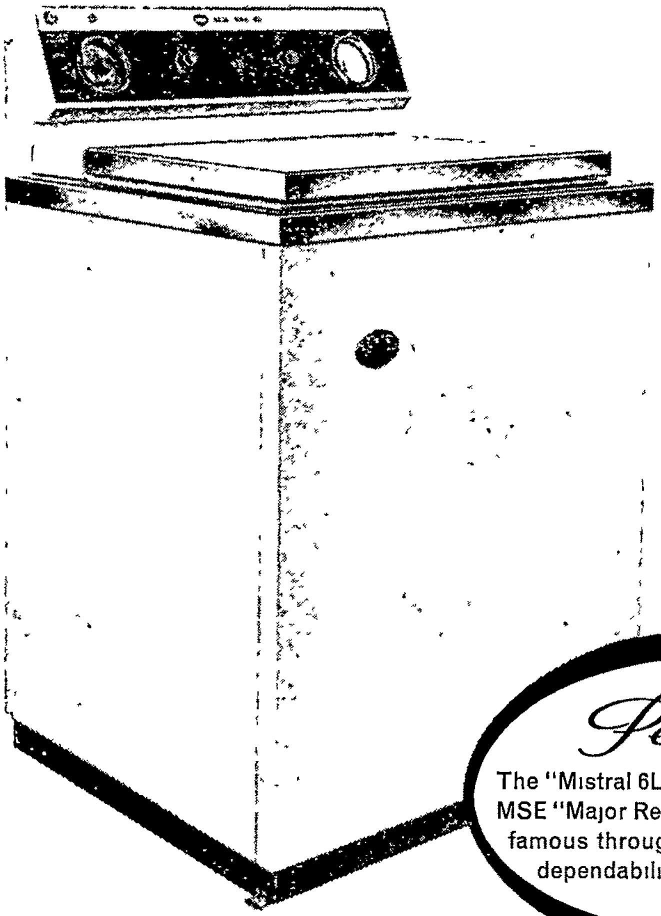

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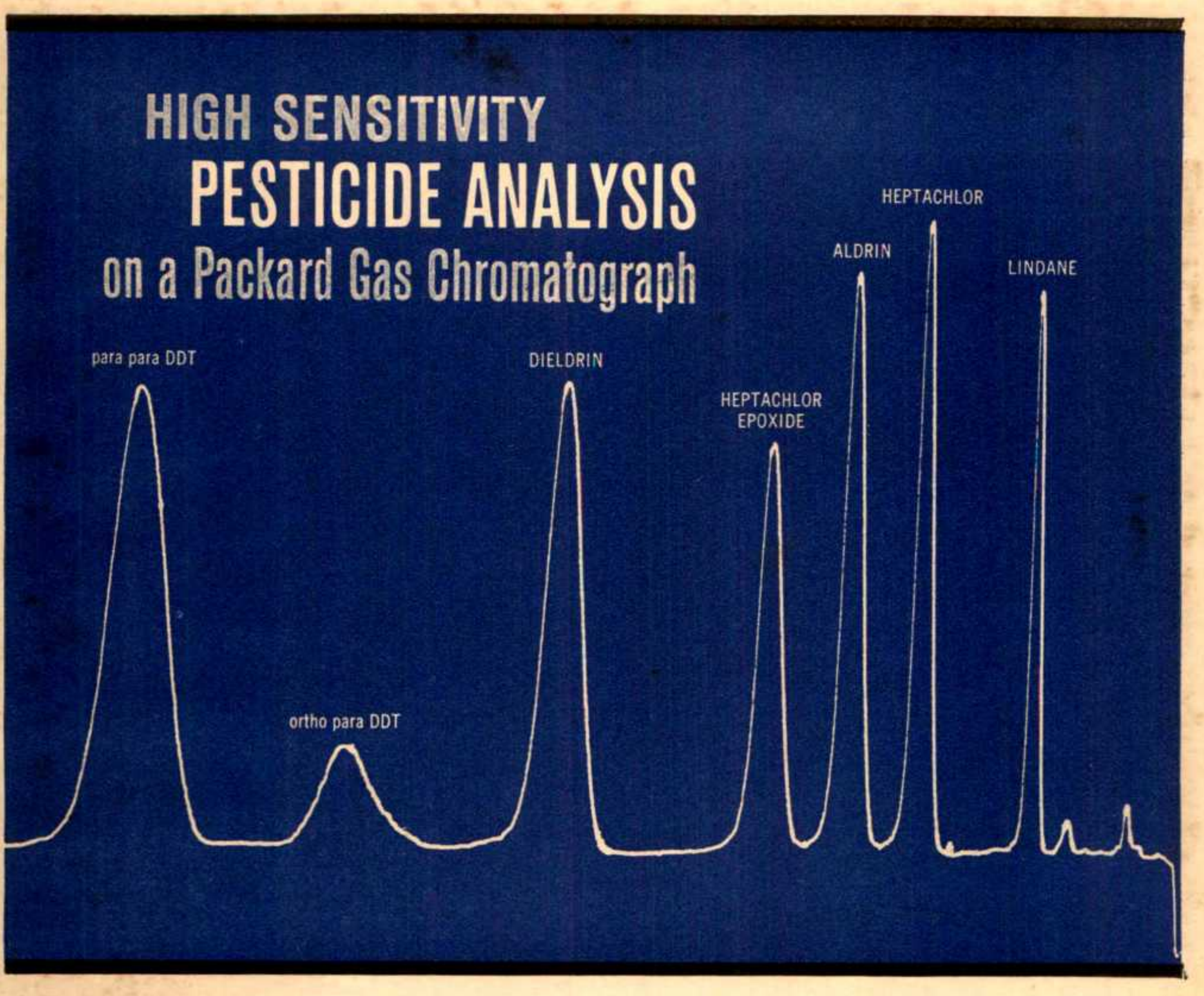

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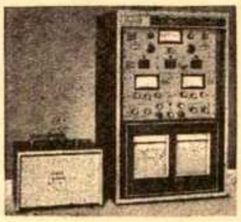

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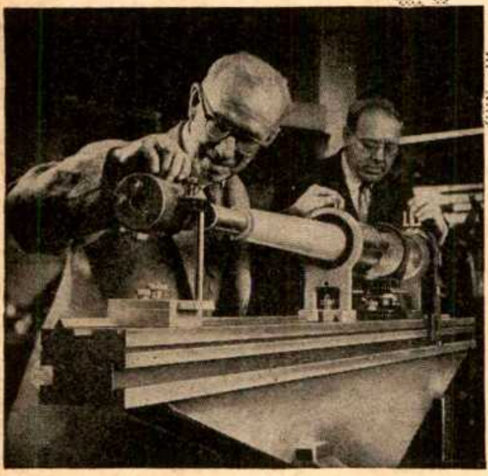

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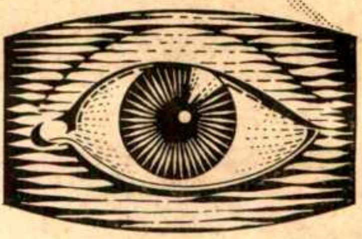

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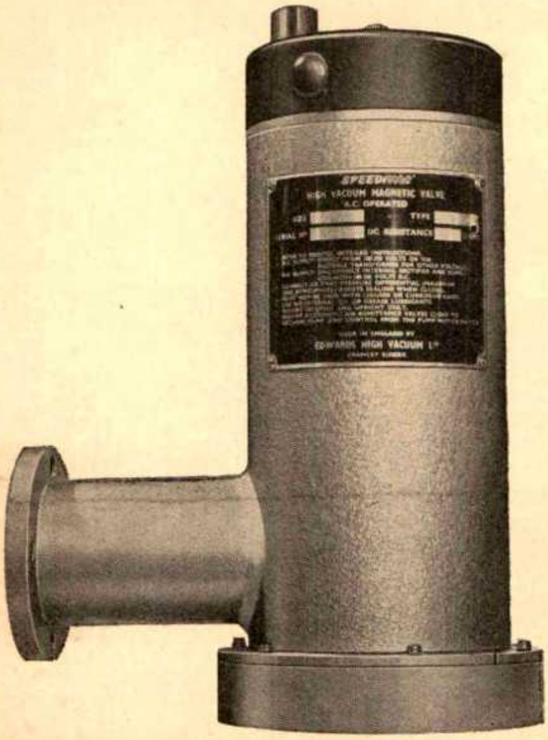

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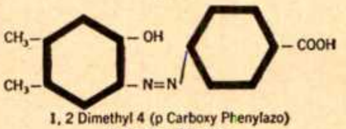

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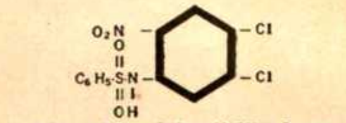

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(1) D. W. Woolley and J. M. Stewart, Biochem. Pharm. 11, 1163, (1962). (2) D. W. Woolley, Proc. Nat. Acad. Sci. Wash. 39, 6, (1953). (3) D. W. Woolley, Ibid. 41, 111, (1955). (4) D. W. Woolley, Cancer Res. 13, 327, (1953). (5) D. W. Woolley and G. Schaffner, Ibid. 14, 802, (1954).

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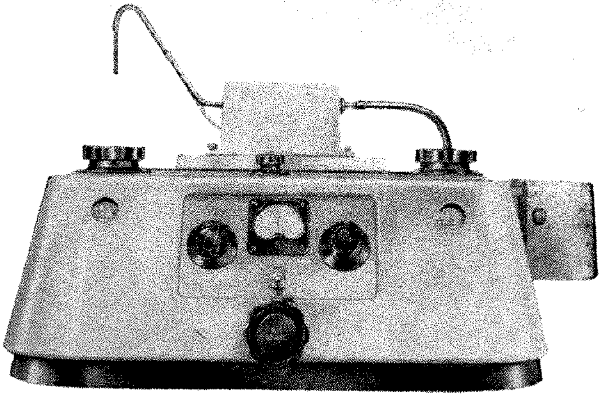

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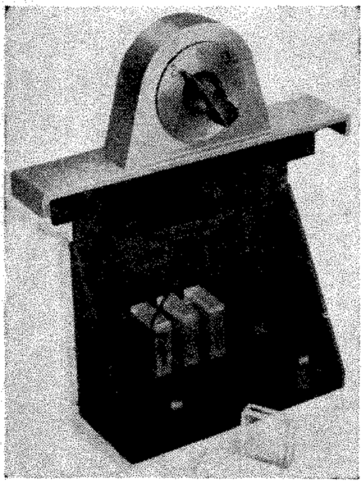

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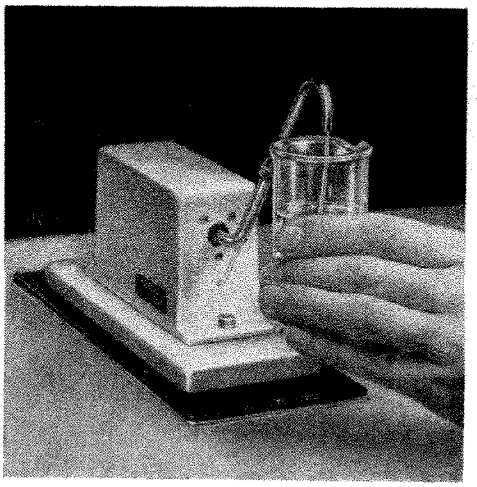

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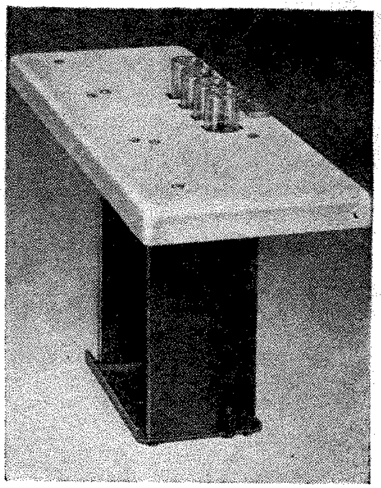

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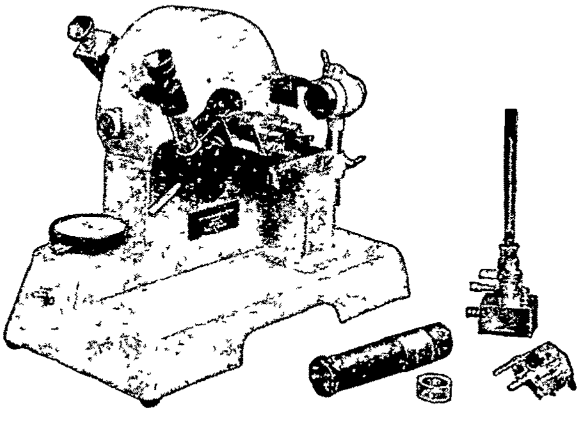

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SCIENCE AND THE METAMORPHOSIS OF GOVERNMENT

THE background paper*, which examines the links between scientific research and economic growth and discusses the implications of those relations for the policies for science adopted by the member countries of the Organization for Economic Co-operation and Development, was prepared at the request of the Committee for Scientific Research for the Ministerial Meeting on Science in Paris during October 3–4, and covers much ground in common with the report on *Science and the Policies of Governments*†, in which the meeting of Ministers for Science was specifically recommended (see *Nature*, 200, 199, 1963) The latter report, prepared by an *ad hoc* Advisory Group on Science Policy appointed by the Secretary-General of the Organization, discusses the implications of science and technology for national and international affairs. It was, in fact, intended to stimulate the discussions at the meeting recommended on national science policy, international scientific co-operation and on the role of the Organization itself in furthering the development of scientific policies which could contribute effectively to economic growth It is concerned with outlining the factors involved, including the extent to which the development of a sufficient supply of trained manpower, including managers, effective communications and statistics and the like were contributory factors

This background paper deals more specifically with economic aspects of scientific research and seeks to display some of the directions in which further knowledge is desirable It is more detailed than the report from the Advisory Group on Science Policy, and, while it insists on the importance of scientific research as a contributory factor in economic growth and on the necessity for a sound balance between investment in real capital and investment in the advancement of knowledge, it is careful not to claim too much Readers will be struck by the extent to which the argument follows that developed in two successive presidential addresses to the British Association for the Advancement of Science. investment in science and investment in man (see *Nature*, 195, 875, 1962, 199, 877, 1963) They will also note the admission that it is still impossible to estimate with any precision the return on investment in research and development, in the form of increased output The evidence is there of a connexion, but all the factors of economic growth are intimately interrelated, and while the paper is concerned primarily with the contribution from the advance of knowledge it does not neglect other aspects It points out, moreover, that the available stock of knowledge does set a limit on economic growth, particularly in countries which are already using the most advanced techniques, and also that there are grounds for believing that, in a market economy, the allocation of resources to the advance and spread of knowledge tends to be below the level required for the most efficient long-term growth of the economic system as a whole Further, there are social and welfare benefits from the advance of knowledge which may affect economic growth indirectly, and formulation of a national research policy should take into account such non-economic objectives as well as economic ones

While investment in capital equipment may be regarded as one immediate cause of the increase in productivity, that increase depends largely on the new technology built into new capital equipment, and this technology in turn is the result of research, invention and development, springing in their turn largely from education in the widest sense of the word Attempts made to measure the contribution to economic growth of changes in the input of the principal factors of production usually suggest that in mature industrial countries the rise in the capital/labour ratio accounts for only a small part of the long-term increase in labour productivity, and efforts are now being made to assess quantitatively the contribution of other factors. Here, it may be noted again that while there is some evidence of a statistical association between the research intensity of an industry, as measured by the ratio of research activity to output, its rate of productivity change and its rate of growth, there is no evidence of causality.

Much that is in this paper is not new, but national trends are brought together at an international level. For example, the high ratios of research and development expenditure to gross national product in the United States and Great Britain are clearly related to the high level of expenditure on military research and development Only a part of such expenditure appears to be included in the French estimates Again it is emphasized that economic growth is accompanied by a transformation of the production system, in which process technical innovation plays a predominant part Consequently, the relative growth of the manufacturing sector within the economy usually entails a rise in the research ratio. There also now seems to be a general tendency towards increased research and development expenditure in all industries because of the introduction of new equipment, materials and methods affecting all types of production The pattern of industrial growth has become increasingly complicated and there seem to be strong technological, economic and social forces tending to create a similar industrial pattern for research and development irrespective of national peculiarities

Empirical evidence on the relation between monopoly and research is inconclusive, and the paper suggests that the attitude of management may be much more important than the particular competitive situation of the firm. Furthermore, many of the returns on research and development investment may be social and cannot be pre-empted by the individual firm, being spread over all firms in the industry or even throughout the economy In spite, however, of the great increase in research and development activity, the report concludes that there are good reasons for believing that this activity is often still below that desirable for efficient and sustained economic growth It seems inherently improbable that the scale on which Governments supplement civil research and development in sectors other than nuclear energy is anything like sufficient, moreover, it appears reasonable to undertake a diversion of resources which would yield greatly increased returns, in terms of economic growth and social progress

---

* Organization for Economic Co-operation and Development Ministerial Meeting on Science Agenda Item 111 (Background Paper)—*Science, Economic Growth and Government Policy* Pp 66 (Paris Organization for Economic Co-operation and Development, 1963)

† Organization for Economic Co-operation and Development *Science and the Policies of Governments The Implications of Science and Technology for National and International Affairs* Pp 55 (Paris Organization for Economic Co-operation and Development, 1963)

This is the angle from which, in contrast to the Advisory Group's report, this background paper approaches the problem of Government policy, and in this second part of the survey the discussion is supported by a short bibliography and some further figures comparing the finance of research and development by Government in various countries In all the countries considered, research in the universities draws its funds mainly from Government sources either by direct grants or, particularly in the United States, from contracts However, on the whole, Governments have been loath to recognize their responsibilities concerning the level and balance of national effort in research and development Government policies have evolved somewhat haphazardly, sometimes influenced by the special interests of Government departments and sometimes by lines of thought advocated in influential scientific circles

The apparently unco-ordinated growth of research and development carried out or financed by Governments is attributed to the interaction of varying and sometimes divergent needs· it is over a multiplicity of motives that the difficulty of formulating a national strategy for science and technology arises. The spontaneous human need for wider and deeper knowledge, the need to solve specific social problems, as in health, nutrition. atmospheric pollution or road safety, and the needs of national defence may have points in common with needs related to the promotion of economic growth, but may also sometimes conflict It is suggested in this paper that practically there is no such thing as a science policy but rather a series of separate science policies aimed at different objectives The real question is whether these different policies can be co-ordinated, or at least worked out so as to support each other

It could, of course, be argued that this is a terminological quibble and that to achieve such co-ordination is in effect to formulate a scientific policy. or rather a policy for science and technology What lies behind it all is the allocation of resources and the choice of objectives as, in fact, in foreign policy, which could equally be said to represent the summation of a series of foreign policies towards different countries which likewise has regard to available resources The pragmatic test is inescapable and there is a good deal in this background paper to support the attitude which Lord Hailsham has consistently adopted. Promotion and co-ordination at Government-level of scientific research present an infinite complexity of problems which no group of men, however experienced. can handle effectively without being able to rely on basic and comprehensive data

These data, the paper points out, are often lacking, and it is regarded as one of the great merits of the new scientific councils that they have shown that no efficient work can be done without an overall view and without accurate and searching analyses of the state of scientific research and of the conditions for its development That touches one of the weak points of Lord Hailsham's position, for it can scarcely be claimed that he has provided sufficient drive to secure the data that are lacking It will be noted, too, that the paper insists that the value of the councils on general science policy which a number of countries have already set up depends largely on the efficiency of the permanent departments which support them—staffed not only with scientists but also with economists, financial experts and administrators, and competent to compile the basic data, conduct the enquiries and present the general synthesis and analyses needed

This whole concept is diametrically opposed to Lord Hailsham's conception of the Office of the Minister for Science and its staffing, though it should also be noted that the paper appears to look to the National Economic Development Council to co-operate with the Office of the Minister for Science in this field That, however, is a matter which raises rather large issues, both in respect of the place of the Council in the structure of Government and in relation to Parliament While the view could reasonably be held that the same staff could appropriately serve the Council and the Minister for Science in many matters in this field, there is rather more involved, and the practicability of such a proposition clearly depends on the views that may be taken as to the permanent position of the Council in the structure of Government

These implications, which go far beyond scientific or even economic policy, were put fairly enough by Prof W J M Mackenzie in his two broadcast talks on administrative reform early this year Prof Mackenzie fully recognized the decisive importance of the Director of the Council and his staff and also seemed more disposed than Prof Brian Chapman in his recent book, *British Government Observed* (see *Nature*, 200, 198; 1963), to welcome the development So far, however, as a scientific policy for economic growth is concerned, some such permanent staff must be provided at the most appropriate point if that policy is to achieve the two main objectives desiderated in this background paper first, assessing the possibilities and needs of private and public enterprises with respect to research and development and defining the lines on which Government action is likely to be most effective, secondly, choosing the overall priority objectives which should determine Government action

In this context, the paper makes some comments which are highly pertinent in considering the various statements that have recently been put forward, such as that from the Federation of British Industries on *Civil Research Policy* (see *Nature*, 199, 725, 1963) and the more recent statement, *Labour and the Scientific Revolution*,* which was discussed at the Labour Party Conference at Scarborough It is urged, for example, that Government, by an appropriate policy, can provide encouragement and effective aid, not only to particular enterprises or economic sectors but sometimes to the economy as a whole Above all, Government policy should seek to create a psychological climate favourable to research and innovation Experience has shown that campaigns designed to inform and convince business enterprises of the importance and profitability of research are often effective in converting even the most reticent managements to the idea that research is a paying proposition Here it would seem that much could be done without party politics entering into it There is much common ground in the ideas advanced in the Labour Party's statement of policy and those to which Lord Hailsham has already committed himself

The background paper also makes the important point that the Government should never bear the entire risk in development work and equally that in the event of success the Government should share the profits It enters a caution as to the extent to which the considerable tax concessions in respect of fundamental research which many countries make to private enterprise are important the real problem is to enable business enterprises to finance applied research and development On the role of education in science policy, it emphasizes many of the points which are made in the Labour Party's statement and in the report from the Federation of British Industries, some of which found expression also in Sir Eric Ashby's presidential address to the British Association It stresses also a point in regard to fundamental research that is sometimes overlooked—that the multiplicity of sources of finance is to some extent a prerequisite for the birth of new ideas and should not be eliminated.

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* *Labour and the Scientific Revolution* (A Statement of Policy Submitted to the Annual Conference of the Labour Party, Scarborough, 1963) Pp 4 (London Labour Party, 1963)

On the priorities of a scientific policy for economic growth, it reiterates that the first step is to make as thorough an analysis as possible of each economic sector with regard to its needs for research and development, taking into account the objectives of the economic plan From these sector analyses a synthetic view should be worked out by the bodies responsible for the co-ordination of scientific research, in co-operation with the economic planning authority and also with industry This view would guide the choice of methods or forms of Government action and determine any necessary programmes of research and development, and finally lead to the decision concerning what research training programmes are required

This emphasis on the importance of the economic element is particularly interesting in view of a similar emphasis placed on that factor in the papers contributed to a symposium which have recently been published under the title *Public Expenditure Appraisal and Control* (see p 724 of this issue of *Nature*) The symposium attempted more particularly to demonstrate how economics can be employed as a useful method for gauging the efficiency of Government spending Several of these papers are highly relevant to the kind of appraisal that is required to-day in assessing the allocation of resources Taken, moreover, with the work of Stone and Brown at Cambridge, whose attempt to devise a mathematical model of the whole economy Dr Patrick Rivett described in his address to the Operational Research Society last November as potentially the most important demonstration yet made of what modern computing devices can contribute to the study of the problems of national growth, they provide compelling evidence of the need to bring much wider resources to the work of Government.

The background paper is concerned much less than the report—*Science and the Policies of Governments*—with the actual organization required for the formulation and implementation of policy, leaning to the view that the structure of Government in any country must reflect the national characteristics as well as the purposes it is required to serve What needs to be stressed at the moment is that those purposes must affect the organization of Government, particularly so far as science is concerned, more than Britain has hitherto been disposed to admit Lord Hailsham's more recent utterances seem to indicate acceptance of this, and it seems implicit in the Labour Party statement without being elaborated Both also appear to recognize the very great importance of investigations in applied sciences and engineering attracting the best possible brains—as is emphasized in the background paper

The Labour Party proposes to build on the experience of the National Research Development Corporation, through the creation of large Government organizations for applying science on a massive scale in civil industry, and would appear to go beyond anything contemplated by the group which prepared the paper on *Science, Economic Growth and Government Policy* On the other hand, the proposal to strengthen the scientific staff of Departments, such as the Post Office, the Ministry of Works the Ministry of Transport and the Ministry of Education, seems to be much more in harmony with the ideas expressed in the paper It is also interesting to note the recognition that the opportunities which technological development offers will not be realized without a new climate of opinion both in industry and in the nation generally

This is a matter of education and will require more than the expansion of facilities for adult education and industrial training. The statement fully recognizes, too, that the Trade Unions have a crucial part to play and that a new mobility and readiness to change both skills and jobs will be demanded For all that, the attitude of the Trade Unions to trade union reform as manifest at the last Trades Union Congress scarcely warrants optimism, but with so much ground in common it should not be impossible for the political parties to co-operate in this immense task of education which is prerequisite if the challenge of the scientific revolution is to be met The meeting in Paris of the Ministers for Science itself contributed both to the task of education and to the accurate assessment of the recommendations of the Robbins Committee and the Trend Committee

THE WORSHIPFUL SOCIETY OF APOTHECARIES OF LONDON

A History of the Worshipful Society of Apothecaries of London

Vol 1 1617–1815 Abstracted and arranged from the manuscript notes of the late Cecil Wall by the late H Charles Cameron Revised, annotated and edited by E Ashworth Underwood Pp xiv + 450 (27 plates) (Published for the Wellcome Historical Medical Museum by the Oxford University Press, London, New York and Toronto, 1963) 55*s*

A M. A MOORE, Master of the Society, 1961–62, writes in the foreword to this *History* "The late C R B Barrett published, in 1905, a *History of the Society of Apothecaries*, which has hitherto done good service as a readable and generally available source of information on its subject It was obvious, however, that much additional and important matter for an adequate history of the Society was bound to be discovered by a systematic and adequate examination of the great wealth of documents and records, which it has inevitably accumulated during the nearly three and a half centuries of its continuous activity The Society was fortunate in having as Archivist one of its Past-Masters, the late Dr Cecil Wall When Dr Wall died in 1947, he left in typescript an exhaustive document of more than 300,000 words, dealing with every aspect of the Society's activities from its first separation, as a distinct 'mistery', from the Grocers' Company in 1617, down to its specially important period of constructive and controversial effort, in the middle of the nineteenth century Dr Wall's death, when his labours had reached this stage, left the Society in some doubt concerning his intentions At this junction, Sir Hugh Lett, then Master of the Society, approached the Wellcome Trustees with an appeal for their assistance in dealing with the problem The Trustees after examining Dr Wall's material with expert advice from Dr Ashworth Underwood, Director of the Wellcome Historical Medical Museum, offered to make the financial provision required for such an expert re-arrangement, condensation, and substantial rewriting of Dr Wall's material, as would produce an authoritative history of the Society, in a book of the dimensions and character likely to meet with general appreciation They were fortunately able to enlist the interest in this project of Dr Hector Charles Cameron, whose experience and achievements in medical and scientific history were already well known The Trustees' offer, and Dr Cameron's suggested services in giving effect to it, were gladly accepted by the Society's Officers and the Court of Assistants

"The Wellcome Trustees had further suggested, for the Society's consideration, that Dr Cameron's manuscript, when completed, might be submitted to Dr Underwood, for inclusion if acceptable in the Main Series of Publications of the Wellcome Historical Medical Museum, and this proposal, which would relieve the Society of any expense and trouble contingent on the publication, was also accepted "

I would merely like to add to the foregoing that, in my view, the book would be the gainer by a colour photograph of the Courtyard of Apothecaries Hall, London, such as was specially painted for the July 1954 issue of *The Glaxo Volume* by I C Moody Moreover, the pictures of King James I and of Francis Bacon could add to the value of the volume by being reproduced in colour

K J FRANKLIN

SOLID-STATE PHYSICS

Solid State Physics

Advances in Research and Applications Vol 12. Pp xvi+459 114*s*. 6*d* Vol 13 Pp xv+482 103*s* 6*d* Vol 14 Pp xv+519 114*s* 6*d* Edited by Frederick Seitz and David Turnbull (New York and London Academic Press, 1961–63)

Theory of Lattice Dynamics in the Harmonic Approximation

By A A Maradudin, E W. Montroll and G H Weiss (Solid State Physics Advances in Research and Applications, Supplement 3) Pp viii+319 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1963) 80*s*

THE general excellence of the *Solid State Physics* series scarcely needs to be recorded. it can by now be taken for granted

The 'substantive' articles in Volume 12 include a review by H Inokuchi and H Akamatu of electrical conductivity in organic crystals The conduction in such materials is, of course, not done well, and one is surprised—as Dr. Johnson would have been—to find it done at all It seems, however, that 'organic semi-conductors' are by no means rare forms of molecular crystals and that they are beginning to be understood K Mendelssohn and H M Rosenberg achieve the useful and apparently difficult feat of writing about thermal conductivity of metals at low temperatures in a way which is at once lucid, readable and complete Finally, G Leibfried and W Ludwig survey anharmonic effects in crystals in a way which will not be very helpful to the general reader but invaluable to research workers in lattice dynamics Their article includes quite a lot of original work

There are two articles on 'techniques' in Volume 12 an experimentalist's vade-mecum on the subject of hydrothermal crystal growth by R A Laudise and J W Nielsen (complete with beautiful pictures of growing crystals, diagrams of autoclaves and so forth), and a theoretician's potted guide to group theory and crystal field theory by C M Herzfeld and P H E Meier A feature of this article is the index of references to character tables, tables of direct products of irreducible representations and tables of explicit base functions for the point groups

Volume 13 exhibits a similar balancing of topics The semi-conductor lobby provides us with a comprehensive account of electron spin resonance in semi-conductors by G W Ludwig and H H Woodbury; this tool appears to be particularly powerful when applied to the investigation of impurities J J Gilman and W G Johnston explain, with the help of illustrations, how dislocations in lithium fluoride may be investigated by means of etch pits The pictures look rather like rills on a lunar landscape. C. K. Jørgensen writes about chemical bonding (as inferred from visible and ultra-violet absorption spectra) from a point of view which will be unfamiliar to most physicists Is he a missionary talking to cannibals or a cannibal talking to missionaries? There is little doubt that S S. Mitra who writes about the vibration spectra of solids, is a cannibal His feast included, among others, C. V Raman, and has proved somewhat indigestible

The 'techniques' part of Volume 13 involves a fascinating article by F P Bundy and H M Strong, well known for their work on diamond synthesis at the General Electric Co , on the behaviour of metals at high temperatures and pressures. There is indeed much more than techniques to be found here known data on resistivity, compression, phase diagrams, thermoelectric power and the curious catalytic properties of transition metals which enabled the diamond synthesis to proceed Naturally perhaps, the last topic is skimpingly done There is no reference to Henry Eyring. Theoretical technicians will be well pleased by E I Blount's account of the 'formalisms' of band theory This article is centred on the Wannier representation It draws together scattered results in an intelligible and intelligent way and includes some original work

The 'mixture' of Volume 14 is not the standard one There are three high-level articles on special problems, namely, "g Factors and Spin-Lattice Relaxation of Conduction Electrons", by Y Yafet; "Electron Spin-Resonance Spectroscopy in Molecular Solids", by H. S Jarrett; and "The Theory of Exchange in Insulators and Semi-Conductors", by P W Anderson. Each of these articles is authoritative, critical and complete, but they demand much background knowledge from the reader The fourth article—a book within a book—is an account of molecular motion in solid-state physics by N Saitô, K. Okano, S Iwayanagi and T Hideshima It reviews competently theory and experiment in a field which is (literally and figuratively) amorphous

Theory of Lattice Dynamics in the Harmonic Approximation, by A A Maradudin, E W Montroll and G H Weiss, is a masterly account of the 'active centres' of a popular and important field The topics covered include the role of critical points in phonon spectra, the effects associated with defects, surface effects and atomic correlations There is no other convenient account of these subjects available, and they are here treated with clarity and elegance The theory of ionic crystals is the only obvious 'gap' Is it too much to hope that it will be filled by someone like Rosenstock ?

R O DAVIES

ANIMAL PATHOLOGY

Pathology of Domestic Animals

Vol 1 By K V F Jubb and P C Kennedy Pp xv+477 (New York and London Academic Press, 1963) 128*s* 6*d*

FOR long a notable omission in the ever-expanding veterinary literature has been the absence of a modern authoritative text, in English, on morbid anatomy and histopathology This book, the first of a two-volume work on the pathology of domestic animals, goes far towards filling the gap It is particularly opportune that it should come at the present time when there is a growing interest in, and awareness of, the importance of comparative pathology Although the work has been designed primarily for the teaching of veterinary students it will also serve as a useful guide to those medical pathologists who turn their attention to apparently analogous conditions in man and animals It will be useful to the research worker in experimental pathology, and non-veterinarians in particular will find useful the brief descriptions of some of the normal variations which occur in different species of animals Equally valuable to all are the side-by-side illustrations of some normal and diseased tissues

Volume 1 presents a fairly detailed account of disease processes affecting bone, joints and synovial membranes Organ pathology is discussed on a systematic basis and here includes the respiratory, hæmopoietic, endocrine, circulatory, and the genital systems Volume 2, which is to be published shortly, will deal with the remaining systems and organs of the animal body There is, rightly, no account of general pathology because, as the authors point out, this subject is adequately covered in many standard text-books The gross and microscopic structural changes in the various tissues are presented, together with sufficient information on altered function and consequent clinical signs to stimulate and interest the student The text is well illustrated with photographs and photomicrographs of excellent quality which clearly indicate the essential features of the lesion under discussion A minor criticism here might be that some of the illustrations are wasteful of space, for example, two full-page photographs to show adventitial placentation in the ox.

The excellent bibliography is a major strength of the book. Appended to each chapter are many selected references which will be useful to a wide range of readers References are given to key papers in the related fields of virology, bacteriology, parasitology, oncology and genetics where these abut on to the main subject of pathology It is, however, unfortunate that some, at least, of the references are not 'tied in' to the text, because the authors in some of their discussions do not always distinguish between what is fact and what is speculation This is an obvious drawback from the point of view of the student and the non-specialist reader The references are well set out and there is a good and accurate index Typographical errors are rare, the book is well produced and of the high standard associated with the publishers It is probable, however, that the cost will be prohibitive for many students

Inevitably in a work so extensive as this there are bound to be omissions, and the relative emphasis placed on some subjects will not meet with everyone's approval In the chapter on respiratory disorders, a brief résumé of the modern concepts of the structure of the alveolar epithelium would have helped to clear some of the confusion in the text, and the section dealing with the interstitial pneumonias could, with advantage, be amplified and more attention directed to the pathogenesis of these diseases Students of comparative pathology will be disappointed with the section on arterial disease There is no discussion of atherosclerotic lesions in pigs or cattle (but, in fairness to the authors, they do point out that arterial disease is of no economic importance in domestic stock) In their introductory remarks, the authors state that all they have to say on a specific disease is said in one place under the organ system which is most appropriate They waive this general rule in several places but chiefly in the sections devoted to the genital systems It is, however, very surprising that in the chapter on the cardio-vascular system no mention is made of the virus of swine fever ('hog cholera') as a major cause of vascular damage in swine and hence as the prime cause of the protean lesions of this important disease It is probable that swine fever will be described in Volume 2, but the essential lesion is one affecting the small blood vessels and as such should receive attention in this section

In their preface, the authors do not attempt to justify their book because, as they say, a good book is its own justification This is such a book; it will find and retain its place on the shelves as an indispensable standard work of reference A R JENNINGS

THE DEVELOPING WORLD

Our Developing World

By L Dudley Stamp Pp. 195. (London: Faber and Faber, Ltd, 1963) 8s 6d

THE population of the world to-day is more than 3,000,000,000, probably almost double the population in 1900 This means that the past sixty years have seen a growth equal to that in the entire previous history of mankind This sobering thought becomes frightening when we look into the future In 1951 the United Nations estimated the world population for 1980 as between 2,976,000,000 and 3,636,000,000 They revised these figures in 1954 to give estimates between 3,295,000,000 and 3,990.000,000 and again in 1958 to between 3,850,000,000 and 4,280,000,000

These figures are quoted by Prof Dudley Stamp in *Our Developing World*, and he points out that they show not only that our knowledge of the numbers of mankind is improving, but that they are constantly showing previous estimates to be too conservative By 1980 world population will probably be considerably more than 4,000,000,000 and, by the end of the century, between 7,000,000,000 and 8,000,000,000 It is difficult to write about the future relationship between population and resources against so dynamic a situation, and Prof Stamp's experience shows this The core of his latest book was a series of talks (the Patton Foundation Lectures at Indiana University) published in 1952 as *Land for Tomorrow* This was brought up to date for an English edition, in 1953, called *Our Undeveloped World*, a book which, he says, should now be read "almost as historical documents" It had to be re-written in 1960, when it was called *Our Developing World*, and some of the figures had to be brought up to date again for this paper-back edition

Prof Stamp sets the population explosion against a more stable background, that is, the amount of land available and the basic facts of climate and soil these are the given elements from which man must obtain his food But the relationship of man and land is by no means simple The history of mankind is that of developing techniques to produce more and more food cultivation, domestication, the invention of the hoe, the plough, the harnessing of power, rotation, irrigation, fertilization—all these have dramatically changed the relationship, and Prof Stamp discusses the latest advances, mechanization and 'chemicalization', to show that potential changes are as great as those of the past But another fact is equally evident that this progression has been most uneven Whereas revolutionized techniques enable advanced communities to produce the food they need with only a small percentage of primary producers, others are still at subsistence-level Indeed, the majority of people are still setting the balance between Nature and society at starvation-level Strangely enough, the more technologically advanced countries depend to an increasing degree on these same underdeveloped countries which supply, for example, much of the world's oil, most of its copper, tin and bauxite The table of their contribution in this respect to the world's economy makes thoughtful reading

Meanwhile, the problem of equating an exploding population with food supplies is of paramount importance, and demands all the skills of the most advanced technologies Prof Stamp's admirable little book puts these problems succinctly and clearly without pretending to attempt the answers It gives the more general reader a real appreciation of the situation against a more geographical background than is usual It cannot fail to make the reader conscious of the great dilemma of the age, and for this we are in debt to Prof Stamp for one more expression of his concern with these problems

EMRYS JONES

Public Expenditure

Appraisal and Control. Edited by Alan T. Peacock and D J Robertson. Pp viii+168. (Edinburgh and London Oliver and Boyd, 1963) 21*s*

THE publication as a separate volume of the ten papers contributed to a symposium on the appraisal and control of public expenditure is timely when not merely the efficiency of Government control in general has been brought in question, for example, by Prof Brian Chapman in his recent book, *British Government Observed* (*Nature*, 200, 198, 1963), but also the effectiveness of Government organization has been re-examined by the Trend Committee

Originally, the papers were published in the *Scottish Journal of Political Economy*, and while books by Basil Chubb and S H Beer have in recent years discussed the control of public expenditure from the administrative point of view, these papers concentrate on the economic side and indicate, for example, the value of Dr R McKean's systems analysis in decisions on Government spending The subject is one which is likely to grow in importance, and as Prof A T Peacock shows in his paper on ' Economic Analysis and Government Expenditure Control", while the way forward has been pointed by the Plowden Report, for example, extensive adoption of economic analysis is likely to await a major change in the attitude of the electorate to public spending—or, it might be suggested, some rational understanding between political parties

The specific problems of defence expenditure, health service policy, the allocation of expenditure in the electricity supply industry and the control of subsidy expenditure in agriculture are discussed by Dr McKean, Mr J Wiseman, Dr R L Meek and Mr G F B Houston, respectively Others deal with the appraisal of Government purchasing, controlling the Government's wage bill and the growth of Government expenditure in the United Kingdom since 1790 In discussing problems of expenditure control in local government, Mr L Boyle comments that many universities appear to have been singularly remiss in providing facilities for studying public finance and political science He suggests that a much closer contact is desirable between the academic world and the field of practice so that senior officials in the public service can communicate their problems to the universities, enabling research to be conducted into those problems, and thereafter benefiting from the results of such research.

Such a remark itself attests the significance of this volume to which Mrs Ursula K Hicks contributes an epilogue on choice, efficiency and control in the public services, which has some sound comments to make on the Plowden Report

Experiments in Physical Chemistry

By J M Wilson, R J Newcombe, Dr A R Denaro and Dr R M W Rickett Pp xii+290 (London and New York Pergamon Press, 1962) 21*s* net

EXPERIMENTS in Physical Chemistry is intended for use by students reading chemistry to honours degree standard The form of presentation is most attractive. Each experiment is assigned to one of three sections, the sections differing in the degree of complexity of the operations and the associated theory

Each experiment is prefaced by a discussion of the theory involved The discussions are incomplete but serve to relate the book with theoretical texts Next, the apparatus required is described Diagrams are useful, and firms manufacturing the equipment to be used are listed The method to be followed is then given, and here the text is weak More detail is required, too much initiative is required of the student For this reason the book might prove a valuable addition to the teacher's library, the student reaping the benefit second-hand

The book has an efficient index and the contents are set out very clearly One hundred and two experiments are presented and they are selected to cover the wide range of physical theory and technique Although most of the experiments are of college standard, there is a great deal here to interest the very able sixth-form pupil, and the book would be a very useful additional source of ideas for end-of-term laboratory work D C FIRTH

Organic Chemistry Laboratory Operations

By W B Renfrow and P J Hawkins Pp viii+216 (New York The Macmillan Company; London The Macmillan Company, New York, 1962) n p

THIS book covers a very wide range of techniques Its introductory chapters on distillation, extraction, melting and boiling point determinations are familiar ground for a pupil in the lower sixth form The final chapters on high-pressure hydrogenation and infra-red spectroscopy are the province of an honours student in his final year.

A range of preparations are given and these illustrate well the basic reaction types of organic chemistry Many of the experiments are done using unfamiliar materials For example, *p*-xylene is sulphonated and not benzene Saturation and unsaturation are illustrated using *cyclo*hexane and *cyclo*hexene and not the more familiar methane and ethylene The general theme, worthy of close attention, is that reactants must be easy to handle, reactions must work quickly, and the products be easy to isolate

Each chapter includes a discussion illustrated by clear diagrams, also an equation and a suggested mechanism for the reaction A problem is given at the end of the chapter and is designed to test the student's understanding of the theory Between theory and problem there is a method This is adequate in earlier chapters on elementary experiments, but quite the reverse later in the book when advanced work is under discussion Students are asked to see the instructor for working instructions!

The early part of the book is well worth reading at the bench, the latter part has no place in the laboratory

D C FIRTH

Mild-Moderate Forms of Protein-Calorie Malnutrition

Symposia of the Swedish Nutrition Foundation, Bastad, August 29–31, 1962 Edited for the Swedish Nutrition Foundation by Gunnar Blix Pp 159 (Stockholm Almqvist and Wiksell, 1963) Sw Kr 35

TOWARDS the end of 1961, the Swedish Nutrition Foundation came into being, mainly through the financial support of the Swedish food industry Among its projected activities is an annual symposium The first of these was held in August 1962 This book records the papers read then, and a summary of the discussion There were 13 contributions, all by workers who had a direct concern in the problem of protein-calorie deficiency These included research workers from the United States of America, Africa and Central America, as well as a representative from the World Health Organization and one from the Food and Agriculture Organization The contributions dealt with the clinical and biochemical features of the condition and with related questions such as protein and calorie requirements, methods of assessment of physical condition, the use of protein-rich foods for prevention, and an exhaustive and provocative article on the evaluation of the nutritive value of protein

Together these papers form an excellent review of the present-day situation The discussion is especially valuable in emphasizing how much we still have to learn about this, the commonest of all forms of malnutrition The book is beautifully produced and a pleasure to read There are very few and quite unimportant misprints, a most praiseworthy effort for a book produced and printed in Sweden

JOHN YUDKIN

TELEVISION IN MEDICAL EDUCATION

By C. E ENGEL

Department of Medical Illustration, Guy's Hospital Medical School, London

IT is perhaps reasonable to ask why television should be considered at all seriously in the context of medical education, it is still a relatively novel technological development and primarily applied to mass entertainment. As television reproduces movement accompanied by sound, it could in any case be argued that the motion picture film should fulfil whatever useful functions are claimed for television However, the film, like a paper in a journal or a chapter in a text-book, must be complete and able to stand on its own Once projection has been started, it is difficult to stop the film and repeat sequences already viewed Thus the ideas, concepts, phenomena or procedures to be portrayed by the film must be developed most carefully, so that even the less well-informed members of an audience do not lose comprehension, as the remainder of the film might otherwise be devoid of meaning for them For this reason the production of films is a time-consuming and not inexpensive undertaking Films are frequently not only inflexible but also impersonal and tend to convey a sense of unreality Because academic teaching is essentially individual and adaptable to the varying needs of each individual teaching situation and audience, few lecturers are prepared or indeed able to use the conventional full-length film in their teaching[1]

Television, however, offers a number of potential advantages These may justify serious investigation into its effectiveness in the context of the present situation where a rapidly increasing volume of information has to be imparted, in a restricted period of time by a limited corps of teachers, to undergraduates, to those reading for higher degrees and to practising graduates It may, therefore, be helpful to consider the educational problems in which television might possibly be of assistance Students in the medical school represent a captive audience, as it were, for they can be reached by the lecturer at a time and place of his choice General practitioners, however, work as individuals and are widely separated from each other, so that a lecturer finds it much more difficult to meet a number of them all in one place and at the same time These are, therefore, two fundamentally different situations which might be considered separately as intramural and extramural problems

Intramural Problems

All learning relies to a large extent on repetition, and, particularly in medicine, a great deal of learning is concerned with objects rather than with concepts, so that visual experience must play an important part Yet an adverse staff/student ratio constitutes a serious obstacle to the ideal where each student in the medical or dental school can see what is being demonstrated not only really well but also frequently and repeatedly

Image Magnification and Optimum Perception Many objects, phenomena, reactions and clinical appearances are quite small so that adequate demonstration would require individual presentation to each student or in any event to very limited groups of students Restricted teaching staff makes this impracticable and repetition by one lecturer soon becomes tiring and uninspired Television, because it can reproduce an optically magnified image on a virtually unrestricted number of receiver screens in one or several rooms simultaneously, may help to solve this problem The lecturer can still be in the same room or ward, as his students where he can now teach with the aid of the enlarged image The recording television camera can also be in the same room and, indeed, be operated by the lecturer himself The receiver image is so bright that daylight or artificial light need not be excluded

This situation offers each student the same magnified view from the 'ideal' observer's position The lecturer is able to switch on or off as he thinks fit, so that he need show such images and associated sounds only at the instant when they serve his exposition. The students can interrupt if they cannot see clearly enough, or require further explanations This active participation by the students is likely to help the learning process, and it enables the lecturer to repeat parts of a demonstration, possibly in a modified form, according to the students' needs

Numerous examples could be quoted and a few of those which have been reported in the literature may here be of interest Small television cameras have been attached to microscopes, so that the lecturer can point out specific structures which the students can then identify under their own microscopes during classes on histology[2]

Cameras with lights attached have been suspended above a demonstration table[3], set up to record from surface-silvered mirrors or fitted to a mobile support for attachment to dissection tables for macroscopic demonstrations during courses on anatomy At Guy's Hospital Medical School such macroscopic demonstrations have been used to give students advance information or pre-knowledge before they proceeded to carry out their own dissections This approach was subjected to a series of objective tests in collaboration with the Institute of Education, University of London[4].

Both microscopic and macroscopic television can be used for better vision during the demonstration of animal experiments in physiology[5] and pharmacology It is, therefore, easy to imagine the uses of television during clinical rounds and clinico-pathological joint meetings when relatively small lesions, either on the patient or in a histological preparation, are to be demonstrated to a large number of students Equally, a television camera can offer a close-up view to the students, however many may be assembled to watch a post-mortem examination

Although students may not be required to watch a large number of surgical procedures, television can provide a better view than the conventional observation gallery Ruhe[6] has reported on a well-planned installation where the lecturer and his students are given a close-up view on television receivers in the observation gallery, while they can also see and hear what is going on in the theatre below A by-product of such an arrangement is that every member of the surgical team, not only the surgeon and his assistants, can see what is going on[7], quite apart from the obvious reduction in the risk of cross-infection which results from keeping all but essential personnel out of the operating theatre

Apart from the foregoing instances, which represent largely passive learning, there are many situations when students are taught essentially practical manœuvres and techniques While it may well be impracticable to assign one demonstrator to each student, a single teacher can demonstrate to a television camera, so that the whole firm or class can see At least one film has been tested successfully in the role of simultaneous multiple-student technique-instruction[8] This film may have become somewhat outdated through subsequent changes in the technique itself, television, however, can always be up to date and has the additional advantage of complete flexibility. Merrill[9] has proved by experiment that the time taken by students in preparing a tooth for an inlay was reduced by almost 40 per cent and that the quality of their preparations was considerably better, when compared with the work produced by more orthodox methods of instruction Best results and the greatest saving in time were achieved when the students were given an opportunity to complete a discrete stage of the practical work before the next manœuvre was demonstrated and explained to them on the television screen.

Image Transportation One problem, then, to which television may be applied is visual magnification, so that every student can see as well as the demonstrator, examining physician or surgeon A further problem is how to demonstrate related events, or phenomena which are separated in space This is normally difficult to organize or, indeed, impracticable because of time available, number of students, possible risk of infection or restricted space as in a research laboratory[10], sometimes the teaching situation might be adversely affected, as during a psychiatric interview, if too many observers are present

The television camera can be called on to bring images to the lecture theatre, classroom or laboratory from as widely separated locations as other laboratories, wards, out-patient departments, X-ray department, delivery room, operating theatre and post-mortem room Only the number of cameras available for distribution to these locations and the versatility of a switchboard restrict the possible sources from which the lecturer can show one or more remote but relevant events to his students Which locations are connected to the students' receivers and the sequence in which events from different locations are shown is entirely at the discretion of the lecturer, so that he can make use of these facilities just once or repeatedly during his exposition He can also use the televised picture and associated sound to make comparisons, either in sequence or by juxtaposition on adjacent receivers This need not, of course, exclude the complementary use of the more conventional aids such as lantern slides, blackboards or short film units

Apart from helping to solve the problems already mentioned, this use of television may claim other advantages When the transmitted sound is reduced in volume, the lecturer can add his own explanations while the visual information continues to be received by the students· his comments and active participation by the students do not, of course, interfere with the event which is being televised. Particularly extensive use has been made of this method in the teaching of psychiatry[11] Markee[12] has shown that the possibilities offered by this ability to bring information to the assembled students have by no means been exhausted yet On the premise that knowledge largely acquired from visual information ought to be tested by posing visual problems, he has televised dissections, specimens, photographs, drawings and patients, with superimposed written questions to test his students of anatomy The results were statistically evaluated in comparison with the performance of the same students in other, more orthodox, tests; he found that this method could also be used successfully to establish a student's knowledge and his ability to apply such knowledge appropriately Markee satisfied himself that television could be more versatile, more searching and less time-consuming than the usual method of letting students file past a series of exhibits annotated with questions

Image Multiplication One more problem, that of bringing the same visual information to students who are not assembled in one room, may be solved by television This method is already used in other academic subjects where the student audience is too large for one lecture theatre, and experimental data on its relative efficiency are now available[13] In medicine those schools which have adopted the unit laboratory system have found the transmission of visual and verbal information from one room to the various laboratories both efficient and time-saving[14]. A two-way sound-link between students and teacher is a necessity in this situation

The Clinical Center of the National Institutes of Health at Bethesda, among other American teaching centres, has installed the necessary wiring, so that cameras and receivers can be connected in a large number of rooms[15] This makes it possible to show in study rooms, library cubicles, teaching rooms attached to wards and in operating theatre galleries anything deemed of interest to students—from simple references to relevant, up-to-date information in journals to interesting radiological examinations, rare or unusual conditions seen in out-patients, a birth or parts of a surgical operation This offers visual experience and learning by repetition without having to call the students together or away from their places of work

Image Transformation Television, with specially sensitized tubes and in conjunction with other devices, can convert invisible radiation into visible images In this way, radiological examinations[16,17], differential ultraviolet transmission by cell components[18] and infra-red penetration of the skin to reveal the subcutaneous venous system can be demonstrated to students Infra-red sensitive television tubes can also be used to demonstrate events and processes which can only take place in the dark. Of these potential applications, television radiology is likely to establish itself most readily

Endoscopy The observation of endoscopic appearances by the student has so far been largely restricted to a quick, and possibly displaced, glance through the endoscope, to a photograph or, though rarely, to endoscopic motion picture films The French endoscopes[19] which make use of intense light piped to the distal end through a quartz rod, and such recent developments as a miniaturized television camera[20], which is no bigger than an electric shaver, will make television endoscopy both simple and safe The reproduction of colour must unfortunately wait until smaller, simplified and much less expensive colour equipment becomes available

The further development of fibre optics, where each glass fibre in a flexible bundle conducts a fragment of the image by internal reflexion[21], may before long make it possible to show to students structures and processes[22] on the television screen which are inaccessible to the rigid endoscope Even now television linked to periscopes[23], ophthalmoscopes and operating microscopes[24] may help to increase the student's visual experience well beyond that of his immediate predecessor

Extramural Problems

The needs of the graduate in general practice are different from those of the undergraduate The almost explosive progress of medical science makes it desirable that up-to-date information should be available to the graduate as soon, and as readily, as possible No one journal could attempt to offer such a service and it is doubtful whether this channel of communication would prove entirely acceptable The spoken word alone, as, for example, over the sound radio system, is likely to prove less effective than a combination of sound and sight Not only experiment but also common experience has shown that the face-to-face or 'fireside-chat' situation provides the most effective learning situation Films could be considered in this role, but they are likely to lack spontaneity, the mechanics of production and duplication would lead to undesirable delay, and the additional apparatus in the home might prove a severe handicap

Television has already been considered seriously in the United States[25], and a number of experiments are being staged at the present time What, then, are the possibilities offered by television ?

Open broadcasting, as used in Scotland[26] and by several American centres[27], incorporates a number of obvious advantages and disadvantages Medical practitioners receive the same information simultaneously and thus have a common subject for discussion. They are able to watch and listen either in their own homes, at a society meeting or at a local hospital. Some evidence is already available from the United States[28], and several experimental series of broadcasts are now in operation. The inherent disadvantages are not inconsiderable. The lay public is able to receive the same information, so that the spoken word and the transmitted image must of necessity be rendered unobjectionable and so neutral as to leave the doctor-patient relationship unaffected. Perhaps equally serious is the objection that the doctor must make himself available at a specific time if he is not to miss what may be an essential part of a series of broadcasts. The possibility of repeating each transmission may become impracticable due to cost and lack of broadcasting time, as soon as other professions begin to ask for similar facilities. This restriction of air space and time is not likely to be overcome even if the one remaining free channel were to be allocated exclusively to education.

Scrambled or coded broadcasting offers the advantage of excluding the lay public, so that verbal and pictorial information is freed from any inhibitions imposed by open broadcasting. The disadvantages, however, are the same as for open transmission, with the additional difficulties associated with any special licences which may be required for scrambled broadcasts to minority groups and the need for a special decoding device in each receiver. One experiment is at present in progress in the United States.

Rediffusion, or transmission through land-laid cables, can be organized regionally, so that special local needs can be catered for. This facility and the privacy it offers can be regarded as potential advantages only, as the practical implementation, certainly for non-commercial purposes, is not likely to be realized within the present decade. It is equally unlikely that Great Britain can offer in the foreseeable future the extensive system of coaxial cables and microwave relay circuits which school education makes available to doctors in South Carolina[29].

Video tape in cassettes may eventually prove a more realistic and practical means of up-to-date dissemination of information to a widely scattered group of specialists, in this case, to doctors.

It will then be possible to plan a postal service, so that new information can be presented once fortnightly or once monthly. One or more medical schools could be asked to accept the responsibility for producing the master tape from which the copies are prepared, and as soon as the first set of copies is returned a further programme can be recorded on the same magnetic tape. As tapes will be interchangeable, regional as well as national services will become possible. The doctor who participates in such a scheme will be able to look at and listen to the recorded information with his own television set at a time or at times most convenient to him. He will be able to start, stop and, of course, repeat the tape or any part of the tape whenever he wishes. Eventually complete courses made up of a series of tapes could be added, so that an individual doctor can select the refresher course in which he is most interested. Contrary to film production, video tapes can be re-recorded more readily to keep the information up to date, and particular importance should be attached to this facility if the medium is to be exploited fully.

Cost will be a prominent factor in any discussion. The initial capital outlay will have to consider the necessary television recording equipment at a medical school or other medical centre and the bulk acquisition of the magnetic tape, which can, within reasonable limits be used over and over again. The cost, in thousands rather than tens of thousands of pounds, might be met by the authorities responsible for continuing education. Clerical and postal expenses, as well as the cost of the individual play-back instrument, might well be recovered by a reasonable subscription and hire fee.

However, the technical and financial difficulties are still so considerable that it may be advisable to consider motion picture film as a possible alternative, at least for the immediate future. The 'Technicolor 800*E*' rear projector at a cost of £55 already offers facilities for showing an 8-mm film loop in a plastic cassette which will run for a maximum of 4½ min before it repeats itself. Daylight viewing, the absence of any necessity for threading into the machine or for rewinding, and extraordinary cheapness—approximately £2 for 4½ min of film—are the major advantages of the system. While the film for this apparatus is silent, equipment for the showing of sound film is also becoming available.

Conclusion

Closed-circuit television, whereby electric signals are passed along a cable to reproduce a visible picture, has been shown to possess several properties which may be called on to help in the solution of a number of problems associated with the instruction of students in a medical school.

Television can offer a close-up view from the best possible observer position to large numbers of students; it can bring related visual information to the assembled students from other locations; it can act as the privileged observer and reporter where space is too restricted or when the presence of too many observers is not desirable, it can present the same visual problem to all students during an examination which sets out to test the application of knowledge which has been acquired from essentially visual information, it can distribute visual data from one room to many others so that students need not be assembled but can watch from wherever they happen to be working, it can make use of invisible X-ray as well as ultra-violet and infra-red radiation to present visible images, and it can, with the help of additional devices, such as endoscopes, permit all students to see structures and processes which until recently were seen only by the experienced physician and surgeon.

Although only a restricted number of critical experiments have been carried out so far, notably in the United States, the practical application of closed-circuit television has shown that it can materially help the student to enrich his visual experience and to learn by repetition. However, it must be acknowledged that, like any other device, its effectiveness is closely related to the manner in which it is used. Thus further experimentation will be necessary to establish really effective techniques and to determine the precise teaching situations where it can be expected to contribute most.

As programmed learning, or scientifically planned self-instruction, matures and therefore becomes more universally applied, television will also be called on to make its contribution in the form of stored visual information on video tape or similar media.

The dissemination of up-to-date information to medical practitioners can be achieved by broadcast television. Open transmissions are also received by the lay public, so that the contents and presentation of such programmes are likely to be handicapped. Open broadcasts share with scrambled and rediffusion transmissions the disadvantage of fixed broadcasting times when the doctor must make himself available or miss the programme. The first two methods would involve competition with other potential users for air space and time, and rediffusion is not likely to materialize as a practical reality for some time to come.

Video tape, television from recordings on magnetic tape, may become available at a realistic price. It may then be possible to distribute duplicate recordings to individual doctors for study and re-study in their own homes and in their own time. The ability to erase and record fresh information on the same tape repeatedly should make up-to-date continuing education through television more practical and perhaps more acceptable than present methods through journals and isolated postgraduate courses.

In the meantime, an 8-mm loop film cassette loading rear projector with sound may be a more practicable alternative

It is to be hoped that sufficient funds might be made available to conduct a critical pilot investigation which could be regarded as a reliable guide for the further development of continuing education

I thank Mr P V. Reading and Prof R. Warwick for their advice

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CHANGING FOOD HABITS

By Prof JOHN YUDKIN

Department of Nutrition, Queen Elizabeth College, University of London

SINCE the Department of Nutrition was instituted in Queen Elizabeth College, University of London, in 1954, and the courses began for the degree of B Sc (Nutrition), it has emphasized the importance of the social aspects of nutrition In particular, it has felt that we need to know very much more about the factors which determine people's choice of food, in the hope that we may be able to identify those through which nutritionally desirable change may be effected

When, with the aid of a grant from the Leverhulme Trust, a research programme in social nutrition was instituted, it was soon found that one needed advice not only from social scientists engaged in academic studies, but also from those in contact with the market-place The sociologist, anthropologist and psychologist who worked with the food industry had most useful and relevant experience, as had also the food chemist, the food manufacturer, the advertising agent and the market research worker Many informal discussions showed most gratifyingly that there was no reluctance at all from all these to share their knowledge and views with an academic group From this, the idea emerged of a more formal symposium, to which were invited some speakers representing many of the wide range of interests in patterns and trends in food consumption

The result was a symposium, held at Queen Elizabeth College on September 24, 1963, at which Mr James Van den Bergh took the chair, and where eight papers were read, representing the views of the nutritionist, the economic historian, the social anthropologist, the sociologist, the social psychologist, the experimental psychologist, the food chemist and the food manufacturer. The title of the symposium "Changing Food Habits" was deliberately ambiguous, and was meant to convey an interest both in the ways in which food habits were changing, and in the methods which might be effective in inducing change The surprisingly large number of 180 participants attended The eight papers were circulated before the meeting, speakers spent only a short time introducing their papers, so that most of the time was occupied by a very full and interesting discussion

The first paper, by John Yudkin, discussed "The Need for Change" Most people recognized that improved nutrition in the developing countries, especially of the tropics, involves not only an increased supply of food but often a change in the sorts of food which were eaten Whole populations, or groups within them such as women or pregnant women or children, often had habits and social customs which prevented the consumption of nutritionally desirable foods even where they existed But it was necessary not only to assure the consumption of desirable foods but also to try to prevent the consumption of undesirable foods There is evidence from the African countries that increasing industrialization in the developing countries might lead them straight from one sort of malnutrition to another This is because the food manufacturer has two separate functions First, he preserves food, and in so doing increases greatly the availability of food But as well as this, he is able to make completely new foods, based on his ability to separate palatability from nutritional value. Thus, palatability which normally is a guide to nutritional value is so no longer The ability to separate sugar, especially, has been responsible for the vast increase in sugar consumption in the developed countries in the past two hundred years, and may well contribute to the increased incidence not only of obesity but also of coronary thrombosis and diabetes

John McKenzie spoke on "Food Trends the Dynamics of Accomplished Changes". In Britain, there have been many changes in the pattern of food consumption, of varying magnitude Though it took some two hundred years for potatoes to become popular, they are now a basic part of our diet Yet we are not certain of the reasons for this change, nor for the differences in regional consumption Why, for example, do people in London eat three times as much poultry as those in the North of England ? Yet the experiences of some of these changes, such as the enormous increase in sugar consumption, suggests that increasing prosperity in the developing countries will lead to a similar rise We do not know exactly what effect advertising, education and price have on food consumption, except that there is a tendency to exaggerate their influence Other social factors certainly play a part in determining the foods which we eat Yet with the scant knowledge we have, and by the use of the techniques of social investigation, it is now possible to devise programmes, both of investigatory research and of experimental research, which should give us a much better understanding of the determinants of food habits

Margaret Read described "The Role of the Anthropologist" and spoke from her considerable experience of tropical countries She directed attention to the vicious circle of malnutrition → disease → decreased food production → aggravated malnutrition Though traditionally the social anthropologist has worked little in the field of food habits, he has recently been brought into consultation, in many parts of the world, to help in the elimination of the widespread and serious disease of protein deficiency especially in its form of kwashiorkor in children In order to be able to help in such work, an anthropologist needs to know something of the environmental factors affecting a community, its social structure, its domestic life, and its ideas on and attitudes towards food With this knowledge he can proceed to investigate the changes in food habits which are occurring in the country It appears that there are three motivations for such change, prestige, the wish to exchange foods and other products for money, and attempts to ease the burden of work for the women The details of these value systems, however, will differ in different communities

"The Role of the Sociologist in Inducing Changes in Food Choice" was described by David Pickard and Ray Corl People live in groups which each have a particular culture, that is, a particular set of usages, customs and institutions However, an individual does not belong just to one group, but can at any one time be a member of several groups, such as a teenage, working-class army officer The relationship between the individual and his group is expressed as the part which he plays according to his position in the group Change is slow because it tends to be a threat to the stability of the cultural system It can be accelerated, perhaps, by education and propaganda, but only in the direction in which it is already going Much can be done, however, through the innovator, the person who more readily adopts change and whose lead can be accepted While these general statements about the part of the sociologist have meaning in the context of changing food habits there has in fact been very little work done on the sociological background to eating

Cyril Sofer, I Janis and R L Wishlade gave an account of "Social and Psychological Factors in Changing Food Habits" They referred especially to some pilot studies which they had carried out at the Tavistock Institute of Human Relations, designed to reduce the consumption of carbohydrate in a group of housewives Among other factors, the effectiveness of the change in diet could be assessed in those who were overweight, since the change would lead to reduction in weight The method included group discussions, intensive individual interviews and an experiment in which attempts were made to persuade some of the overweight women to adopt low-carbohydrate diets The results, though tentative, suggested that group sessions, and 'working through' the difficulties of dietary change in advance, might be useful for changing food habits

According to Colin Golby, a psychologist engaged in academic research is more concerned with analysing a problem than with its solution, conversely, in commercial research he is more concerned with finding the solution and often has to do this without an adequate analysis of the problems Describing "The Role of the Experimental Psychologist", he pointed out that the problem of inducing changes in food habits was but one example of the problem of persuasion It was necessary to appreciate the parts that different foods played This knowledge was useful as a basis of a persuasion campaign, which itself could well be modelled on the methods of product-testing used in commercial promotions The next step would be a search for a main appeal for the product Finally, there is much to be said for attempting the actual persuasion by the use of the 'theory of dissonance' This is based on the drive for consistency, and the conditioning of an agreement response With appropriate attention to technique, it might then be possible to carry a group through the four stages of persuasion, these are communication, conditioning agreement, attitude change and finally action.

Arnold Bender spoke on "The Nutritionist in Industry" Perhaps 98 per cent of the food we eat passes through the hands of the food processor, who consequently has considerable responsibility for our nutritional health It is certainly true that the public will buy and eat what it likes, but the food manufacturer can sometimes mitigate the harm of nutritionally inferior choice as, for example, in the fortification of white bread with minerals and vitamins In the opposite direction, there is no point in nutritionally desirable food being made without adequate attention to attractiveness Yet there are examples of food preparations which are sold in vast quantities, on the basis of quite unsubstantiated nutritional claims While this is less true in Britain than in the United States, we too can be persuaded into buying and consuming such useless commodities as cider vinegar or royal jelly, and especially into buying preparations for slimming In spite of this, we do not know whether or to what extent even genuine nutritional claims—high protein, or the presence of vitamins—influences consumption of particular foods Nor is it usually possible to forecast whether a new manufactured food will or will not be a commercial success, and in particular to forecast whether the success will be sufficient, as with breakfast cereals, to induce a major change in food habits

The final paper, "The Food Manufacturer in a World of Economic Reality", was presented by Edgar Graham From the commercial point of view, the food market has two desirable qualities continuous demand and inherent growth It has, however, also to contend with the conservatism of the consumer The manufacturer must nevertheless be concerned with inducing change, either for improving nutrition in the developing countries, or in taking account of changing social attitudes in the wealthier countries In the former, as well as in the latter, change can be successfully introduced only when commercial principles are used It is true that these may sometimes be misused, but without their use the most desirable change may be difficult or impossible to produce. On the other hand, the use of commercial principles will not be able to produce a change in food habits unless they are in the direction of both general nutritional opinion, and of changing social and cultural patterns. In order that a new food should 'sell', it must be readily available to the housewife at a time when she is in the mood to buy it, and there should be adequate communication to her of the reasons why she should buy it In spite of these general statements, there are, however, still great areas of ignorance where much more research is needed before we can be certain of success

The discussion through the day was intense and varied, but two or three major points emerged One was that, from the point of view of the nutritionist, the difficulty was how to make one's food wants coincide with one's food needs It appeared to be accepted by most people that what they like to eat must be what they need—that "a little of what you fancy does you good" This perhaps explained the lack of interest in nutrition education, even among those who were in general interested in health education Though many suggestions were made as to the value of different methods in changing food habits, it was agreed that we are in fact not very knowledgeable, and that much more fundamental research needs to be done

There was also general agreement that the meeting was an extremely useful beginning, in particular in bringing together the academic and the commercial interests in this problem The papers, with an introduction and a summary, will be published by MacGibbon and Kee early next year, under the title of "Changing Food Habits"

THE YARD UNIT OF LENGTH

By P. H. BIGG and PAMELA ANDERTON

National Physical Laboratory, Teddington, Middlesex

THE Weights and Measures Act, 1963, passed by Parliament on July 31, 1963, lays down that the yard shall be 0.9144 metre exactly, the definition will come into force on January 31, 1964.

As the National Physical Laboratory is to be called on to state the current values of the former Parliamentary yard standards in terms of the metre in connexion with the adoption of the new definition, they have once again been determined. At this juncture, when the material yard standards are ceasing to have their former significance, it is appropriate to sketch briefly the history of the yard standard as a background to setting on record the recent comparisons and their outcome.

The British measures of length are thought to have been derived initially from a very ancient and widespread measure known as the 'northern cubit', rather more than 2 ft. long, which can be traced back to Mesopotamia and Egypt. The half cubit, or foot, was recognized in 12 B.C. as a standard for land measurement in Lower Germany, and its length was recorded by the Romans. With the coming of the Saxons in A.D. 410 the foot became established in England. William I in 1066, succeeding sovereigns and Magna Carta in 1215, maintained the existing measures and weights. Edward I in 1305 ordained that "three grains of barley, dry and round, make an inch, twelve inches make one foot, three feet make an ulna . . ."[1], etc.* His original 'iron ulna' or 'ell', later called the yard, has long since disappeared, but from a study of various bronze yards still in existence it seems it is unlikely to have differed from our current yard by more than about 0.04 in. (1 part in 1,000). By present standards the primary standard of Henry VII (1497) is only 0.04 in. in error, while that of Elizabeth I (1588, standard until 1824) is correct to 0.01 in. Both these last-named standards are preserved in the Science Museum, London, with other historical yards.

The first scientifically constructed standards were commissioned in 1742 by the Royal Society. Two brass bars, 42 in. × 0.5 in. × 0.25 in., on which were engraved the lengths of the existing Tower Standard Yard (Rowley, 1720) and Elizabeth's 1588 yard were sent to the Academie Royale des Sciences and marked with the French half Toise of three Pied du Roi, one bar was retained in Paris and the other returned to the Royal Society, but both have been kept for reference.

A Royal Commission (1758–1760) under Lord Carysfort prepared a new bronze standard (the maker was Bird) 1 in. square in section with inset gold studs 36 in. apart, the yard being indicated by fine indentations. This standard bar became the legal primary standard in 1824, but it was ruined in 1834 when the Houses of Parliament were destroyed by fire, and was not replaced until 1855.

All but one of the present yard standards were designed, constructed and compared with existing standards by Sheepshanks and Bailey, acting as members of the Royal Commission of 1843–55 (ref. 2) and were legalized in 1855. They are bronze bars (copper 16, tin 2½, zinc 1) 1 in. square in section, cast by Troughton and Simms, London. At the bottom of a hole near each end of the bar is a small gold plug inserted with its flat surface approximately in the neutral plane of the bar. On each gold plug three fine lines 0.01 in. apart traverse the axis of the bar, and two lines 0.03 in. apart, parallel to the axis of the bar, intersect the three lines. The length defined by the bar is the distance at 62° F, under certain conditions of support of the bar, between the middle transverse line at one end and that at the other, the part of the line utilized being that between the longitudinal lines. One of the bars was selected as the Imperial Standard Yard and designated No. 1, and four others were regarded as copies, to be used for the replacement of the primary standard should it ever be lost or damaged. Copy No. 4 was immured in the Palace of Westminster and has rarely been disturbed. It is required that the copies should be intercompared every 10 years and compared with the Imperial Standard every 20 years, the immured copy being expressly exempted from these requirements.

The Weights and Measures Act, 1878, which was not superseded until this year, authorized the construction of a further copy (No. VI), which was brought into use by 1886.

It is convenient to turn now to the metre. When the metric system was set up at the end of the eighteenth century the metre was intended to be one ten-millionth part of a certain polar quadrant of the Earth, and the Mètre des Archives, a platinum bar, was constructed on this basis. It soon became clear that the basic definition was too inconvenient for practical purposes, so the Mètre des Archives came to be regarded as having primary status. In 1889 this standard was superseded by the International Prototype Metre, an X-section bar of platinum–iridium, the distance at 0° C between certain lines on the exposed neutral plane of which defined the metre without altering the length of the unit in relation to that defined by its predecessor. The prototype metre is at the head of a hierarchy of platinum–iridium metre standards, including the national copies, and the prototype and certain other important copies are preserved with every care at the Bureau International des Poids et Mesures at Sèvres, near Paris, under the auspices of the Metric Convention and the surveillance of successive General Conferences of Weights and Measures.

With the advent of the possibility of using wave-lengths of light for the measurement of length, first Michelson and Benoît and later other experimenters in various standards laboratories (including the National Physical Laboratory) determined the length of the metre in wave-lengths. Nine determinations were made during the period 1892–1940 (ref. 3) and the mean departure from the average result was only 1 part in 6 million, and there was no evidence of a secular change of length, it was therefore clear that the length of the prototype metre was extremely stable. Further, this and other research work at the National Physical Laboratory and elsewhere proved beyond doubt that progress in length measurement would best be served by re-defining the metre in terms of wave-lengths of light. Consequently in October 1960 the value 1 650 763·73 wave-lengths in vacuum of monochromatic orange light radiated by the krypton-86 atom was adopted for the definition.

Returning now to the yard, as a result of comparisons with the metre it was suspected by the National Physical Laboratory as long ago as 1932 that the Imperial Standard was not stable and in consequence the value then found was not put into effect for purposes of science and technology. The results of the 1947 comparisons[4] confirmed these suspicions, it was clear that there had been a fairly uniform shortening of the Imperial Standard during this century the total decrease in this period being nearly 2

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* Further details may be found in "The English Yard and Pound Weight", by F. G. Skinner, *Bull. British Soc. Hist. Sci.*, 1 (May 1952), from which this historical information is taken.

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parts per million This lent powerful support to those who wished to unify the British and American yards (the latter had been linked to the metre in 1893), and in 1959 it was decided by the national standards laboratories in the English-speaking world to adopt the definition 1 yard = 0 9144 metre for scientific and technological purposes This definition is now legal in the United States and Canada, and will shortly be so in all the countries of the Commonwealth, the exigencies of legislation had delayed legal action in Britain, but, as already stated, the Weights and Measures Act, 1963, received the Royal Assent in July 1963 and the provision that the yard shall become 0 9144 metre will come into force on January 31, 1964 An outcome of this Act and the re-definition of the metre is that the yard is now related precisely to wave-lengths of a standard radiation

The National Physical Laboratory was in a position to apply the new definition of the yard as soon as need be, indeed, for science and technology it had been using this definition since 1959 But because it is to be called on to pronounce on the present value of the former Imperial Standard Yard (now to be called the United Kingdom primary standard of the yard and referred to here as the Yard No 1) and its copies in terms of the yard unit equal to 0 9144 metre, and for reasons of history, it was decided to re-compare these standards and to assess all of them directly in terms of the metre so that the results could be held in readiness

Through the courtesy of the Standard Weights and Measures Department of the Board of Trade the various yard standards were made available to the Laboratory, which already has the custody of the national copy (No 16) of the former international metre The yard standards were intercompared in the National Physical Laboratory Standards Division transverse comparator following the methods described in the Board of Trade Reports 1950 (ref 4) and 1957 (ref 5) Included in the comparisons was a nickel transfer standard No 184, bearing both yard and metre intervals related so as to facilitate linkage with the metre[6]. This same nickel standard was then compared with the national metre No 16 and a number of National Physical Laboratory working standard metres which it was convenient to include in order to ascertain their present values The value of the metre No 16 was known both on the basis of comparisons with other metre standards at the Bureau International des Poids et Mesures and through direct determinations in wave-lengths of krypton-86 at the National Research Council, Canada[7] It was, therefore, possible to assess the present values of the yard standards in terms of the metre on both bases and to add a further point to the graph relating the Yard No 1 and the metre The results in Table 1 have been obtained in terms of the international metre defined in wave-lengths in 1960 and the 1963 yard equal to 0 9144 metre

Table 1

Values of the national yard standards in May 1963, in terms of wave-length international metre and the 1963 yard

Yard standard	Value at 62° F (16 667° C)	
	Metre	Deficiency in relation to the 1963 yard
No 1	0 914 396 9	0 000 12 in
No 2	0 914 396 2	0 000 15
No 3	0 914 395 1	0 000 19
No 5	0 914 395 9	0 000 16
No VI	0 914 389 8	0 000 40

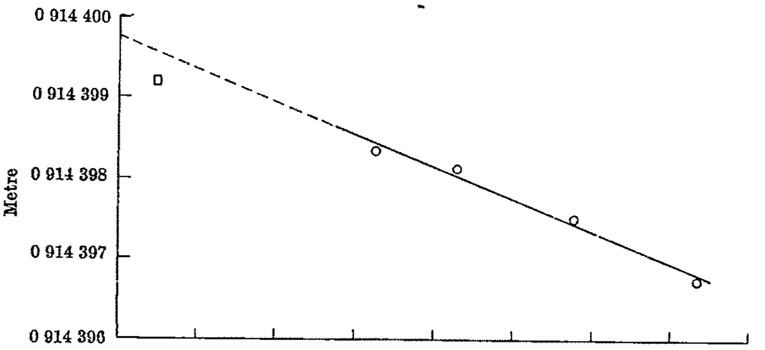

Fig 1 Values of Yard No 1 in terms of the metre as defined in 1889

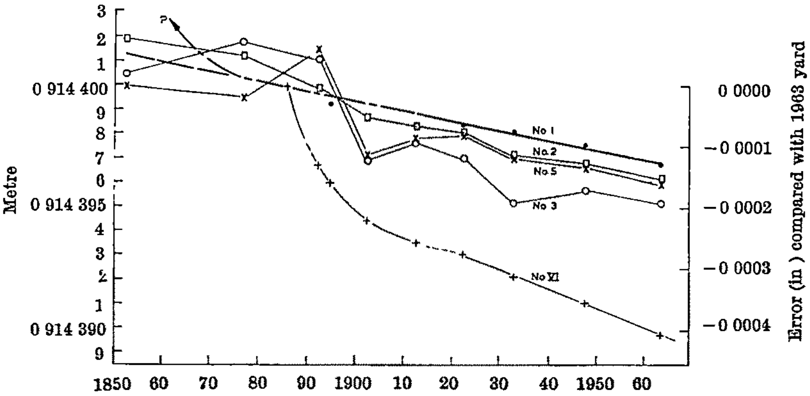

Fig 2 The yards in terms of the former International Prototype Metre and the 1963 yard Basis 1963–1922, yards compared with metre No 16 (via National Physical Laboratory Standard 184) 1922–1852, extrapolation of Yard No 1 and comparisons between yards

Fig 1 gives values of Yard No 1 since the turn of the century For consistency these values are based on the values of metre No 16 derived from the international prototype metre (used before the wave-length definition was adopted) The 1895 value was obtained at the Bureau International des Poids et Mesures by comparing Yard No VI and another yard (No 12) in all combinations with two subdivided metre standards, the comparisons being carried out by means of calibrated micrometer eyepiece microscopes, Yard No 12 had previously been compared with Yard No VI which itself was known in terms of the Imperial Standard.

The method used was not comparable with that later adopted, and though the result helped in 1948 to establish for the first time the instability of the yard system it is regarded as too old in relation to the present behaviour of the yard and too uncertain in relation to recent results to warrant its being given much weight now The line in Fig 1, which is accordingly based on the values found over the past 40 years only, shows that the Yard No 1 has decreased in length at the rate of 1 part per million in twenty-three years, corresponding closely to 0 0001 in so far this century

Fig 2 includes the information in Fig 1 on a reduced scale and shows the corresponding values for the other standards. Prior to 1922 no comparison of equal reliability exists, so the 1963/22 line has been extrapolated backwards, and this extrapolation, together with the observed errors of the copy yards in relation to Yard No 1, yielded the plotted values for this period

It may first be noticed that on this basis the value of Yard No 1 was exactly 0 9144 metre, that is, the 1963 legal yard, in 1884 The new definition of the yard therefore lays down a value equal to that defined by the Imperial Standard Yard in the early days of modern metrology Secondly, while at first sight it is curious that the values for Yard No 1 lie much more closely on a straight line than do those for the standards 2, 3 and 5 this is probably due to the slightly better quality of the fiducial lines on this standard This supposition is supported by the results for Yard No VI, on which the lines are perhaps a little better still, and for which over the period 1922–63 consistency of behaviour in the region of about 0 1 µm, or about 1 part in 10 million, has been experienced

The early history of Yard No. VI is one of rapid shortening. This standard was initially in extremely close agreement with Yard No. 1, but it is now 7 µm (about 2 5 ten-thousandths of an inch) shorter than No 1 and 10 µm (0 0004 in) shorter than originally. But for a slight discontinuity between 1912 and 1922 (due perhaps to a minor shock) it might have been even shorter now

The results on Yards 2, 3 and 5 have been much more irregular Those for Yard No 3 are particularly erratic, and it is interesting that this standard has been kept with the Pound No 2 which has also been a little unstable[4] Both the Yards 3 and 5 changed considerably in relation to Yard No 1 between 1892 and 1902 Incidentally, while Yard No 1 may have decreased similarly to No VI (Fig 2) in its early days, the spread of the values at any given time would be unaffected if this were allowed for. Broadly, Fig 2 shows that Yard No 2 has agreed with Yard No 1 very closely throughout their existence

In this article the present values of the yard standards, formerly the Imperial Standard Yard and its Parliamentary copies, have been presented against the background of an indication of the history of the yard from its earliest days to the present time Details of the recent determinations will be published shortly elsewhere It is a far cry from grains of barley to wave-lengths of a particular kind of light as emitted under closely defined conditions, whereas the earliest yard standards were probably not consistent to better than 1 part in 1,000 the present definition of the yard admits of a precision, through wave-lengths, of 1 part in 10^8 (ref 8) The present definition is likely to meet the requirements for accurate measurements for a long time to come For how long, it would be rash to hazard.

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OBITUARIES

Dr. Graham P. DuShane

GRAHAM PHILLIPS DUSHANE, editor of *Science* during 1956–62, died of a heart attack on July 19, 1963 Earlier in the spring he had been in hospital for five weeks recuperating from a mild coronary attack Recovery was apparently thoroughly satisfactory, the intervening period had been one of partial rest and gradual resumption of his duties, at the time of his death he was on a leisurely trip from his home in Nashville, Tennessee, to Palo Alto, California The fatal attack thus came as an unexpected shock to family and friends and brought to an untimely end DuShane's career as experimental embryologist, teacher, editor and university administrator

Graham DuShane was born in South Bend, Indiana, on July 20, 1910, son of Donald and Harriette Graham (McLelland) DuShane He received the Bachelor of Arts Degree from Wabash College in 1930, which awarded him an honorary LL D degree in 1958 From Wabash College he went to Yale University to become a student under Prof. Ross Harrison, through whose guidance he received the Ph D degree in 1934 The summer of 1931 was spent in Munich and the year 1934–35 as a research associate at the University of Iowa Receipt of one of the highly coveted National Research Council fellowships allowed him to spend the following year at Stanford University During 1936–46 as instructor, assistant professor and then associate professor of biology in the University of Chicago he continued his research in experimental embryology of the amphibians, the development of pigmentation and the nervous system, and the induction of melanin by strange ectoderms His most significant research contribution was the finding in amphibians that cells migrate outward from the developing neural tube to become pigment cells in the ectoderm.

At the University of Chicago he also developed a reputation as an outstandingly good teacher, and was honoured by the University in 1945 with an award for excellence in undergraduate teaching In 1946 he accepted a professorship at Stanford University, where his research interests were primarily carried out by graduate students, while his own time was largely devoted to developing and supervising the excellent general biology course which was taken by most Stanford undergraduates and to the revision of the undergraduate curriculum in biology that he left as a legacy to Stanford when he became editor of *Science*

At the beginning of 1956 he joined the staff of the American Association for the Advancement of Science as editor of *Science* and *The Scientific Monthly* The six and a half years of his editorship were years of steady and major improvement in the quality, size and circulation of *Science* The merger of *The Scientific Monthly* with *Science* at the beginning of 1958 allowed DuShane and his editorial staff to concentrate on the one journal During this period he instituted the series of articles entitled "Current Problems in Research", each of which summarized for a general scientific audience the current state of an active field of research Started also as an important addition to *Science* was the section entitled "News and Comment", which quickly became the most widely read and useful source of information for scientists about governmental, economic and social actions and forces influencing scientific activities Under DuShane's editorship, the circulation of *Science* grew from 32,000 to 76,000

During his years as editor, DuShane received a number of invitations to return to a university post In 1962 one came that he decided he could not decline Vanderbilt University offered him the triple responsibility of a professorship of biology, chairmanship of the biology department and, in the newly created post of Dean of the Graduate Sciences, responsibility for graduate research and education in the basic sciences, medicine and engineering

He was a member of the American Association for the Advancement of Science, the American Society of Zoologists, the American Association of Anatomists, Sigma Xi, and Phi Beta Kappa He was a consultant to the President's Science Advisory Committee and a member of the Board of Trustees of *Biological Abstracts* At Stanford he served as president of the local chapters of Sigma Xi and the American Association of University Professors He is survived by his wife, Susan Elizabeth (White) DuShane, three daughters, and two brothers

DAEL WOLFLE

Dr Don E. Eyles

DR DON E EYLES died on October 4, 1963, of coronary thrombosis on board ship in Penang, Malaysia, a few hours before he and his family were due to return to the United States This sudden death of an outstanding scientist and excellent colleague was particularly tragic, as Dr Eyles was about to retire after twenty-four years' work for the U S Public Health Service to join the Lahore (Pakistan) Unit of the Institute of International Medicine of the University of Maryland School of Medicine

Dr Eyles was born in 1915 in Atlanta, Georgia, and obtained his M S (Biology) at Emory University and his Sc D at the Johns Hopkins University in Baltimore

During the early period of his academic life he was interested in ornithology and medical entomology, but later much of his work was connected with investigations on malaria imported into the United States by returning Service-men, and with curative action of drugs against relapsing malaria infections The difference between the effect of pyrimethamine and primaquine on the tissue forms of malaria parasites became clear as a result of this work The curative action of pyrimethamine and sulphadiazine, and the synergistic effect of these drugs in toxoplasmosis, were reported by Eyles *et al* in 1952, and these findings were promptly confirmed in acute and chronic forms of the disease Much knowledge of the epidemiology of toxoplasmosis was due to the work of Eyles on the relationship between the infection in domestic animals and its transmission to man

In 1960 Dr Eyles, taking a clue from his accidental laboratory infection, showed that *Plasmodium cynomolgi bastianellii* of monkeys can be transmitted to man through a mosquito This started much new and still expanding research on the possibility of simian malaria as an anthropozoonosis

In 1961 Dr Eyles was given the task of establishing a research unit of the U S Public Health Service in Malaya and he went to the Far East accompanied by his wife and three children The research unit was set up at the Institute for Medical Research in Kuala Lumpur and within less than three years Eyles and his Malaysian, American, British and Australian colleagues produced a remarkable series of investigations which have greatly extended our knowledge of simian malaria Five new species of malaria parasites of Malaysian monkeys and of a moose-deer were discovered and their relationship to a number of anopheline vectors was established

A new area for further research of considerable fundamental and practical importance was thus opened in a field that until recently seemed to be uninspiring and well-trodden The impetus given in this way to the investigation of simian malaria was characteristic of Eyles's vision, determination, leadership and phenomenal energy During the past year Eyles was greatly interested in the problem of resistance of human plasmodia to synthetic drugs and particularly 4-aminoquinolines He left a number of papers which are now awaiting publication

Some of us who saw Eyles in September at the International Congresses of Tropical Medicine in Rio de Janeiro and who heard his summary of the work carried out in Malaya could not help saying that he seemed to be in a hurry to complete one job to start another He was in a hurry, indeed, for his "appointment in Penang"

Eyles's work resulted in more than a hundred publications He has demonstrated his qualities as an administrator of a research laboratory and his brilliance and versatility as an investigator of general and specific problems in parasitology One of Eyles's most important assets was his ability to work with a team, he has undoubtedly stimulated in others as much research as he has been personally responsible for His proficiency in experimental work in parasitology was equalled by his general knowledge of ornithology, botany and entomology He was an intensely live individual—an inveterate collector of almost anything, biological or otherwise—universally liked and admired by those who have had the good fortune to know him during his short, happy and fruitful life

L J. BRUCE-CHWATT

Mr. Douglas P. R. Petrie

DOUGLAS PETRIE died at his home suddenly on October 13, aged fifty-two He was born in Australia, graduated in Melbourne in 1931 in physics, took his M Sc under the late Prof Laby two years later, having worked on X-ray vacuum spectroscopy and on this gained an 1851 Exhibition Overseas Scholarship which he held in the Cavendish Laboratory Here he worked with Dr M L E (now Sir Mark) Oliphant, developing the electron optics for a hydrogen ion gun and for a very high voltage discharge tube, one of the first essays in electrostatic focusing to be performed on ion accelerators He was awarded the M Sc for this work

In October 1937, he joined the research staff of the Valve Department of Standard Telephones and Cables, Ltd Like most of this staff, he was soon brought into the development of decimetre- and centimetre-wave oscillators for airborne radar sets and worked on this throughout the War Petrie's great physical and mathematical skill played an extremely important part in the development of practical continuous-wave oscillators for various wave-lengths from 6–20 cm which were used for a variety of operational and instrumental purposes The development of these required much detailed calculation of the distribution of high-frequency electric fields He discovered and proved a new theorem in electrostatics, which made it possible to replace intelligent guessing by accurate quantitative design

Little of his war-time work could be published at the time it was performed, but at the end of the War he collaborated with other members of the team to write a major paper on the principles of velocity-modulation which received the Institution of Electrical Engineers Radio Section premium award for 1946–47

After the War he joined the Associated Electrical Industries Research Laboratory as a section leader, jointly with Dr D R Chick, to develop the Company's interests in nuclear physics, and together they were responsible for designing and constructing a 4-MeV electrostatic generator operating under high gas pressure, it being the Company's intention to exploit the patents of Dr van de Graaff in Great Britain under licence The construction and use of the machine served to train the first generation of scientists in Associated Electrical Industries for the coming nuclear power developments, but in addition very many new ideas were incorporated into this machine, in particular, Petrie's experience of ion focusing Before the idea of tandem generators had been developed, Petrie and Dr Chick in 1948 began the investigation of a helical slow-wave structure to accelerate ions emerging from the van de Graaff generator then being built, and, with others, he designed and constructed a pilot length of 1 metre, into which 2 5-MeV protons were injected and accelerated successfully to 4 MeV This would certainly have been continued but for the proved success of the tandem generator, the first of which to be built by the Associated Electrical Industries incorporated a number of significant improvements due to Petrie

Petrie then turned to electron optics and took charge of the Section dealing with this subject He developed the lens designs for a one-million-volt electron microscope which might have given a resolving power of 1–2 Å and worked out the theories of quadruple and octuple systems for correcting spherical aberration, he set up an experimental electron-optical bench for the measurement and correction of spherical aberration and devised computer programmes to solve related problems His great mathematical ability coupled with a keen physical insight kept his work in the forefront of what was required by the industry, and he keenly wanted to join the engineering team responsible for instruments based on electron optics

He was of a quiet disposition, and thought deeply before he spoke, his pronouncements were always appreciated by his colleagues and staff In earlier life he had composed music which had been played in public, and he was a good tennis player

He leaves a widow and one son

T E ALLIBONE

NEWS and VIEWS

The Royal Society Awards: Royal Medals

H M THE QUEEN has approved recommendations made by the Council of the Royal Society for the award of the two Royal Medals for 1963 as follows to Prof H H Read, emeritus professor of geology in the University of London and Senior Research Fellow in geology, Imperial College of Science and Technology, London, for his outstanding contributions to the understanding of the processes of rock metamorphism and the origins of granite, to Dr Robert Hill, member of the staff of the Agricultural Research Council, for his distinguished work in the biochemistry of plants, especially for his contributions to knowledge of photosynthesis

Mathematics in the University of Warwick:

Dr E C. Zeeman

DR E C ZEEMAN, whose appointment to the chair of mathematics in the new University of Warwick has recently been announced, has, since the War, been one of the leaders of the school of topology in Cambridge, where he is a University lecturer in mathematics and a Fellow of Gonville and Caius College His work has been mainly in the geometrical theory of manifolds and he has taken a leading part in the recent revival of purely combinatorial methods in this subject He had a large share in the work which led to the proof two years ago of the Poincaré hypothesis for spaces of dimension 5 and upward, a famous conjecture which had been outstanding for many years in spite of many attempts on it He has also recently put forward an interesting theory of the functioning of the brain, and especially of the processes of vision, based on concepts taken from those of general topology Dr Zeeman's loss is bound to be keenly felt in Cambridge, where he is the centre of an active research group He will have the good wishes of all for his success in building a new school at Warwick

Inorganic Chemistry at Monash University:

Prof B. O West

DR B O. WEST has been appointed to the newly established chair of inorganic chemistry at Monash University Dr West, who was born in 1928, was educated at Adelaide Boys High School and the University of Adelaide After graduating with honours in physical and inorganic chemistry in 1949, he joined the staff of the Chemistry Department of the University of Adelaide, where he held the positions of demonstrator, lecturer and senior lecturer and was, in 1962, appointed reader in inorganic chemistry During this period, in 1953–54, he spent two years at the University of Cambridge, where he held a Rhondda research studentship at Gonville and Caius College, and worked in the inorganic chemistry research laboratories Dr West's main research interest has been in the chemistry of co-ordination compounds and he has made significant contributions to our knowledge of the preparation and reactions of the inner complexes More recently, he has also become interested in the chemistry of organometallic compounds and has developed new methods for the synthesis of compounds containing both fluoroalkyl and alkyl or aryl groupings attached to the same element

Bio-Engineering at the Royal College of Science and Technology, Glasgow

THE Council of the Royal College of Science and Technology has announced the formation of a Bio-Engineering Unit within the Department of Mechanical Engineering, Dr R M Kenedi has been appointed research professor, as from October 1, 1963 Work in this field first commenced in the Department of Mechanical Engineering in the College some six years ago with experimental studies of skin tension in the human body and with the application of refrigeration techniques in hypothermia Since then, the Department's research activity in this field has greatly intensified in close co-operation with Glasgow's teaching hospitals Co-operation takes the form of direct collaboration between surgeons, physicians and engineers, working as combined teams in the College engineering laboratories, the hospital wards and operating theatres and the various medical research departments as required Thus the medical side is allowed every opportunity to become familiar with the available relevant engineering techniques, while the engineers are taken directly into the hospitals to become fully acquainted with particular medical problems under consideration in relation to the human patient The Unit will be administered by the Department of Mechanical Engineering in the College in association with a Steering Committee The primary duty of the Steering Committee will be to screen the projects put forward for investigation and to authorize work on such as seem suitable and practicable.

Dr R M. Kenedi

DR R M KENEDI graduated B Sc with honours in civil engineering at the University of Glasgow in 1941, and was awarded the degree of Ph D (Glasgow) in 1947 He was awarded the honorary degree of C H of the National University of Engineering, Lima, Peru, in 1958, and is an Associate Member of the Institution of Mechanical Engineers and an Associate Fellow of the Royal Aeronautical Society He joined the staff of the College in the Department of Civil and Mechanical Engineering in 1941 and was appointed to a senior lectureship in 1947 He became reader in strength of materials in 1956, a post which he has held until his present appointment In addition to his academic duties, Dr Kenedi has pursued a wide range of research interests, many connected with industrial problems In 1957 he carried out a two-month tour of universities and industrial research centres in the United States, surveying current research on cold-forming processes and products for the Cold Rolled Sections Association, and in 1958, as a result of a joint invitation from the Universidad Nacional de Ingenieria, Lima, and the British Council, he spent some six weeks in Peru, lecturing and advising on the contents of engineering courses and on the planning of a materials testing laboratory Dr Kenedi was born in Hungary and became a naturalized British subject in 1947

Nuffield Unit of Medical Genetics at the University of Liverpool

The Trustees of the Nuffield Foundation are to make a grant of up to £350,000 to the University of Liverpool in support of the capital and running costs of a proposed Unit of Medical Genetics This Unit will be established under the ægis of the Department of Medicine It is anticipated that the grant from the Nuffield Foundation will meet the major part of the cost of the Unit for a period of nine years The University has undertaken to assume full responsibility for the Unit from the beginning of the academic year 1972–73 Since 1953 an increasing amount of work in human genetics has been carried out in Liverpool At the present time there are ten workers investigating human genetics problems in the Department of Medicine under Lord Cohen of Birkenhead The emphasis throughout has been on investigation into the causes of common diseases, among them duodenal ulcer, rheumatic fever, rheumatoid arthritis, Rh hæmolytic disease and its prevention, leukæmia and other cancers, thyroid disease and schizophrenia Dr C A Clarke, reader in medicine at the University, will direct the Unit of Medical Genetics, which will work in close association with Prof P M Sheppard, head of the Department of Genetics at Liverpool, with Prof E B Ford, of the University of Oxford, and with other universities

The Imperial College of Science and Technology and the Indian Institute of Technology (Delhi)

The Imperial College of Science and Technology, University of London, has entered into special relationship with the Indian Institute of Technology (Delhi), formerly the Delhi College of Engineering and recently raised by the Government of India to the status of an institute of national importance The British Government, in collaboration with British industry, has given substantial financial assistance towards the new Institute's full development Parallel academic assistance will be provided through the special relationship with the Imperial College This will be made possible through financial help from the Department of Technical Co-operation Discussions are now proceeding in London between Dr Thacker, chairman of the Board of Governors of the Indian Institute, Sir Patrick Linstead, rector of Imperial College, and Sir Willis Jackson, chairman of the British Technical Committee The scheme will involve long-term secondments of Imperial College staff, training of Indian staff at the College and short visits to Delhi by senior College staff Academic assistance is already in being for example, the chair of mechanical engineering at the Indian Institute has been filled for more than a year by Prof N P W Moore He is on long-term secondment from Imperial College, where he holds the post of reader in the Department of Mechanical Engineering

The Council of Scientific and Industrial Research, New Delhi. Publications

The present publications of the Council of Scientific and Industrial Research, New Delhi, include the *Journal of Scientific and Industrial Research*, a monthly general science periodical, replacing the *Journal of Scientific and Industrial Research Section A—General*, the *Indian Journal of Chemistry*, a monthly research periodical devoted to original communications in chemistry (including biochemistry), replacing the chemistry part of *Section B—Physical Sciences*, *Indian Journal of Pure and Applied Physics*, concerning original communications in physics, replacing the physics part of *Section B—Physical Sciences*, the *Indian Journal of Technology*, again a monthly research periodical dealing with applied sciences and technology, replacing *Section D—Technology*, and the *Indian Journal of Experimental Biology*, a quarterly research periodical embracing original communications of an experimental nature in the biological field, replacing *Section C—Biological Sciences* The *Indian Journal of Technology* (1, No 6, June 1963) gives details of the aforementioned changes and coverage of the subjects sponsored by the Council This particular issue is notable for the range of subjects discussed, including investigations on activators for vulcanization of rubber, oxidation of cellulose by chromic acid in presence of hydrated oxides of metals, modification of lac esterification of lac with allyl alcohol, investigations of stamped charging of coals for coking, utilization of lignite dust for making granular products, observations on the theaflavin gallate and thearubigins of black tea, chemical composition of mackerel (*Rastrelliger canugurta*) changes in the nutritive value during storage, dehydration of green peas These papers are followed by short communications traversing an equally versatile range of subjects

The British Glass Industry Research Association

The eighth annual report of the British Glass Industry Research Association presents brief accounts of seven major projects, largely carried out in what is clearly the closest co-operation with the industry itself (Pp 44 Sheffield The British Glass Industry Research Association, 1963) Among some highly interesting operational work is an inter-firm comparison of the performance of container furnaces, from which differences in British and American practice were brought into prominence American practice leans towards higher temperatures and higher melting rates, and it is interesting that of 142 American furnaces noted, none was now using producer gas, while a third of the 100-odd British furnaces were still on this fuel, although its use has fallen by a half in the past ten years Other operational exercises dealt with batch mixing and handling in the founding problem and with inspection and handling of glassware There are some interesting and unsolved problems in the loss of the strength during storage, a phenomenon which is also observed for some ceramic products Fundamental work on strength has continued with particular reference to the possible application of ion-diffusion techniques to conventional mass-produced glassware, and it has been demonstrated that ion-diffusion processes can increase the strength of abraded unetched samples from about 30,000 lb /in ^{2} to 90,000 lb /in ^{2} On the technological scale, stress configurations on large glass panels under uniform loading are being investigated by strain-gauge techniques Considerable progress has been made on the examination of heat transfer from glass through the mould, and the condensation aspect has been solved An analogue computer has been built to extend the investigation to take account of heat transfer by radiation The report concludes with a note on the information service and a list of reports and publications It is depressing to read that "the rising costs of running the Association have not been matched by any increase of income" and that a planned reduction of staff is contemplated One must deplore that a massive national asset like the family of research associations should have to see any of its members financially embarrassed

History of Science

Volume 2, 1963, of *History of Science*, an annual review of literature, research and teaching, is noteworthy for F Maddison's survey, with bibliography, of sources and modern studies of early astronomical and mathematical instruments, W A Smeaton's paper, "New Light on Lavoisier the Research of the Last Ten Years", and R E Schofield's article on the needs and opportunities for research into the histories of scientific societies Under the title "Merton Revisited", A R Hall contributes a critical re-examination of R K Merton's article in 1938 on science, technology and society in seventeenth-century England, while A H Ewen describes a sixth-form course in the history of the philosophy of science Among the ten essay reviews may be mentioned D T Whiteside's discussion of the scientific papers of Newton; R W. Southern on technology and history, and D S L Cardwell on science in the nineteenth century *History of Science* is edited by A C Crombie and M A Hoskin and published by W. Heffer and Sons, Ltd , Cambridge (paper bound, 21*s*. net, cloth bound, 30*s* net)

The Society for the Promotion of Nature Reserves

THE 1963 *Handbook* of the Society for the Promotion of Nature Reserves includes, besides the forty-ninth annual report covering the year ended March 31, 1963, and the accounts and balance sheet, a report on the Council for Nature, membership of which has risen to 339, and lists of the Society's reserves and local committees, officers and Council, Naturalists' Trusts and members and associates (Pp 44 London The Society for the Promotion of Nature Reserves, British Museum (Natural History), 1963 10*s*). Annexes to the report list purchases completed during the year with the aid of the Nuffield Loan Fund The report includes brief accounts of activities during the year on the Society's own reserves, and in that relating to Dancer's End, Buckinghamshire, reference is made to the pillage of wild flowers and the difficulty of restricting such pillage

African Engravings and Decorations

THE Museu do Dundo, run by the Portuguese Diamond Co of Angola (Diamang), has long been famous for its splendid publications concerned with the cultures, past and present, of the country The latest, *Cabaças Gravadas da Lunda*, by Mário Fontinha and Acácio Videira, is concerned with the engravings and decorations on calabashes, which are used to-day as drinking-vessels, mugs, funnels, boxes, etc (*Publicacoes Culturais* No 57 Pp 178 (34 plates) Lisboa Companhia de Diamantes de Angola, 1963) The engravings are made with a sharp knife, the blade being thick or thin according to the lesser or greater delicacy of the strokes to be made The volume is largely a picture book illustrating a considerable number of these engravings For the most part, conventionalized human beings are depicted, some of them very amusing to look at But animals, equally stylized, and patterns also occur One interesting figure represents a man driving a motor-car The economy of line would probably please many modern European artists For those interested in pre-historic and primitive art it is always well to take note of a modern art group made by modern primitive peoples This work can be strongly recommended, both for its scientific value and as an intriguing picture book.

Distance of the Andromeda Galaxy

THE late Dr W Baade was interested in extending our knowledge of the stellar populations and obtaining a more accurate distance of the Andromeda Galaxy. For this purpose he selected a number of fields at varying distances from the centre of the Galaxy and obtained a large number of photographs The results for one field have now been completed by his associate, Miss H H Swope (*Astronomical J* , 68, 435, 1963) They refer to a field 96′ south preceding the nucleus in a very distant outer spiral arm, selected for its low content of absorbing material More than 150 variable stars have been found, and. of these, twenty Cepheids have been investigated in detail, obtaining brightnesses and periods of variation The Andromeda cepheids are systematically reddened in comparison with those in our own Galaxy, but it is thought that this reddening arises mainly in our Galaxy and not in the Andromeda Galaxy The Cepheids are further from the centre of the Andromeda Galaxy than the Sun is from the centre of our own Galaxy The unreddened distance modulus turns out to be +24 20 magnitudes. The distance is 690 kiloparsecs (2 25 million light years) with an uncertainty of at least 50 kiloparsecs

Textile Research at the Lódz Technical University

THE theory of flow in the multi-branched siphon is developed in No 9 of the *Textile Sciences* (*Włókiennictwo*) series of the *Scientific Bulletin of the Lódz Technical University* (*Zeszyty Naukowe Politechniki Lódzkiej*) This siphon is the main feature of Prof Atanazy Boryniec's invention for spinning staple viscose fibres in a hydraulically closed system to which gases harmful to health may be confined Other papers deal with the role of sulphuric acid in the acetylation of cellulose; the use of melamine–urea–formaldehyde mixed precondensates for imparting crease recovery to linens and viscose–flax blends; benzylation of cellulose; stability of knots in fishing nets, structural changes in polyacrylonitrile fibres during drawing and heat treatment All papers are in Polish, but there are Russian and English summaries, which are sometimes extensive. The *Bulletin* is mimeographed and appears about once a year. It is edited by Dr Witold Żurek, and copies are obtainable from Redakcja Wydawnictw Naukowych Politechniki Łódzkiej, Łódź, Gdańska 155, Poland

New Coagulant Aids for Removal of Suspended Particles

FLOCCULANTS facilitating removal of suspended solids from sewage and industrial wastes, also in pre-treatment of raw water similarly charged, are now reinforced with what are described as " . new coagulant aids . liquid, water-miscible polyamines (cationic polyelectrolytes)" These products are known as 'Primofloc *C*-3' and 'Primofloc *C*-5' and are now being offered on a commercial scale by Rohm and Haas, Philadelphia, Pa , well known for their manufactures of plastics, synthetic resins and chemicals for many industrial applications A recent issue of the *Rohm and Haas Reporter* (21, No 3; May–June, 1963), a colourful and informative house-organ (distributed in Britain by Lennig Chemicals, Ltd , Bedford Row, London, W C 1), gives full details of these compounds, also of the many technical services offered in the wide field of their international activities

Treatment of Tuberculosis

BEFORE the advent of potent and anti-tuberculous drugs, the recovery of patients depended entirely on natural and acquired resistance augmented by supporting treatment This inevitably resulted in a high mortality With the use of anti-tuberculous drugs, especially streptomycin, isonicotinic acid hydrazide and para-aminosalicylic acid, the prognosis of this formerly deadly disease changed for the better The application of adrenocorticotrophic hormone or adrenal corticosteroids and their analogues achieved equally promising results When hormones were first used, reports were discouraging because the hormone was given alone or for the treatment of another disease without enough knowledge of the underlying tuberculous lesion To-day, combined treatment is increasingly used and often without knowledge of all the facts A communication by Drs Somchai Bovornkitti, Prasert Kangsadal, Pairojana Oonsombatti and Damrong Bejrablaya of the Departments of Medicine and Physiology, Faculty of Medicine, and Siriraj Hospital, University of Medical Sciences, Dhonburi, Thailand, reports their experience on the use of prednisone concurrently with conventional anti-tuberculous treatment in cases of tuberculous pleurisy with effusion (*Japanese Journal of Tuberculosis*, 10, No 1–2, June, 1962) Twenty-three cases of pleurisy with effusion, presumably tuberculous in origin, were treated with a combined régime of prednisone and conventional anti-tuberculous drugs Prednisone was used for a month The results indicate that the combined treatment is superior to the conventional anti-tuberculous remedies used alone The results of treatment were attributed to the desensitizing properties of prednisone on tuberculin hypersensitivity.

Visiting Fellowships in Astrophysics

APPLICATIONS are invited by the Joint Institute for Laboratory Astrophysics, Boulder, Colorado, for visiting fellowships in theoretical astrophysics, low-energy atomic physics and other closely related areas The fellowships, tenable for one year or possibly a shorter period, are awarded by the University of Colorado for the Institute, to provide an opportunity for persons actively contributing to these fields to visit the Institute to continue their studies and research in the field chosen by them The amount of the stipend will normally equal the recipient's present academic salary adjusted to a twelve-month basis The cost of transportation to and from Colorado for the recipient and his immediate family will also be met by the Institute The closing date for application is January 15 Further information and forms of application can be obtained from the Secretary for Visiting Fellows, Joint Institute for Laboratory Astrophysics, University of Colorado, Boulder

The Vernon Prize in Industrial Psychology and Physiology

THE late Dr H M Vernon made a bequest to the National Institute of Industrial Psychology expressing the wish that the Institute should award a prize every second year to the investigator who, being a British subject and under the age of forty-five years, has in the opinion of the Council of the Institute done most valuable research work in service of industrial psychology or industrial physiology The sixth award will be made in 1964, it will consist of approximately £40 in cash and a silver medal Recommendations are invited of the names of workers in this field whom the Council should consider Direct applications for the award may also be made Recommendations or applications, supported by reference to published work and, if possible, copies of unpublished but relevant reports, should be made to the Director, National Institute of Industrial Psychology, 14 Welbeck Street, London, W 1, by February 29, 1964

The David Anderson-Berry Prize

A DAVID ANDERSON-BERRY Silver-Gilt Medal, together with a sum of money amounting to not less than £100 (280 U S dollars), will be awarded by the Council of the Royal Society of Edinburgh during 1964 The Prize will be awarded for recent work on the effects of X-rays and other forms of radiation on living tissues Published work will be taken into consideration if submitted to the Society with the application In addition to direct application for the Prize, proposals can be made on behalf of others Applications and proposals must be sent to the General Secretary, Royal Society of Edinburgh, 22–24 George Street, Edinburgh 2, Scotland, by March 31, 1964.

Courses in Higher Technology

THE wide variety of courses in advanced technology and management studies available to students in the London area is again presented in a bulletin prepared by the London and Home Counties Regional Advisory Council for Technological Education (*Bulletin of Special Courses in Higher Technology, Management Studies and Commerce, 1963–64*. Part 1 Autumn Term Pp 134 London London and Home Counties Regional Advisory Council for Technological Education, 1963 4*s*) The courses range from elements of law relating to civil and mechanical engineering at Battersea College of Technology, hydraulic and electrical control of injection moulding machinery at the Borough Polytechnic, a practical course in Elliott 803 autocode at Brunel College, the economics of tanker operation (shipping) at the City of London College, the Lebesgue integral at Hatfield College of Technology, solar heat gain at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, gyrodynamics and inertial navigation at the Northampton College of Advanced Technology, rapid reading at Slough College, and carpet planning at the Technical College for the Furnishing Trades

Announcements

WE regret to announce the death on November 13 of Dr Margaret Murray at the age of 100 (*Nature*, 199, 119, 1963)

SIR WILLIAM PENNEY, deputy chairman of the United Kingdom Atomic Energy Authority, has been appointed to succeed Sir Roger Makins as chairman of the Atomic Energy Authority when he retires in February 1964 Sir Roger Makins was appointed chairman of the Authority in 1960 Sir William Penney has been a member of the Authority since it was set up in 1954

THE Plastics and Polymer Group has instituted an annual prize of £50 for the best paper submitted on any aspect of pure or applied polymer science The competition is open to all British subjects under thirty years of age Full details can be obtained from the Secretary, Plastics and Polymer Group, c/o Society of Chemical Industry, 14 Belgrave Square, London, S W.1.

THE Yearbook of the National Institute of Sciences of India, 1962, besides the report of the Council, to which are appended brief reports on research in physics, chemistry, botany, zoology and physiology, includes a brief account of the foundation of the Institute together with the lists of Fellows, membership of Council and committees, grants and publications and additions to the Library, as well as the rules and regulations (Pp vi + 251 New Delhi National Institute of Sciences of India, 1963 Rs 15 00)

THE 1963 Arthur Stanley Eddington Memorial Lecture entitled "Mind and Consciousness in Experimental Psychology" will be delivered by Dr R H Thouless in Cambridge on November 29 Further information can be obtained from W. B Harland, Arthur Stanley Eddington Memorial Trust, Gonville and Caius College, Cambridge

THE annual meeting of the American Association for the Advancement of Science will be held in Cleveland, Ohio, during December 26–30 Further information can be obtained from Mr Kneeland A Godfrey, American Association for the Advancement of Science, 1515 Massachusetts Avenue N W , Washington 5, D C

AN introductory course on "The Biology of the Skin" will be held in the School of Clinical Research and Postgraduate Medical Teaching, University of Cambridge, during December 9–14 The course is intended primarily for registrars and postgraduate students at an early stage of their training in dermatology, but is also open to other medical practitioners The fee for the course will be 5 guineas Further information and forms of registration can be obtained from the Secretary, Cambridge University Medical School, Tennis Court Road, Cambridge

THE forthcoming programme of the recently formed Society of Acoustic Technology (see *Nature*, 197, 23, 1963) will include a meeting on "Acoustics", arranged jointly with the Liverpool and North Wales Branch of the Institute of Physics and the Physical Society at the University of Liverpool (December 11), a meeting to discuss "Practical Cases of Noise Reduction" at Salford (January 16), a meeting on "Noise in Ventilation Systems" at Manchester (February 21), a meeting on "Scale Model Techniques" at Salford (April 15), a conference on "Planning and Design for Protection from Noise" at Coventry (June 1–4), a conference on "Medical and Industrial Aspects of Hearing Loss" at the University of St Andrews, Dundee (September 25–27) Further information can be obtained from Dr P Lord, Department of Pure and Applied Physics, Royal College of Advanced Technology, Salford.

THE GEOLOGICAL SOCIETY OF AMERICA, 1888-1963

ON December 28, 1888, the geologists of America, previously forming a group within the American Association for the Advancement of Science, started their own Society, the Geological Society of America, at a meeting in Ithaca The list of Founder Fellows numbered 109

Since that time the United States has progressed consistently in geological research, due mainly to its wealth in geological material The exploration of the West, with the palæontologists reporting on fossils, has led to important discoveries in the structure, the dynamics and the evolution of the vertebrates forming probably the most brilliant chapter in the earlier history of geology The invertebrates, though deprived of a solid structure, left enough imprints throughout the ages to allow the palæontologists to reconstruct a substantial part of their biological and evolutionary past The origin of life and of the Earth are other subjects closely associated with the aims of geology. Coal, ores, petroleum, light and rare metals, radioactive elements, all are the concern of geologists, who must also be credited for advances in prospecting methods, in the study of the ocean floors, of water resources, earthquakes, and in the techniques of surveying and mapping Geological engineering is a new development, and if man sets foot on a new planet, the chances are that this man will be a geologist

The Geological Society of America acts as the academic body, the guardian of the science of geology and related earth sciences Oceanography, seismology, astrogeology, the study of meteors, of the crust and the mantle of the Earth, the behaviour of rivers, wells, lakes, water resources, the construction of dams and tunnels, hydroelectric systems, location of mines, their development and prospecting, all need the helping hand of the expert geologist

The Society soon formed one of the best trained groups of geologists in the world, and its efforts united and encouraged them, and helped them to overcome their earlier provincialism Geology had come of age in the United States

Still, in the early 1930's this was a rather small society (600 members), until one of its Fellows and one-time president, a practising geologist, professor and investor, son of a prominent Philadelphia family which had been better known for its political connexions, bequeathed half his estate to the Society (the other half to the American Philosophical Society in Philadelphia) The benefactor was Dr Richard A F. Penrose, jun, who died on July 31, 1931, leaving to each society approximately 3,900,000 dollars, to be considered an endowment, the income of which was to be used and the capital to be properly invested Probably no other small organization devoted to a relatively humble science was ever before so lavishly endowed Although, in the succeeding years, the Society may have disbursed more than twice the value of the endowment, the capital fund still stands to-day in excess of the original valuation

In the years following this gift, geological science has witnessed an almost explosive evolution to which the bequest has not been foreign It revolutionized the Society, opened new vistas, supplied needed support, and raised the Earth sciences to their rightful place in scientific research About 40 per cent of the income went to publications, and a large share to grants in aid of research The journal of the Society, the *Geological Society of America Bulletin*, a primary objective created in 1890, changed from a quarterly to a bi-monthly and, in 1935, to a monthly It was the first publication in a position to add inserted charts and coloured maps to its text

But the *Bulletin* was not enough to provide an outlet for manuscripts, particularly those too long or unsuited to a journal From this need originated the more extended publishing programme of the Society, and the list of its *Memoirs* and *Special Papers* now numbers about two hundred These volumes, of permanent value, are considered essential tools for research in the Earth sciences

A *Bibliography and Index of Geology exclusive of North America* was authorized in 1932, of which 26 volumes have appeared so far, and in this series of annotated references the foreign geological literature is reported This effort parallels and complements that of the U S Geological Survey, which publishes the *Bibliography of North American Geology*

Another important enterprise is the publication of the *Treatise on Invertebrate Paleontology* in 24 volumes by 180 authors from all over the world 12 volumes have been completed and 12 are in process

Many geologists prefer accurate maps to descriptive texts For their benefit the Society has allocated funds for the production of large wall maps, compiled from many dispersed smaller ones Geological maps of North America, South America and Mexico, and glacial, tectonic, physiographic and ocean maps have been completed. This work continues

Some 1,000 individuals have been assisted in their investigations by grants in aid at an average of about 1,500 dollars a grant

The Penrose Medal (endowed as early as 1927) is the greatest honour bestowed by the Society and is highly prized among the awards to which geologists of the world are eligible

During November 18–20 the Geological Society celebrated its seventy-fifth anniversary when its meeting was held in New York (at the Hotel Commodore). The roster of Fellows, Members, students and guests attending, from the original 109, exceeded 3,000

The Geological Society of America has been fortunate in the continuity of its leadership Its seventy-five years of history have been witnessed by only seven secretaries and six treasurers The officers for 1963 are *President*, Harry H Hess, *Vice-President*, Francis Birch, *Secretary*, Joe Webb Peoples, *Treasurer*, J Edward Hoffmeister

At the time of convening in New York, the Society is leaving its stately home on the Columbia Campus, where for thirty years the welcome mat was out for visiting geologists The mat will be moved to 231 East 46 Street, the new building acquired this year by the Society

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DURING the seventy-fifth anniversary meeting, three general symposia were held (1) Relation of Geology and Trace Elements to Nutritional Problems, (2) Geology of Ocean Basins and Continental Drift, (3) The Principle of Uniformity

Awards and medals were presented, namely the Kirk Bryan Memorial Award, to Arthur H. Lachenbruch (U S. Geological Survey), the Penrose Medal, to William W

Rubey, University of California, Los Angeles, the Arthur L Day Medal, to Keith E Bullen, University of Sydney Associated societies took part in the celebrations, namely, the Mineralogical Society of America, the Paleontological Society, the Society of Economic Geologists, the Geochemical Society and the National Association of Geology Teachers Some of these societies also presented awards at the meeting

PREPARATION AND PUBLICATION OF GEOLOGICAL JOURNALS

A TWO-DAY meeting of editors of British geological journals, sponsored by Messrs Oliver and Boyd, Ltd, was held in Edinburgh during October 11–12 The meeting covered a wide range of topics including instructions to authors, refereeing, preparation of manuscript for printer, relations with printer, design and layout of British geological journals, blockmaking, sales, distribution and costs, index cards

Suggestions as to ways of reducing editorial chores were few. The solution seems to rest largely with the author and the better preparation of his script The *World List* reference style has been adopted by most geological journals in the past few years, but inconsistency in references still remains an editorial problem More uniformity in instructions to authors was welcomed by some, but not all, of the editors present It was understood that the Geological Society of London's detailed notes to authors are due to be revised, and the hope was expressed that much of the material in the new edition would prove to be of general use Certainly the plea for a more uniform treatment and a higher standard of diagrams was welcomed by those present

Mr Thomson (Oliver and Boyd) discussed editorial relationships with the printer A clean copy, as always, is much to be preferred Once the printer knows a journal style, only a small amount of mark-up, such as the centring of headings and indenting, is required He revealed that printers generally keep notes of the style of each journal, including the spelling of scientific words peculiar to that journal, and that uniformity of style from issue to issue is achieved by using the same compositor.

It was agreed that authors did not appreciate the high costs involved in even minor alterations (as was proved by a visit to the compositors' room), and all the editors welcomed the opportunity of acquiring copies of *Authors' Alterations Cost Money and Delay*, produced by the British Federation of Master Printers This pamphlet would be sent out to authors at the proof stage. The suggestion was made that authors should be charged for all alterations, printer's errors apart, that they made to the proofs The advantages of this scheme lie in the saving in costs and in quicker publication since it might be possible to omit the galley proofs and go straight into page proof The disadvantage lies in a less-than-perfect final copy in which the errata are confined to a short appendix

Mr Ian Wilson, chairman of the Scottish Council of Industrial Design, provided one of the highlights of the meeting by making a candid assessment of the design and layout of British geological journals He classified them into three groups, 'Pleasing', 'Nothing special but some care shown', and thirdly, 'Nothing done since first published' It would be invidious to classify them here, but several general criticisms are worth making They included poor type-faces, wrong size of type in relation to the length of line, too yellow a paper, paper too bulky, paper of different colours in the same issue, too many type faces (and therefore added costs), generally poor quality of diagrams, and, with one exception, unnecessarily drab colours of the covers He wondered why journals did not print more frequently on both sides of the art paper used for plates He suggested that some schools of art or of printing would welcome the opportunity to re-design a journal The defence to Mr Wilson's arguments was that more attractive layouts usually cost more money, and if costs increased by, say, 10 per cent, then 10 per cent fewer papers would be published, to which Mr Wilson rejoined that there is little use in printing papers if they are not readable The general feeling among the editors was that presentation could certainly be improved and that this could be done in collaboration with the printer. A useful suggestion made during this discussion was that the page numbers should be printed on the spine of each part

Mr Hislop (Hislop and Day) discussed methods of blockmaking He thought that geological diagrams were better than the average, his main criticism being that originals are generally too large Reduction to less than half size should be avoided He reminded editors and authors of the necessity of marking the top of each photograph, and of having soft rather than contrast prints Contrast can always be increased but not reduced He thought that Letraset was good for lettering and recommended a new photographic process of lettering called Starlettograph-Lichtsatz (Munich)

Mr Douglas Grant (Oliver and Boyd) reminded editors of the necessity of watching costs They should ask for detailed invoices from their printers so that the costs of the different items such as paper, composition, corrections, machining, etc, can be determined Offprints should be ordered before printing so that the required number of plates, and in long articles certain of the printed sheets, can be run off during the main printing Variations in type sizes and type faces can increase printing costs and should be reduced to a minimum a supply of reviews and short articles should be kept so that the part can be brought up to a multiple of 16 pages wherever possible vertical rules in tables should be removed because of the expense of setting

The meeting was told by Mr Inglis (Oliver and Boyd) of discounts, commission and distribution. Individual small costs add up to impressive totals, ranging from invoices at ½*d* each, through envelopes costing 1½*d* to 6*d* for the carton sizes, to postages for larger parts at 2*s* 3*d* Small wonder that some journals (not geological as yet) find it difficult to run to six parts annually and are considering a quarterly issue

Index cards, the last item on the agenda, had been tried out by the editors of the *Transactions of the Edinburgh Geological Society* in a recent issue, with the view of providing subscribers with abstracts for use in filing systems Each copy contained 5 in × 3 in cards, one for each of the papers, bearing the author's name, title of the paper, journal reference and the author's abstract (not exceeding 250 words) The cost worked out at a little less than 1*d* a card and represented some 3 per cent of the total cost of the part Such cards were welcomed, but it was felt that their spread to other journals would be more influenced by subscriber pressure than by editorial decision

The overall impression at the end of the two days of useful discussion was that it was greatly to the advantage of journals that the editors should see more of the printer and that a more satisfactory relationship should be established between author, editor and printer

G Y CRAIG

COAL PETROGRAPHY

THE International Committee for Coal Petrology has recently prepared a second edition of its *Handbook of Coal Petrography* which has now been published by the Centre National de le Recherche Scientifique, 15 Quai Anatole-France, Paris 7^e English, German or French texts are available from this organization and the Russian text from the Academy of Sciences of the U S S R, each volume costs 54 N F Since normal retail channels will not be used for distribution of this work, a member from each nation represented on the Nomenclature Sub-committee of the International Committee has been asked to contribute a short note about the *Handbook* to an appropriate journal in his or her country In this way it is hoped that coal scientists and petrographers working in related fields will be made aware of the existence of the *Handbook* and can obtain a copy, if they wish, from one of the aforementioned sources

The first edition of the *Handbook* was published in 1957 in glossary form The second edition has been considerably expanded and revised It is divided into two parts, the first and lengthier dealing with nomenclature, the second describing methods of petrographic analysis

The original publication of 1957 was concerned mainly with the Stopes–Heerlen system of petrographic nomenclature of coals and their constituents that is used primarily in Europe Further terms have been added to this system in the intervening six years, and the new definitions are now included in the *Handbook* together with a considerable number of terms from the Thiessen-Bureau of Mines system of nomenclature This is the other principal system of petrographic classification and it is used mainly in the United States A brief outline is also given of the important nomenclature recently introduced in the United States by Prof Spackman A summary of the genetic classification of the Geological Institute of the Academy of Sciences of the U S S R is also included as well as definitions of eleven of the more important terms from this system Definitions of a number of general terms used in coal petrology have also been added to the *Handbook*

Only the petrographic nomenclature of the 'hard coals' (bituminous coals and anthracites) is considered in the *Handbook* The petrography of the soft brown coals is still under discussion by the Nomenclature Sub-committee and no terms or definitions have yet been published The *Handbook* is in the form of a strong loose-leaf folder made up of sheets approximately 9½ × 6¼ in. in size In the nomenclature section, 47 of the 149 sheets are plates composed of 108 photomicrographs, 4 sheets contain line diagrams Eighty-one terms have been defined in this section, the text giving details of origin, description and, in the case of macerals, microlithotypes and lithotypes, their occurrence and practical importance Where possible, correlation of terms with those in other systems of nomenclature have been made Photomicrographs that illustrate various features have been taken where necessary by transmitted and reflected light, many are in colour

Methods of analysis have been included in the *Handbook* because they have helped substantially towards better definitions of the petrographic constituents of coals The Analysis Sub-committee was formed in 1955, and the second short section of the *Handbook* presents the findings of the Sub-committee since then Exchange of approximately 100 samples between petrographic laboratories in different countries has led to considerable improvement in maceral and microlithotype analyses, as well as to the formulation of recommendations for the preparation of samples and their examination in reflected light Sixteen sheets, two being plates and one a line diagram, are devoted to general notes on methods of quantitative analysis of polished coal surfaces, maceral and microlithotype analyses on particulate blocks and suggested procedures for the preparation of samples

Although the nomenclature of the 'hard coals' is now well established, the definitions presented in the *Handbook* are not necessarily final Amendments may be made in the future and further terms added to the different systems of nomenclature All terms and definitions do, however, accord so far as possible with the present state of knowledge in coal petrology D G MURCHISON

EXPLORATION OF THE SEA

THE total water-surface of the Earth is estimated at 70 8 per cent of its area, and of this the overwhelming proportion is represented by the oceans Geological science has done much to elucidate the nature, composition and structure of the Earth's surface, aerial reconnaissance and especially modern geophysical techniques have, during the past four decades or so, materially increased our knowledge, but it still remains relatively superficial The nature of the Earth's interior, be it liquid core or otherwise, is largely a matter of hypothesis, although the Operation Mohole project launched in 1961, to drill a hole 7 miles deep into the mantle-rock from a ship stationed north of Puerto Rico, may, if successful with samples from depth, throw further light on the problem By contrast, exploration of space, the atmosphere and beyond, is being pursued on a vast and ever-increasingly costly scale by means of instruments borne by rockets and Earth satellites; in the near future we may find out what is the geology of the Moon

In a recent lecture, Sir Frederick Brundett, referring to the comparative neglect by science of the sea, is reported to have said that "The world must be *mad* to spend more in a year on space research than has been spent on studying the oceans in the last hundred years" In a paper read before the Royal Society of Arts (*Journal*, 111, No 5086; September 1963) Dr T F Gaskell, scientific adviser in the Information Department, British Petroleum Co, Ltd, formerly with the Royal Naval Scientific Service engaged in research in anti-submarine mine counter-measures and beach reconnaissance, pursues this theme, explaining why advances in our knowledge of the sea have been slow, what is the trend of contemporary oceanic investigations and what lies in store economically and scientifically as we learn more of the sea in future

Dr Gaskell makes some pertinent points at the outset The sea is a difficult place in which to work "Sea-water is opaque and this is the first difficulty that faces the oceanographer Most of the tools needed to investigate the sea must use physical principles which are more complicated than the optical methods that are so satisfactory for studying the surface features of the land"

" sea-water contains enough salt to be neither a really good conductor of electricity nor a useful insulator, so that in this respect also it is more difficult to work in than is the atmosphere The salt water also militates against the scientist by being corrosive, and to ensure that experiments shall be conducted with the maximum of difficulty and discomfort, the sea surface is seldom still " Again, "The greater part of the oceans is about three miles deep, so that when apparatus must be lowered to the sea-bed long cables are needed"

Undoubtedly the navies of the world, especially those of the great maritime powers, during and since the Second World War, have contributed much to our knowledge of the sea itself, also of the shape of the deep sea-floor; this is an important factor for submarines, now accustomed to diving much deeper and staying submerged far longer than formerly Detection of submarines demands an understanding of sound-wave propagation in the sea-water medium, which in turn requires more accurate measurements of temperature and density distribution in the oceans Of the more peaceful aspects of future ocean investigations are off-shore drilling for new oilfields or important extensions of known continental developments, these projects are proceeding apace to-day and, apart from oil-finding, every hole drilled provides more information of sedimentary accumulations, past and present, forming the continental shelves concerned In the long run, however, it is the fish in the sea that will take pride of place in future oceanic researches Increase in world population will compel more attention to the sea as a vast food-producer As Dr Gaskell observes. " we hunt the fish that Nature provides, just as our ancestors hunted animals for food We have not yet begun to herd fish or to improve their quality—but one day we shall be forced to farm the seas as we do the land"

H B MILNER

THE ROWETT RESEARCH INSTITUTE

THE report of the Rowett Research Institute for the year 1962–63 * is on the same lines as that for the previous year; that is to say, the main section of the report summarizes the numerous papers which have appeared during the year and supplements these summaries with additional unpublished information so as to bring the accounts of the research more nearly up to date In all, some 150 original papers published by workers in the Institute are referred to, together with references to about 40 papers in preparation and other unpublished observations These considerable numbers give some idea of the great mass of work proceeding in the Institute, and of the magnitude of the task of presenting a succinct and intelligible account of it within reasonable compass In addition to this general account of present research within the Institute, the report contains two special contributions on topics deemed to have reached a stage suitable for review

Both these review articles deal with topics of considerable practical importance in British agriculture The first, by Dr T R Preston, discusses recent work on high-energy barley diets for livestock feeding It has been found that, with suitable management, beef cattle may be reared on a diet of 85 per cent of appropriately treated barley and a 15 per cent supplement containing protein, minerals and vitamins Animals so fed gain weight in the form of lean beef surprisingly rapidly, and it was found possible to bring some dairy-type cattle to a good finish in 10–12 months instead of the usual 18–24 months The régime, therefore, promises to be economic and profitable and has excited considerable interest among the farming community It has been introduced into commercial practice, but the resulting meat has encountered some criticism on the ground of taste and texture Dr. Preston's article surveys the scientific background of this topical and somewhat controversial subject

The other review, by Dr E Cresswell, deals with two topics both in connexion with sheep The first is the problem of the premature dropping of the incisor teeth by certain sheep, more especially mountain breeds on particular diets The causes of this curious defect have been rather obscure, and an account is given of investigations into the course of abnormal development of the teeth and the means which have been suggested to prevent or minimize the trouble The second part of this article deals with the problem of providing suitable shelter for sheep during winter storms on mountains or other exposed places Plans are given of a type of 'stell', which is relatively cheap to construct and which provides shelter for about 100 sheep and at the same time ensures adequate ventilation Animals so protected are able to pass the winter on the more exposed mountain pastures and do not need to be brought to lower ground for protection from storm and cold

In a section of the present report giving a brief survey of the past year, reference is made to the fact that in 1963 the Rowett Research Institute celebrates its Golden Jubilee (see *Nature*, 200, 201, 635, 1963) It was in 1913 that a joint committee of the University of Aberdeen and the North of Scotland College of Agriculture initiated its research programme on the nutrition of farm animals and appointed Dr John Boyd Orr (now Lord Boyd Orr) to carry out this novel enterprise The work on animal nutrition has gone on without interruption ever since, apart from dislocations due to two world wars The contents of this report demonstrate how vigorously the organization then born has grown, and how it has justified and rewarded the efforts of the pioneers who created it fifty years ago

W O KERMACK

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* Rowett Research Institute Annual Report on Animal Nutrition and Allied Sciences Vol 19 Pp 83 (Bucksburn, Aberdeen The Rowett Research Institute, 1963) 10s

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THE MEDICAL RESEARCH COUNCIL

THE report of the Medical Research Council for the year 1961–62*, which follows the general plan of this Council's previous reports, reflects, as they did, the trend of medical research as a whole The volume and wide range of the work being done by the Council's staff and the practical applications of their work are indicated by the fact that their publications have now become so numerous that it is no longer possible to list them in the report, although details of them can be obtained from the Library of the National Institute for Medical Research, Mill Hill, London, N W 7

Apart from the usual summaries of researches in progress, which occupies 140 pages of this report, and the details of the personnel employed by the Council, the report includes, as previous reports have also done, a section entitled "Some Aspects of Medical Research", which is also published separately under the title *Current Medical Research* (H M S O 5s. 6d) This particular section is this year made up of eleven articles, the range and content of which can be only briefly indicated here

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* Committee of Privy Council for Medical Research Report of the Medical Research Council for the Year 1961–1962 Pp viii+284 (Cmnd 2075) (London H M S O , 1963) 16s 6d net

The first article, entitled "The Genetic Code", includes in its scope the work on this subject for which four members of the Council's staff, Dr M F Perutz, Dr J C. Kendrew, Dr F H C Crick and Prof M H F Wilkins, were, together with Prof J D Watson, awarded Nobel Prizes for 1962 (see *Nature*, 196, 319, 519, 1962) The second article, entitled "Chemical Mutagens", discusses chemical substances which may produce mutations and their mode of action and their specificity The third article is on cell transformation by tumour viruses, with special reference to the polyoma virus which is being studied under the direction of Prof M G P Stoker in the Council's Experimental Virus Research Laboratory at the University of Glasgow Next comes an article on "Cancer in the Tropics", and this is followed by a discussion of "River Blindness" caused by the parasitic nematode worm, *Onchocerca volvulus*, in tropical Africa, an article on the demyelinating diseases, and another on the central nervous control of the endocrine glands and the influences exerted by hormones on the central nervous system Then follows a valuable article on the relationship between mental retardation and chemical abnormalities in the body Two articles discuss the metabolism of plasma protein and the biosynthesis of sterols, and finally there is an article on biological engineering, a term of which the author of the article apparently does not fully approve It refers to the various ways in which engineering techniques are nowadays applied to medicine and the biological sciences, and includes such devices as radio-pills and other 'miniaturized' instruments, the whole-body plethysmograph, the conicycle, which measures the content of respirable dust and other aerosols in the air, the breath-alcohol detector, and the remarkable prostheses which are now available as artificial limbs and other artificial aids, such as plastic heart valves, artificial kidneys, heart–lung machines, and aids for the blind and deaf The value of this whole section is increased by photographs of a molecular model of deoxyribonucleic acid, of polyoma virus particles and cells transformed by this virus, of a radio-pill and a prosthesis for the arm.

The rest of the report, apart from the details it gives of the finance and administration of the Council's work, pays a well-deserved tribute to the work of Sir Charles Harington, who retired on July 31, 1962, from his post as director of the National Institute for Medical Research, after serving in that capacity for twenty years (see *Nature*, 195, 228, 1962) During this period the Institute's scientific work was greatly extended, the number of its staff increased from 35 to 125 and the Council enjoyed the great benefit of Sir Charles's advice on both research and policy Everyone interested in the Council's work will be glad to know that Sir Charles will still be available as consultant adviser to the Council's secretary

In another section on the Council's Advisory Boards it is explained that knowledge and research have become so complex that the Council, which has always been averse to "any arrangement which implies that one single body should bestride the field of clinical or any other type of research", has devised a system of Boards which are, in effect, sub-councils The first of these, the Clinical Research Board, was appointed in 1953 Afterwards, a Tropical Medicine Research Board was established, and in 1962 a Biological Research Awards Committee was created By means of these Advisory Boards, which enable the Council to benefit from the advice of many experienced specialists, the Council is able to decentralize effectively the ever-increasing complexity of its work

No one can doubt, after reading these well-written and closely reasoned pages, that here we have an organization of scientists working together and directing their own work free from Governmental control, which ensures, as no other kind of organization can do, that all the sciences, whether they are primarily medical or not, are effectively integrated together for the benefit of man and other animals Long may it continue to produce the remarkable results that, year by year, are recorded in its reports G LAPAGE

THE LIQUID STATE

THE Institute of Physics and the Physical Society held a conference on "The Liquid State" at the Imperial College of Science and Technology, London, during September 9–13

The study of the structure and properties of liquids, like many branches of classical physics, is a subject in which real progress is now slow and irregular There was a burst of activity after the Second World War in which the implications of the integral equations for the distribution functions of Yvon, Kirkwood, Born and Green were studied and in which the limitations of Kirkwood's 'superposition' approximation came to be appreciated This was followed by an advance in 'experiment' when, in the 1950's, computers were used to calculate the pressure and rate of approach to equilibrium of systems of simple molecules by, for example, the solution of the equations of motion of a small representative assembly This work gave an impetus to the study of simple models, and the past two years have seen the development of at least two new sets of integral equations and for one of them, the equations of Percus and Yevick, their exact solution in closed form for a system of hard spheres Meanwhile, there have been substantial advances in the theory of transport properties, although there is still argument about the legitimacy of some of the steps that are necessary to relate these properties to the intermolecular forces These developments were reflected in many of the 68 papers presented at this conference, and a selection of these are described briefly in this report

The first day was a discussion of methods of determining structure In the opening review, Dr P A Egelstaff (Atomic Energy Research Establishment, Harwell) emphasized the power of the method of neutron-scattering Thermal neutrons can show shifts of both wave-vector and energy when scattered by liquids and so can be used to supplement the information from X-ray diffraction and light-scattering experiments Dr S J Cocking (Atomic Energy Research Establishment, Harwell) illustrated this in outlining his investigations of the motion of atoms in molten lead Dr J A Elliot, Prof H E Hall and Dr J M Williams (University of Manchester) described the use of the Mossbauer effect for the study of diffusion in glycerol, and Dr J G Powles (Queen Mary College, London) has used nuclear magnetic resonance to examine rotational relaxation

Prof J D Bernal and Miss S V King (Birkbeck College, London) reported further experimental studies of random close-packed arrays of steel balls, and suggested that such arrays often showed long ordered lines of particles This point was taken up the next day when Dr B J Alder (Livermore, California) discussed his computer calculations on the transition from ordered to fluid phase in a two-dimensional array of hard disks. He suggested that melting might start when lines of molecules could slide past each other Hitherto Bernal's studies have had little contact with more conventional theories of liquids, but an attempt to bridge this gap was made by Prof R Furth (Birkbeck College, London)

PHYSICS DEPARTMENTAL LIBRARY

At the end of the first day, Prof D H Everett (University of Bristol) showed a film of the motion-in-a-plane of an array of cork balls agitated by a stream of air Again, co-operative motion in lines and rings could be seen

The longest session of the conference was on the equilibrium properties of pure and mixed liquids and occupied the second and third days It was introduced by Prof J de Boer (University of Amsterdam), who set out the present position both of lattice theories and of calculations of the pair distribution function In the latter he outlined the 'hyper-netted chain' approximation on which he and other groups have been working recently In the papers that followed, only Dr J A Barker (Commonwealth Scientific and Industrial Research Organization, Melbourne) used the lattice model for liquids, in a version which he calls the 'tunnel' model He uses the fact that combinatorial problems in one dimension present little difficulty to choose a model in which the molecules have complete freedom of motion in one direction only His comparisons of theory and experiment were outstandingly successful

The mathematical properties of the distribution function and some inequalities that they must satisfy were set out by Dr J L Lebowitz (Yeshiva University, New York), Dr J. K Percus (University of New York) and Dr O Penrose (Imperial College of Science and Technology) The first author also presented his solution of the Percus–Yevick equations for mixtures of hard spheres Prof J S Rowlinson (Imperial College of Science and Technology) has obtained numerical results for this model and showed that the excess Gibbs energy is always negative and hence that phase separation cannot occur This conclusion was consistent with the calculations of Mr M Rigby and Dr E B Smith (University of Oxford) on the fourth virial coefficients of mixtures of hard spheres The extensive cancellation of cluster integrals that characterizes both the hyper-netted chain and the Percus–Yevick approximations was, to a degree, justified by the calculations of Dr H N V Temperley (Atomic Weapons Research Establishment, Aldermaston) for molecules with Gaussian repulsions Dr J Walkley (Imperial College of Science and Technology) discussed the quantal treatment of a cell model of an assembly of hard spheres

Papers on the two-dimensional lattice gas were presented by Dr D M Burley (University of Sheffield) and Dr M E Fisher, Dr D S Gaunt and Dr J Stephenson (King's College, London) In some cases such a fluid appears to show a second-order transition to an ordered state

Three of the experimental papers of this section aroused much interest Mr A T J Hayward (National Engineering Laboratory, Glasgow) has started measurements of the equation of state of liquids under tension and is repeating the more common measurements of the maximum tensions that liquid can bear Dr G Casanova and Dr A Levi (University of Genoa) showed that their measurements of the isotopic separation factor at a liquid-vapour equilibrium can be used to measure $\nabla^2 U$, where U is the configuration energy of the liquid, and it was suggested in discussion that their measurements provided also a means of testing intermolecular potentials without any assumptions about the structure of the liquid Dr J J M Beenakker, Dr M Knoester and Dr K W Taconis (University of Leyden) have developed a flow calorimeter for the direct measurement of the heat of mixing of compressed gases, a quantity that has hitherto been obtained only indirectly

The properties of mixtures of linear hydrocarbons are of interest because they are regular functions of the chain-lengths Prof D H Everett (University of Bristol) and Dr Th Holleman and Dr J Hijmans (Royal Dutch Shell, Amsterdam) extended correlations of theory and experiment to high temperatures where the heats of mixing are negative Dr I A McClure and Dr F L Swinton (Royal College of Science, Glasgow) reported volumes of mixing from some well-chosen systems in which the size ratios of the molecular pairs were close to 2 1

Transport properties were discussed on the fourth day The introductory paper was by Prof I Prigogine and Dr P Résibois (University of Brussels), who summarized the statistical mechanics of irreversible processes and, in particular, its application to ionized systems They emphasized the difficulty of treating thermal diffusion in such systems This paper was followed by a substantial contribution from Prof R Eisenschitz and his colleagues (Queen Mary College, London) in which they tested both the theories of Kirkwood (that is, the introduction of a 'friction constant') and of M S Green The former was confirmed by calculations on a simple harmonic model of a crystal with a temperature gradient Rice and Allnatt have suggested that the fundamental dynamical event in a liquid can be represented as a 'hard-core' repulsion of a pair of molecules, followed by their Brownian motion in the field of their neighbours Dr S A Rice and Dr E G Wilson (University of Chicago) tested this assumption by comparing its predictions with four of the transport coefficients of the rare gases Dr E McLaughlin (Imperial College of Science and Technology) emphasized the importance of the coefficient of thermal expansion of a liquid in determining the temperature coefficient of the thermal conductivity He illustrated this by calculations on simple models

Two experimental papers were on the subject of nucleation Dr L O Roellig (Wayne University, Detroit) showed that the probability of a particle of ionizing radiation destroying a super-heated state can be related to the amount of energy it can transfer to a small volume of liquid Dr E R Buckle (Imperial College of Science and Technology) showed, by means of a cloud-chamber, that the freezing of super-cooled ionic melts is critically dependent on temperature, a dependence which he ascribed to the threshold effect of spontaneous nucleation The final paper of this session was by Dr T A Litovitz (Catholic University, Washington), who compared the dielectric (or rotational) time of relaxation with the ultrasonic (or structural) time He showed that the former was the longer and that both changed from a single time to a distribution of times in liquids in which the viscosity did not show a simple exponential dependence on temperature

A widespread dissatisfaction with the present theoretical basis of many of the approximations that are used in discussing the transport properties of liquids led to an unscheduled discussion of fundamentals on Friday afternoon The principal speakers were Dr Résibois, Prof Eisenschitz and Dr Lebowitz

Liquid metals were discussed on the last day, and, of necessity, most of the papers in this part of the conference were concerned with specifically electronic (and hence quantal) effects Dr S F. Edwards (University of Manchester) reviewed the present state of the theory of electrons in liquid metals He applied methods adapted from field theory to the calculations of momentum and energy distributions in disordered potentials, and demonstrated that, while at present there can be no detailed comparison of theory with experiment, this goal is coming within reach Experimental work on soft X-ray spectroscopy, transport and optical properties, positron annihilation, and electron spin resonance all have a part to play An alternative route to some of Edwards's conclusions was provided by Dr D Libermann (University of California)

Two of the papers of this section sought to extend to liquid metals concepts which have been useful in non-electrolytes Dr W B Mott (Atomic Energy Research Establishment, Harwell) examined Hildebrand's rule for the calculation of mutual solubility of two liquids He was able to modify it to produce qualitative agreement for most metallic systems More ambitiously, Dr E Morris (Atomic Weapons Research Establishment, Aldermaston) attempted the calculation of the critical properties of metals from the principle of corresponding states The agreement with experiment for mercury was only fair

Three experimental papers from the University of Birmingham by Dr J R Wilson, Dr G H Laurie and Dr J N Pratt revealed the anomalous dependence on temperature of the resistivity and partial molar properties of the systems Hg + Tl, Mg + Pb, and Sn + Pd + Ag These were ascribed to variations of the degree of local order with temperature, and to the formation of intermetallic compounds Dr R V Hodgson (University of Birmingham) measured the resistivity of five binary semiconductors and showed the change towards metallic properties at high temperatures Finally, Dr D T. J. Hurle, Dr J B Mullin and Dr E R Pike (Radar Research Establishment, Malvern) described the growth of single crystals of semiconductors by a zone-melting process in which a d c electric current passed along the bar

The chairman of the Organizing Committee of the conference was Dr. H. N V Temperley The proceedings are not to be published as a separate volume

J S Rowlinson
E R Buckle

OBSERVED TRIPLICATION OF *PKP*

By Dr R D ADAMS and M J RANDALL

Seismological Observatory, Department of Scientific and Industrial Research, Wellington, New Zealand

DETAILS of the variation of seismic velocity near the boundary of the inner core have always been uncertain, and so, correspondingly, has the theoretical travel-time curve of *PKP* in the distance range immediately beyond 143° On the model of Jeffreys and Bullen[1] a short branch extends beneath the PKP_2 curve from the cusp at 143° to 147° The tables of Gutenberg and Richter[2] incorporate a similar branch extending to 169°, with arrival times between those of PKP_1 and PKP_2 for distances greater than 150° No observations have been reported in confirmation of either of these branches

Bolt[3] has recently reconsidered the velocity variation near the boundary of the inner core, with a view to accounting for Gutenberg's observations of early arrivals of *PKP* at epicentral distances in the range $125° < \Delta < 140°$ (ref 4) His preferred solution shows a branch of the *PKP* travel-time curve, labelled *GH* which not only includes these early arrivals but also continues to a cusp *H* at $\Delta =$ 156 5°, for distances greater than about 146° the travel times are between PKP_1 and PKP_2 We have recently found arrivals which confirm the latter part of the *GH* branch These arrivals, which for convenience we have called PKP_3, have been taken from the seismological bulletins of stations which lie in the distance range $148° < \Delta < 156°$ from certain New Zealand earthquakes New Zealand stations have also recorded the triplication of *PKP* from earthquakes at appropriate distances On these records PKP_3 is a stronger arrival than PKP_1, being comparable with PKP_2 Three branches of *PKP* have also been reported by Hai[5] from deep-focus earthquakes near Fiji

Table 1 EARTHQUAKES USED

No of earthquake	Date	Time h	Time m	Time s	Epicentre Lat S	Epicentre Long E	Depth (km)
1	Mar 23, 1960	01	32	19 8 ±0 4	39° 03′ ±4′	174° 52′ ±8′	607 ±4
2	Mar 23, 1960	01	36	55 7 ±0 9	39° 06′ ±7′	175° 04′ ±12′	612 ±9
3	Mar 27, 1960	23	28	27 1 ±0 6	39° 03′ ±6′	174° 55′ ±11′	228 ±6
4	May 10, 1962	00	27	12 0 ±0 5	41° 39′ ±2′	171° 19′ ±5′	12
5	May 17, 1962	02	19	52 2 ±0 3	41° 48′ ±1′	171° 18′ ±3′	12

Table 2 GEOCENTRIC DISTANCES TO STATIONS RECORDING PKP_3 (Degrees)

Station	Earthquake 1	2	3	4	5
Tromsoe				148 8	148 9
Helwan			148 8		
Kajaani				149 3	149 4
Kiruna				149 4	
Poulkovo			149 6		
Simferapol			150 5		
Istanbul				151 9	
Umea				152 2	152 3
Helsinki	151 5	151 5		152 3	152 4
Nurmijarvi		151 5		152 3	152 4
Reykjavik			153 0		
Skalstugan	153 2		153 3		155 0
Sida			154 0		
Uppsala			154 7	155 6	

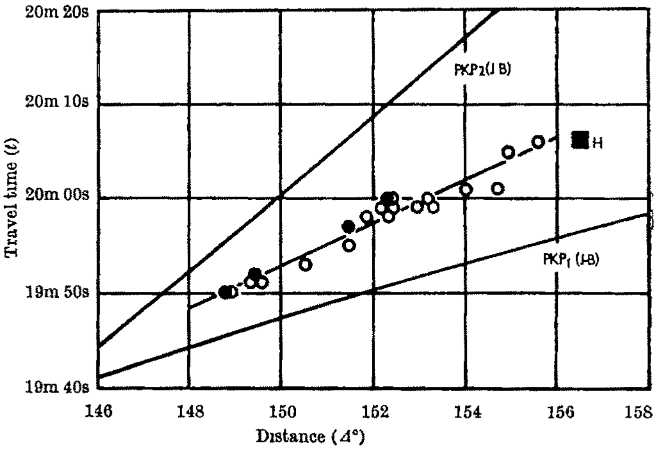

Fig 1 Observed travel times of PKP_3 Solid circles represent two arrivals The position of Bolt's cusp *H* is shown

Additions to the New Zealand network of seismograph stations have enabled the foci and origin times of recent New Zealand earthquakes to be determined with improved accuracy PKP_3 arrivals have been identified from five New Zealand earthquakes for which we have obtained foci and origin times by least squares from numerous observations. Table 1 lists these earthquakes and gives the standard errors of the solutions Table 2 gives the epicentral distances to the stations at which PKP_3 has been found, the increased number of stations now operating in Scandinavia provides more observations of *PKP* in the relevant distance range Arrivals reported from stations at distances less than 148° have been discarded because one cannot be certain which *PKP* branch they belong to

Fig 1 shows the travel times of the PKP_3 arrivals, adjusted so as to correspond to a surface focus. The Jeffreys–Bullen travel-time curves for PKP_1 and PKP_2 are also shown, many arrivals corresponding to these branches were also reported for the same earthquakes All but three of the PKP_3 arrivals have been reported as impulsive, and the standard errors of the epicentral distances and origin times are such that there seems no possibility of these arrivals being either PKP_1 or PKP_2 At some stations there are arrivals corresponding to each of the three branches, for example, Skalstugan reports three impulsive arrivals from earthquake 3, with travel times of 19 min 53 sec, 19 min 59 sec and 20 min 13 sec Uppsala also reports triplication of *PKP* for the same earthquake The largest distance at which we have identified PKP_3 is 155 6°, the branch does not appear to extend as far as 157 5°, where some stations have reported PKP_1 and PKP_2, but not PKP_3 The position of Bolt's

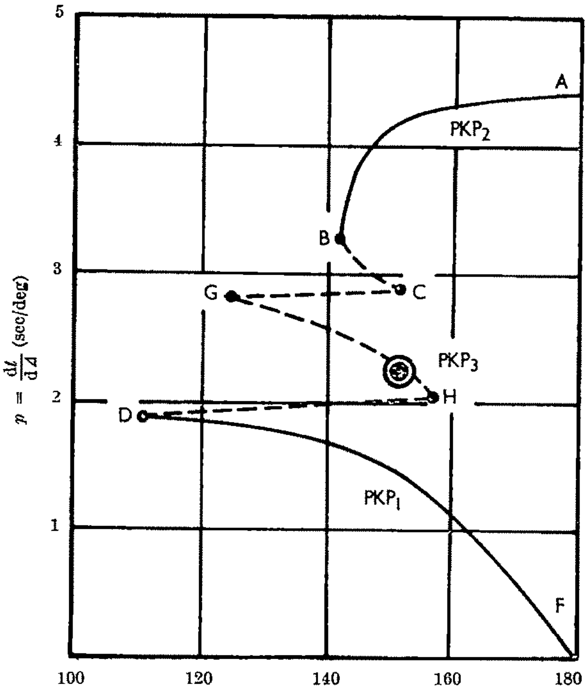

Fig 2 p, Δ diagram corresponding to Bolt's model, showing the observed value of p for PKP_3

predicted cusp H is shown, lying close to the end of the line of PKP_3 observations These observations would thus appear almost certainly to correspond to the upper part of the GH branch of Bolt's travel-time curve Hai's observations[5], when adjusted for depth, appear to be close to ours, though he also suggests a possible extension of the observations to 160°

The 25 observations of PKP_3 shown in Fig 1 were fitted to a straight line as follows

$$t\ (\text{sec}) = 1196{\cdot}9 \pm 0{\cdot}2 + (2{\cdot}24 \pm 0{\cdot}12)(\Delta - 151{\cdot}8°)$$

Adding a quadratic term does not significantly improve the fit Near 151·8°,

$$p = \frac{dt}{d\Delta} = 2{\cdot}24 \pm 0{\cdot}12 \text{ sec/deg}$$

The p, Δ diagram is given in Fig 2 The detail of the curve between adjacent cusps is not readily obtainable from the t, Δ diagram in Bolt's paper, the branches corresponding to PKP_1 and PKP_2 have been derived directly from the Jeffreys–Bullen tables The value of p found from the observations of PKP_3 near 151·8° is shown, and agrees well with that predicted by Bolt Since the slope of the p, Δ curve at a given distance is proportional to the amplitude of the seismic waves, the rather large amplitudes which have been observed for PKP_3 suggest that the slope of the p, Δ curve for the branch GH is greater at the larger distances, as is shown in Fig 2

Thus the observations presented here are in good agreement with Bolt's prediction with respect to travel time, range of epicentral distance, and slope of the travel-time curve We hope to carry out a more detailed investigation of PKP arrivals at relevant distances as further observations become available

We acknowledge the help we received from station bulletins, and we also thank Dr Markus Båth for supplying readings from the Swedish network

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WAVE-LENGTH STABILIZATION OF AN OPTICAL MASER

By Dr W R C ROWLEY and D C. WILSON

National Physical Laboratory, Teddington, Middlesex

THE properties of the gaseous type of optical maser make it a light source of great promise for use in interferometric measurements of length. The coherence in time and the extremely narrow band-width of the radiation enable interference effects to be observed over paths many times greater than possible with conventional sources[1] Moreover, the coherence across the diameter of the light beam leads to simplifications in the construction of interferometers

The use of the 6328 Å line of the helium–neon maser for interferometric length measurements is hindered because, in spite of its narrow band-width at any instant, the wave-length of the light can vary The emitted wave-length is broadly determined by the energy difference of the levels involved in the transition and by the Doppler effect due to movements of the atoms in precisely the same way as is the wave-length of a normal spectral line spontaneously emitted from a discharge tube The precise wave-length of the maser emission, within the wave-length range corresponding to the width of this Doppler broadened profile, is determined by the effective length of the optical cavity This means that the wave-length may lie anywhere within a range of about 2 parts in 10^6 according to the precise length of the cavity, and there is the possibility of several slightly different wave-lengths being emitted simultaneously through multi-mode operation

A simple way of improving the wave-length or frequency stability of the maser is to use the Doppler broadened profile of the transition as a reference and to adjust the length of the cavity so that the maser emission corresponds to the peak of the profile[2] In the technique to be described the length of the cavity of the maser is adjusted continuously by a servo control system through a simple electromechanical coupling With this device the frequency drifts are reduced to only 1 or 2 parts in 10^8

Principle of operation An idealized diagram of the gain coefficient of the maser as a function of the frequency of its emission is shown in Fig 1 This curve is the spontaneous emission spectral profile and its shape is principally due to Doppler effect In practice the curve of maser gain against emission frequency is narrower than the curve of Fig 1 because of the threshold for maser action due to optical losses in the cavity For low input powers to the discharge, only the axial mode[3] nearest the peak of the profile has a gain greater than the threshold level In this condition the maser output intensity is strongly dependent on the operating frequency, with a peak at the frequency of maximum gain

If one of the mirrors of the optical cavity is caused to vibrate very slightly, then the maser frequency oscillates and the maser output is amplitude modulated as indicated in Fig 1 The phase of this modulation relative to the imposed vibration will depend on whether the mean frequency of operation is greater or less than the frequency at which the gain is a maximum When the mean maser frequency is at the peak of the gain curve, the fundamental intensity modulation vanishes, leaving only a slight harmonic component. The intensity modulation can thus be used as the basis of a servo control system to keep the maser frequency centred on the peak of the gain curve.

Mechanical arrangement. The maser to which the control system has been applied is one of the helium–neon type operating on the red line at 6328 Å. It utilizes a radio frequency excited discharge tube fitted with Brewster angle windows which is set between the mirrors of the optical cavity. These mirrors are both concave, having 1 m radius of curvature, and are separated by 750 mm. The framework of the cavity is a structure of four 1.5-in diameter invar rods with several cross-members in addition to the end-pieces. The mirrors are mounted in detachable end-plates which are held by springs in position against their adjusting screws.

The control movements for vibrating one of the mirrors and for adjusting the length of the cavity are both achieved with a single relay coil mounted on the end-piece of the main frame, its armature bearing against the end-plate carrying the mirror (Fig. 2). A current of 50 m.amp through a 200-ohm coil gives almost 2 lbf of thrust, which is sufficient to move the mirror through a distance of several half-wave-lengths by elastic deformation of the end-plate and its supports. This arrangement gives a rotational movement to the mirror rather than a pure translation, but this does not seriously affect the operation of the maser, for the diameter of the maser beam is only about 1 mm, whereas the distance from the mirror centre to the axis of rotation is about 60 mm and so a mirror movement of a few half-wave-lengths corresponds to a misalignment of only a small fraction of a wave-length across the beam diameter.

A direct current is passed through the relay coil, thus biasing it so that there is always a force holding the armature in contact with the end-plate, and to this current is added the control current which keeps the maser length correct. At the same time a small alternating current at 350 c/s, passed through the coil, causes the mirror to vibrate with an amplitude of a small fraction of a wave-length. The intensity variation of the maser beam resulting from this vibration is detected with a photo-diode and, after amplification, is passed into a phase-sensitive rectifier. The rectified output, after smoothing, is a direct current proportional to the input, and is either positive or negative according to the phase of the input relative to that of the alternating current fed to the relay coil. This direct current is the control current which is added to the steady bias current through the coil. The control system is thus a feedback arrangement. Should the maser frequency not be centred exactly on the profile, then there is an intensity modulation which is detected, amplified and fed back to reduce the amount of the departure. The gain of the feedback loop determines by what factor the maser frequency drifts are reduced.

Performance. The stability of the controlled wave-length of the maser has been measured by comparing it with the orange line of krypton-86, which is the primary wave-length standard. The comparisons have been made using a Fabry–Perot interferometer with photoelectric detection in which spectral lines from two sources are effectively compared simultaneously and their interferograms are recorded automatically on punched paper tape. A digital computer analyses the results and can detect relative changes in wave-length down to 2 parts in 10^9. The absolute accuracy within which one source can be compared with another is, however, limited by geometrical differences in the optical paths to only one or two parts in 10^8.

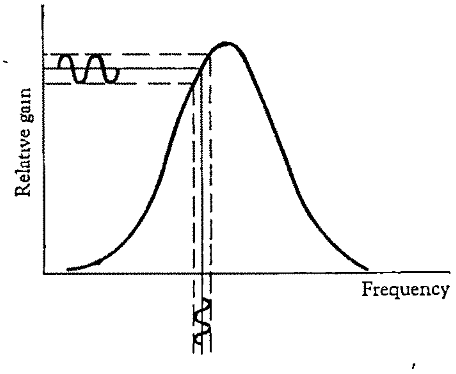

Fig. 1. An idealized diagram of the gain of a maser as a function of frequency, showing how a frequency modulation results in a modulation of the gain and hence of the output intensity

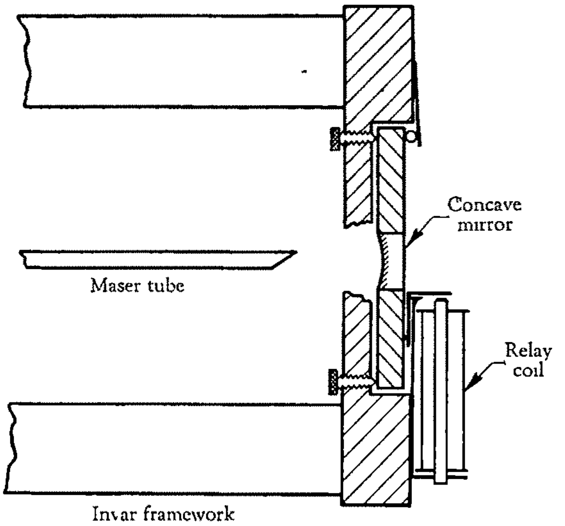

Fig. 2. Diagrammatic view of one end of the maser frame. The armature of the relay coil bears against the mirror mount which moves by elastic deformation, even though the mounting has not deliberately been made flexible

With a loop gain of the stabilizing system of about 100, the measured drifts of maser wave-length are less than 1 part in 10^8, provided that manual readjustment of the cavity length is made from time to time if thermal expansion causes the framework to change length by much more than a wave-length. Any change in the length of the framework is apparent as it causes a corresponding change of control current through the relay coil.

The maser frequency, being controlled to the peak of its intensity profile, is altered by any effect which influences the shape or shifts the frequency of this peak. Any change in the pressure of helium and neon in the discharge will shift the peak frequency, but as the pressures involved are low the effect amounts to a few parts in 10^8 at the most. This has been confirmed by measuring the wave-length emitted by the maser when fitted with different discharge tubes. With an old discharge tube in which the gas pressure was much reduced through use, the emitted wave-length was identical, within the accuracy of the experiment (2 parts in 10^8), to that emitted when the discharge tube was replaced by a new one.

The effective intensity profile for maser action is influenced by maser action itself. The effect known as 'hole burning'[4] distorts the profile particularly in the region of the peak. This effect can lead to an actual dip at the centre of the profile[5,6], but it is not readily observable. There is some asymmetry of the spectral profile owing to the isotopic content of natural neon, and hole burning thus gives rise to an asymmetrical effect which shifts the profile peak towards higher frequencies. The shift appears to be proportional to maser intensity, and a total frequency change of 2 parts in 10^7 is observed over the intensity range from threshold to the maximum intensity at which single mode operation can be maintained with the maser being utilized.

As this latter intensity is ten times greater than that normally utilized when using the maser for interferometry in this Laboratory, the uncertainty of frequency arising from the effect need not be more than 1 part in 10^8. It does, however, mean that some control over the mean maser intensity is advantageous. A portion of the maser beam is reflected from a glass plate on to a cadmium sulphide photo-conductive cell for this purpose. The output from this cell is used to control the power supply of the discharge tube oscillator and thus stabilizes the maser intensity. The maser at present controlled as described here has a vacuum wave-length of 6329·9141 Å at threshold level for maser action.

Because the vibrating mirror imparts a frequency modulation to the maser output, the band-width of the emission is effectively broadened. The amount of the modulation is, however, small and does not upset interferometric work over paths of several metres. For radio frequency beat experiments or for interferometry over longer paths the modulation would be troublesome, but it could be overcome by a more elaborate servo system in which the mirror vibration could be stopped for short periods without affecting the average length of the optical cavity. The length of time for which control could be interrupted would depend on mechanical and thermal stability, but might well be longer than a minute. Alternatively the frequency modulation could be eliminated by using a second maser, not being vibrated, which was controlled relatively to the first using their beat frequency as the control signal.

Experience over a period of several months with the simple control system described has shown that a reproducibility of wave-length to a few parts in 10^8 can be readily achieved with a particular maser system. Because the wave-length is affected by the conditions of operation, the system is not suitable at present for a primary wave-length standard. If, however, its wave-length is occasionally verified against the krypton-86 standard, then the light from a controlled maser forms an excellent secondary standard suitable for precision interferometric measurements of length over long paths.

We thank the Services Electronics Research Laboratory for providing the optical components of the maser. The work described here has been carried out as part of the research programme of the National Physical Laboratory and is the subject of patent applications. This article is published by permission of the Director of the Laboratory.

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SHAPE, INTENSITY AND PRESSURE BROADENING OF THE 2·53-MILLIMETRE WAVE-LENGTH OXYGEN ABSORPTION LINE

By A. E. SCHULZE and C. W. TOLBERT

Electrical Engineering Research Laboratory, University of Texas, Austin

INCREASING demands for broad-band-width communications channels have led to the investigation of propagation characteristics through the atmosphere of the Earth of signals in the 10^{11}–10^{12} c/s frequency range. The resonant absorption of energy at selected wave-lengths by oxygen and water vapour causes the atmosphere to be partially opaque to the transmission of signals in this region of the electromagnetic spectrum.

The microwave absorption spectrum of molecular oxygen consists of many fine-structure spectral lines. The absorption is usually represented by approximately 25 major lines in the 5-mm region and a single isolated line in the region of 2·5-mm wave-length.

Spectroscopists have been concerned primarily with the measurements of oxygen line-centre frequencies, however, propagation of signals in the atmosphere in the vicinity of the line frequencies requires a knowledge of the shape, intensity and pressure broadening associated with the lines. Although sufficiently monochromatic signal sources have been available for several years to allow the measurement of the characteristics of the 2·53-mm line, the difficulties of measuring the low intensity of absorption because of the magnetic coupling of energy to the oxygen molecules, together with the limited interest of spectroscopists in pressure broadening, have delayed accurate investigations of the line shape and intensity.

Although several mathematical models have been proposed to describe the shape and intensity of a spectral line, the Van Vleck–Weisskopf equation[1] has been found to describe best pressure broadening in the microwave region. In this theory the period of oscillation of the incident electromagnetic energy is assumed to be much longer than the mean duration of a molecular collision. In the microwave and longer wave-length regions, this condition is approximately true, however, the approximation becomes worse as the frequency of the incident electromagnetic energy used to observe the transition is increased.

The Van Vleck–Weisskopf theory also assumes that intermolecular collisions constitute a random interruption of the phase of a molecular harmonic oscillator, such that after collision each molecule assumes an independent phase. The mathematical expression for the line shape involves the half-intensity, half-width ($\Delta\nu$) of the line which is treated as a semi-empirical parameter predicted to be a direct linear function of the gas pressure. The peak intensity of the line is predicted to remain constant over the domain of pressure broadening. There is a dearth of accurate measurements of line shape and intensity over a wide range of pressures for comparison with the mathematical model.

The object of the investigation reported here was to measure the shape and intensity of the 2·53-mm wave-length oxygen absorption line over a range of pressures corresponding to elevations in the atmosphere from sea-level to approximately 170,000 ft. The measurements were to ascertain the absorption due to dry oxygen alone, with provisions for extrapolating the data to fit atmospheric absorption due to a mixture of dry atmospheric gases.

A 500-ft long, 6-in diameter, outer copper conductor of a coaxial line was used as the absorption cell. Two glass-supported 'Teflon' windows, experimentally found to approximate an integral multiple of a half-wave-length thick, were used to seal the cell. Transmission through the cell was accomplished by propagating a wave from a conical horn antenna such that it was incident on the dielectric window essentially as a plane wave. The coherent signal between 114 Gc/s and 123 Gc/s used for the measurements was derived from a crystal harmonic generator excited by a variable-frequency Raytheon *QKK* 864 reflex klystron.

The power lost in the free-space coupling of the transmitting and receiving antennæ to the absorption cell, together with the relatively low level of 118-Gc/s signal output from the crystal harmonic generator, required the

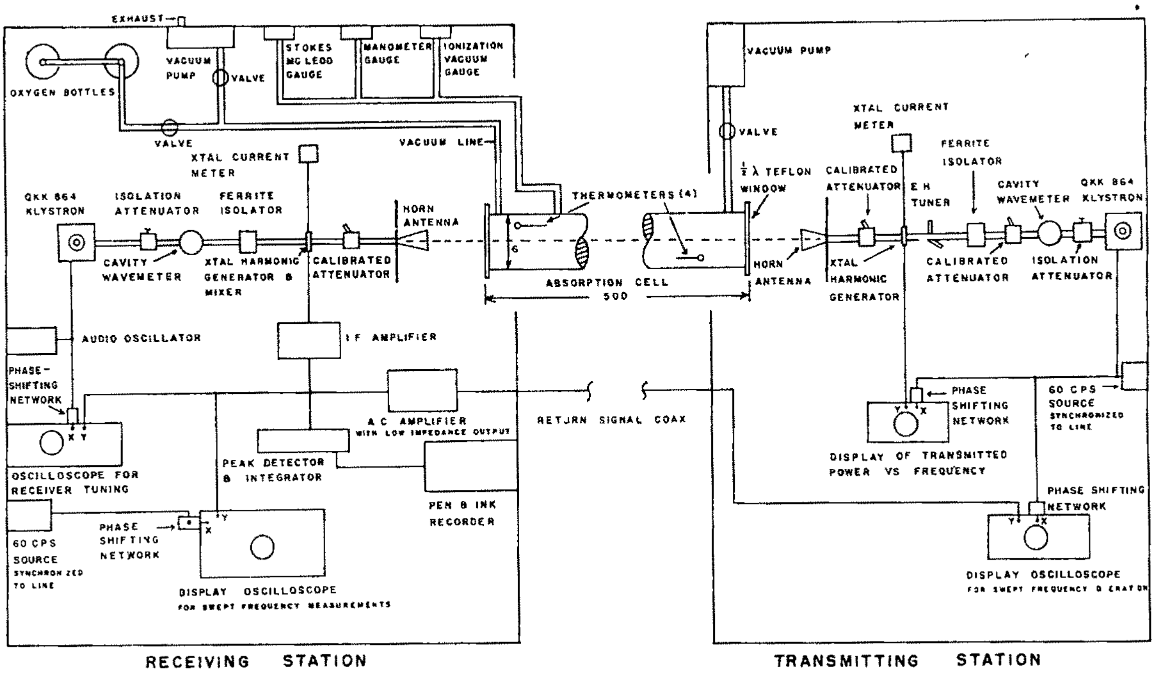

Fig. 1. Block diagram of the microwave spectrometer used to measure the 2·53-mm oxygen absorption line

use of heterodyne detection techniques. The configuration of the absorption cell made Zeeman modulation impractical.

The spectrometer (Fig. 1) is a data-sampling system in which the superheterodyne receiver samples the transmitted signal at an audio rate.

Two types of measuring techniques were necessary to evaluate the line shape and intensity over the wide dynamic range of values associated with gas pressures between 0·5 mm and 760 mm mercury.

Between pressures of 0·5 mm and 4·0 mm mercury, where the significant features of the line could be encompassed by frequency modulating the signal source, swept-frequency measurements were taken. For these measurements, the transmitter was modulated with a 60 c/s sinusoidal signal synchronized to the primary 115-V a.c. power line frequency. A similar signal with adjusted phase was used to scan the X-axis of an oscilloscope, while the output of the intermediate frequency (IF) amplifier of the receiver was displayed on the Y-axis of the oscilloscope. Thus, the X-axis was a frequency scale which was calibrated by a knowledge of the bandpass of the narrow-band IF amplifier. The Y-axis was a signal-level scale which was calibrated by the use of the calibrated attenuator at the receiver.

Swept-frequency measurements of the peak intensity and the width of the absorption line were taken by photographing the signal-level versus frequency data displayed on the oscilloscope at pressures of 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5, 4·0, 4·5, 5·0 and 6·0 mm mercury.

Between the pressures of 4 and 760 mm mercury, the width of the line required the use of a fixed-frequency measuring technique. For these measurements, the frequency of the transmitter was set to a selected fixed-frequency while the pressure of the gas within the absorption cell was varied.

Fixed-frequency measurements of oxygen attenuation at pressures of 8, 15, 30, 60, 100, 200, 300, 400, 500, 600, 700 and 760 mm mercury were taken at frequencies of 30, 60, 120, 240, 480, 1,000, 2,000 and 4,000 Mc/s on both sides of the line-centre frequency. Measurements were taken by evacuating the absorption cell to a pressure sufficiently low to provide an unmeasurable amount of oxygen absorption at the pre-set frequency, calibrating the chart of a pen and ink recorder with the calibrated attenuator, and injecting oxygen into the cell while the attenuation as a function of pressure was recorded. The 30-Mc/s centre frequency of the narrow-band IF amplifier was utilized to precisely set the desired transmitter frequencies between line-centre and 1,000 Mc/s on either side of line-centre.

To reduce each measured value to the equivalent value at a common temperature, a temperature-adjustment coefficient explicit in the Van Vleck–Weisskopf theoretical equation was applied to the data. In this manner, the data were adjusted to a common temperature of 300° K, the mean temperature at which measurements were taken.

The Van Vleck–Weisskopf shape factor was first assumed adequately to describe the shape of this pressure-broadened spectral line, and the value of the line-centre frequency was taken to be 118·745 Gc/s as measured by Anderson, Johnson and Gordy[2]. It was then necessary to determine the required constants of the shape factor in order best to describe the experimental data over the entire range of pressures of 8–760 mm mercury. The assumed expression for attenuation is as follows:

$$\gamma = K_0\nu^2\left[\frac{\Delta\nu}{(\nu_0-\nu)^2+(\Delta\nu)^2}+\frac{\Delta\nu}{(\nu_0+\nu)^2+(\Delta\nu)^2}\right]$$

where γ = attenuation in db/km for 100 per cent oxygen at 300° K, ν_0 = line-centre frequency in Gc/s (118·745 Gc/s), ν = frequency in Gc/s, K_0 = a constant for a given pressure and temperature (related to the peak intensity and $\Delta\nu$ by $K_0 \cong \frac{\gamma\text{ peak }\Delta\nu}{\nu_0{}^2}$), $K_0 = C\frac{P}{T^3}$ (ref. 3).

A digital computer was used to determine K_0 and $\Delta\nu$ for the best fit of this Van Vleck–Weisskopf equation to the experimental data at all measured pressures. Thus, the computed curves accomplished the necessary averaging and weighting of the data.

Fig. 2 shows an example of the calculated curves representing the best fit to the experimental data of the Van Vleck–Weisskopf line shape. The equation for the calculated curves is:

$$\gamma = 24{\cdot}9\,\frac{P}{T^3}\,\nu^2\left[\frac{\Delta\nu}{(118{\cdot}745-\nu)^2+(\Delta\nu)^2}+\frac{\Delta\nu}{(118{\cdot}745+\Delta\nu)^2+(\Delta\nu)^2}\right]$$

The curves (Fig. 2) are within the root mean square experimental accuracy of the data.

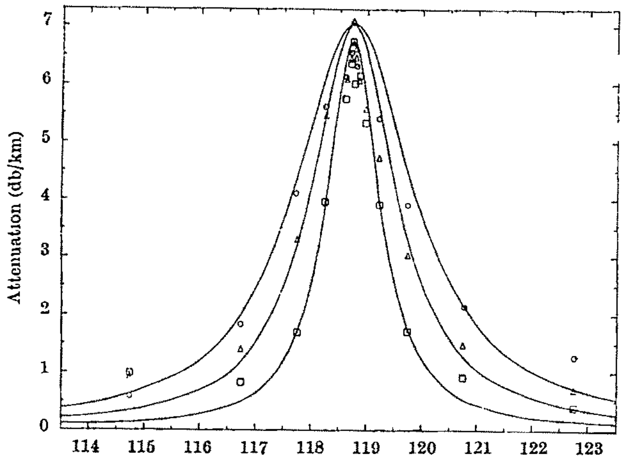

Fig 2 Attenuation versus frequency for pressures of 700 (○), 500 (△) and 300 (□) mm mercury Oxygen 100 per cent at 300° K

The discrepancies existing between the average of the measured values and the calculated curves are associated with the measuring uncertainty, the inadequacy of the line shape factor, and the assumed temperature-adjustment coefficients

It is probable that the Van Vleck–Weisskopf shape factor does not exactly describe the line shape of this oxygen absorption line[1,3] This is evident from the failure of the Van Vleck–Weisskopf line shape factor to describe exactly the experimental points near line-centre frequency and the wings of the absorption line If the shape factor is calculated to fit the measured values of attenuation near line-centre frequency, it fails to fit the wings, and vice versa Thus, the best description of the experimental data using the Van Vleck–Weisskopf shape factor represents a compromise between a description of the attenuation in the vicinity of the peak intensity and a description of the attenuation in the wings of the line

The curve of Δν versus pressure (Fig 3) was derived using the average value of Δν obtained from the calculated curves best describing the data The slope of this line is 1·90 $\frac{\text{Mc/s}}{\text{mm Hg}}$ for 100 per cent oxygen at 300° K The slope

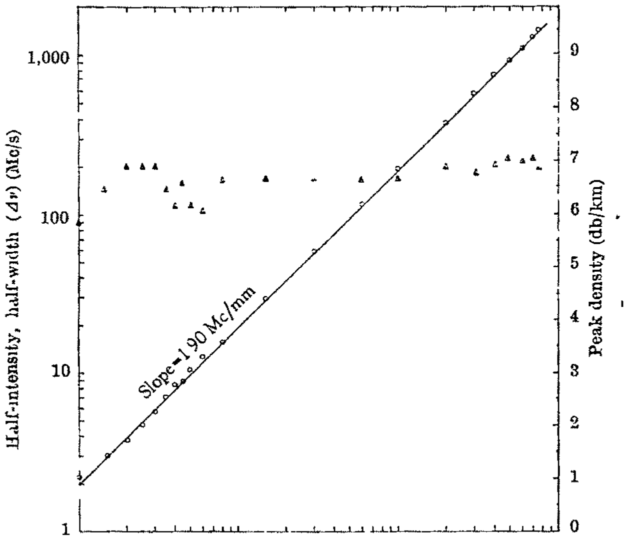

Fig 3 Half-width at half-intensity (○) and peak intensity (△) of the 2·53-mm wave-length oxygen absorption line versus pressure Oxygen 100 per cent at 300° K

of the Δν versus pressure curve obtained by the dynamic-frequency technique for a range of low pressures is 2·08 $\frac{\text{Mc/s}}{\text{mm Hg}}$ The difference between these two values is attributed to experimental uncertainty The mean of these two values, 1·99 $\frac{\text{Mc/s}}{\text{mm Hg}}$ at 300° K, is in excellent agreement with the value of 1·97 $\frac{\text{Mc/s}}{\text{mm Hg}}$ at 300° K measured by Hill and Gordy[4] It is also in good agreement with the theoretical value of 2·064 $\frac{\text{Mc/s}}{\text{mm Hg}}$ calculated by Artman and Gordon[5] The agreement of this measurement with atmospheric propagation measurements made by this Laboratory[6] supports the argument that nitrogen–oxygen collisions and oxygen–oxygen collisions cause approximately an equivalent amount of pressure broadening[3]

Although very few measurements have been made of the peak intensity of this oxygen absorption line, atmospheric propagation measurements by this Laboratory at frequencies below line-centre frequency indicate a peak intensity of 1·71 db/km for air at 291° K and 740 mm mercury[6] Assuming the atmospheric gases to be composed of 21 per cent oxygen, this value yields 7·66 db/km for the peak intensity of the line in 100 per cent oxygen at 300° K From Fig 3, the peak intensity at this pressure is approximately 7·0 db/km The difference between these two values is within the combined experimental uncertainty of the two measurements

Measurements taken with this spectrometer using air show the air/oxygen broadening ratio to be approximately unity and the air/oxygen attenuation intensity ratio to be approximately 0·21 Thus, the approximate horizontal attenuation in the atmosphere due to oxygen absorption alone can be obtained by multiplying the values of attenuation due to 100 per cent oxygen by 0·21

In order better to evaluate the line shape, all data were converted to the equivalent values at 760 mm mercury This conversion was accomplished by assuming that the line expands linearly with increasing pressure about the line-centre frequency For constant values of attenuation, this assumption may be expressed as

$$\left.\frac{f_1}{P_1}\right|_{\gamma = a} = \left.\frac{f_2}{P_2}\right|_{\gamma = a}$$

where f_1 and f_2 are the frequency deviations from line-centre for which $\gamma = a$, a is a constant, and P_1 and P_2 are the pressures corresponding to f_1 and f_2, respectively This condition is also explicit in the Van Vleck–Weisskopf line shape equations for small frequency deviations from line-centre

The result of the conversion of these data by the linear transformation is shown in Fig 4 Although the measured line shape is approximately—but not exactly—described by the Van Vleck–Weisskopf shape factor, the measurements are better described by modifying the exponents of the numerator and denominator of the shape function

At a pressure corresponding to sea-level, 760 mm mercury, all the data converted to this equivalent pressure by the linear transformation are more accurately described by

$$\gamma = 6·69 \times 10^{-4}\nu^2 \left[\frac{(\Delta\nu)^{0·65}}{(|\nu_0 - \nu|)^{1·65} + (\Delta\nu)^{1·65}}\right]$$

where $\Delta\nu$ = 1·45 Gc/s for 100 per cent oxygen at 300° K This modification is of the general type required to partially compensate for the fact that the reciprocal of the average time between intermolecular collisions is less than the average of the reciprocals of these times

Although the Van Vleck–Weisskopf shape factor does not exactly describe the shape of the 2·53-mm wave-

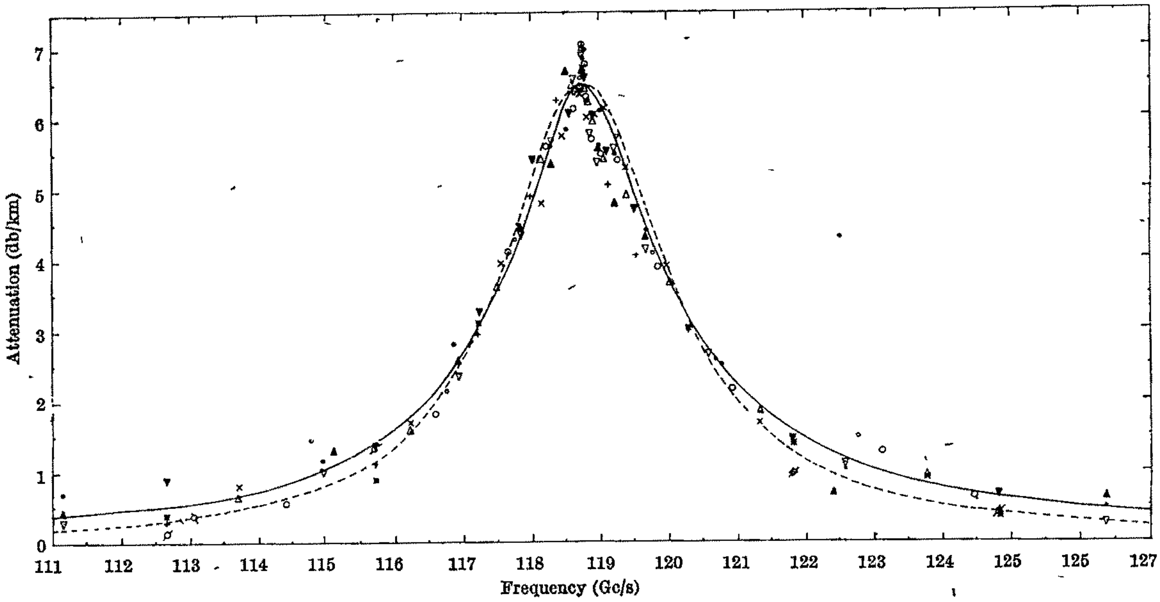

Fig 4 Measured values of attenuation converted to a common pressure of 760 mm mercury by the use of a linear transformation in frequency ∘, 760 mm, ○, 700 mm, △, 600 mm, ▼, 500 mm, ●, 400 mm, ×, 300 mm, ▽, 200 mm, ▲, 100 mm, +, 60 mm, ★, 30 mm, ↗o↗, 15 mm, ↘o↘, 8 mm 100 per cent oxygen at 300° K – – –, Van Vleck–Weisskopf line shape, ——, modified line shape

length oxygen absorption line, it is a good approximation of the shape except in the far wings A better fit of the wings is obtained by modifying the exponents of the numerator and denominator of the shape factor

The Van Vleck–Weisskopf shape of the absorption line as measured at pressures of 8–760 mm mercury is given by the equation

$$\gamma = 24{\cdot}9\,\frac{P}{T^3}\,\nu^2\left[\frac{\Delta\nu}{(118{\cdot}745-\nu)^2+(\Delta\nu)^2}+\frac{\Delta\nu}{(118{\cdot}745+\nu)^2+(\Delta\nu)^2}\right]$$

for 100 per cent oxygen near 300° K.

The measured line-width parameter, $\Delta\nu$, is best described by

$$\frac{\Delta\nu}{P} = 2{\cdot}08\ \frac{\text{Mc/s}}{\text{mm Hg}}\ \text{for } P \leqslant 4 \text{ mm Hg}$$

$$\frac{\Delta\nu}{P} = 1{\cdot}90\ \frac{\text{Mc/s}}{\text{mm Hg}}\ \text{for } 4 \text{ mm Hg} \leqslant P\ L\ 760 \text{ mm Hg}$$

$$\frac{\Delta\nu}{c} = 0{\cdot}481 \text{ cm}^{-1}\text{/atmosphere}$$

for 100 per cent oxygen at 300° K

The measured peak absorption of energy by 100 per cent oxygen at 300° K increases very slightly with pressure from a value of 6·5 db/km at 3·5 mm mercury to a value of 7·0 db/km at 500–700 mm mercury The increase in the peak intensity is due almost entirely to the wings of the pressure-broadened 5-mm wave-length oxygen line complex

This work was supported by the Aeronautical Systems Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio, under contract *AF* 33(657)–8716

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RADIOACTIVE DECAY OF ANTIMONY-124

By A. SESHAGIRI RAO, V. LAKSHMINARAYANA and PROF. SWAMI JNANANANDA

Department of Nuclear Physics, Andhra University, Waltair, India

THE importance and usefulness of a Compton scintillation spectrometer for the investigation of complex γ-ray spectra were first pointed out by Hofstadter and McIntyre[1] A Compton scintillation spectrometer suppresses the Compton continuum thus enabling the low-intensity, low-energy photon peaks, previously masked by the high-intensity high-energy components, to appear prominently in the spectrum Experimental, as well as theoretical, investigations of the efficiency of a fast–slow Compton scintillation spectrometer were made by Howland, Scofield and Taylor[2] They

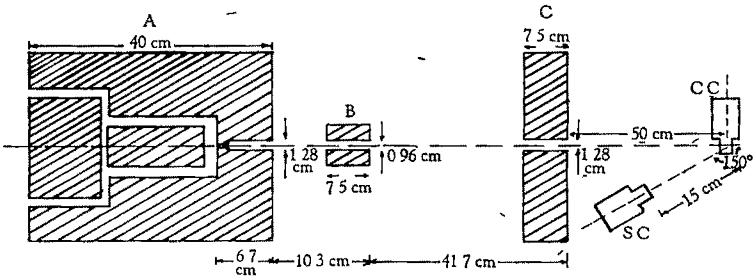

Fig 1 Geometrical arrangement *A*, source-holder, *B* and *C*, lead slits, *C C* , central crystal scintillation head, *S C* , slide crystal scintillation head

Table 1. γ-LINES OCCURRING IN THE DECAY OF ANTIMONY-124

Serial number	Recoil electron energy (keV)	Corresponding photon energy (keV)	Values of energy (ref. 4) (keV)
1	415	603	603 (632, 646)
2	520	719	714, 723 ± 1
3	750	964	970 ± 2
4	830	1,047	1,047 ± 3
5	1,070	1,296	1,298 ± 4
6	1,160	1,388	1,326 ± 4, 1,361 ± 5 1,370 ± 4
7	1,280	1,512	1,450 ± 14, 1,505 ± 15 1,540 ± 16
8	1,455	1,691	1,692 ± 2
9	1,650	1,889	1,900 ± 20
10	1,850	2,092	2,088 ± 4
11	2,030	2,274	2,260 ± 15

investigated the variation of efficiency of their experimental arrangement as a function of incident photon energy. Mono-energetic sources were used in their work, and the experimental results agree with theoretical estimates. The fast–slow Compton spectrometer has since become an important tool for accurate spectroscopic work, and hence was used in the investigation reported here to examine the complicated decay of the radioactive isotope antimony-124.

The decay scheme of antimony-124 was extensively examined and the fairly well-established features were summarized in the work of Dzhelepov *et al.*[3] Later work by Girgis *et al.*[4], using a conventional scintillation spectrometer, pointed out the necessity to introduce three new levels at 1,350, 1,900 and 2,865 keV, to accommodate the new γ-lines detected in their work. Recent work carried out in this laboratory[5], utilizing an electronic summing spectrometer, confirmed the three new levels introduced. However, the 2,260-keV line was shown to be the cross-over transition from the 2,295 keV-level and not from the 2,865 keV-level, as inferred by the complete absence of the cascade 2,260–603 keV in the sum-coincidence spectrum, when the sum gate was set at 2,865 keV. In the present investigation the decay of antimony-124 was examined with a fast–slow type of Compton scintillation spectrometer.

A Compton scintillation spectrometer comprising a 1.5 in. × 1 in. sodium iodide (Tl) crystal as the direct crystal and a 1.75 in. × 2 in. sodium iodide (Tl) crystal as the side crystal, both coupled to DuMont 6363 phototubes, was assembled. A fast–slow coincidence arrangement was made with an overall resolving time of about 120 mμsec. The fast coincidence circuit used was a modification of that reported by Green and Bell[6] and was operated with pulses derived from linear amplifiers following the photomultipliers. A geometrical arrangement similar to that of Davisson and Evans[7], with about 37′ as the semi-apex angle, was adopted, as shown in Fig. 1.

The antimony-124 source of strength about 10 mc. is introduced into the source-holder, and the singles spectrum as observed in the direct spectrometer is first recorded and shown in Fig. 2. In addition to the strong lines reported earlier, indications of peaks at 2,260 keV and 1,900 keV are obtained in the spectrum. The side spectrometer is next used to gate the main spectrometer with a channel width between 170 and 250 keV. The resultant coincidence spectrum is recorded and shown in Figs. 3*a* and 3*b*. The high-energy part of the spectrum is recorded in a greater detail by adjusting the gain of the amplifier and bringing the spectrum to a convenient level. The energies of the prominent peaks are estimated, and from these recoil electron energies the corresponding photon energies are computed and given in Table 1, along with the energies reported by Girgis *et al.*

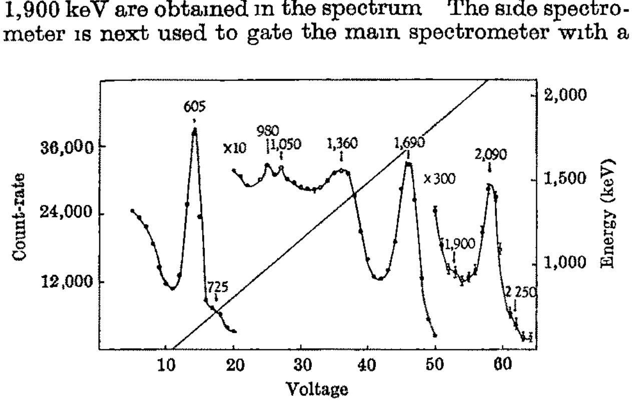

Fig. 2. Singles spectrum of antimony-124

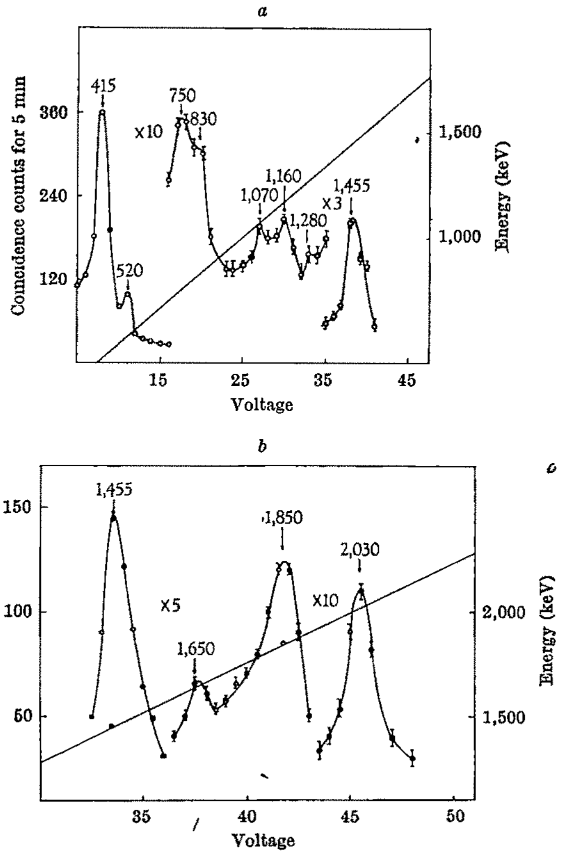

Fig. 3*a*, Coincidence recoil electron energy spectrum of antimony-124 (low-energy portion); 3*b*, coincidence recoil electron energy spectrum of antimony-124 (high-energy portion)

It can be seen from Table 1 that the present γ-lines at 603, 719, 1,388 and 1,512 keV are composite. Because of the small intensities of the closely lying energy components they could not be separated in the present work. In all other cases, the agreement between the present values of energy and those of Girgis *et al.* is satisfactory. Clear indications of peaks corresponding to 1,900 keV and 2,260 keV are observed supporting the existence of these two lines in the decay of antimony-124 as proposed by Girgis *et al.* Although the present work supports the existence of the new lines at 1,900 and 2,260 keV, it is not possible to fix their positions in the level diagram based on the results of this work. Most of the lines occurring in the decay of antimony-124 and not appearing in the singles spectrum could be recorded in the coincidence spectrum because of the Compton suppression.

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A MECHANISM FOR THE DIRECT ACTION OF IONIZING RADIATIONS

By Dr REINIER BRAAMS

Department of Radiobiophysics, University of Utrecht, Bijlhouwerstraat 6, Utrecht, The Netherlands

EXAMINATION of the effect of ionizing radiations on dry proteins[1-4] can be considered of importance for an explanation of the mechanism through which the absorption of radiation energy leads to chemical changes in proteins In the past twenty years a number of interesting experimental results have been reported, but there appears to be no model for the action of ionizing radiation on proteins which explains more than a few of the known facts

From recent investigations with quantitative electron spin resonance spectroscopy[5-10], it has become evident that a close relation exists between protein damage and the behaviour of protein radicals Of these correlations the following are of outstanding importance

(1) The radical yields in amino-acids, peptides and proteins are of the same order of magnitude[5-9] as the G-values for loss of amino-acid residues in irradiated proteins[11-13]

(2) Radical yields for irradiations at 77° K are lower[7,9] than for irradiations at about 295° K This parallels the decrease in radiation sensitivity of enzymes when the material is irradiated at liquid nitrogen temperature instead of at room temperature[2,3,14,15]

(3) Certain SH compounds that can partly protect proteins[16-18] against radiation inactivation when present with the protein in the dry state during irradiation can also receive unpaired spins which initially were induced outside the SH moiety[10,18,19]

(4) Interaction of radicals in irradiated protein with NO leads to changes in the observed radical pattern and strength, and to changes in the degree of inactivation of the irradiated protein[20]

If one accepts the importance of radicals as chemical intermediates, then, with the aid of proposals by Voevodsky[21] and Varshavskii *et al* [22], a scheme can be developed which will explain a number of effects observed in proteins and which can be extended to nucleic acids and to organic material in general

The first step in this chemical reaction scheme is the dissociation of the irradiated macromolecule into a large and a small radical fragment

$$MR_i \rightarrow M + R_i \qquad (1)$$

Here MR_i represents a protein macromolecule composed of a main chain with many different side-chains R_i, M is a relatively stable radical state of the macromolecule and $\dot{R}_i$ is a small diffusible radical Recombination of the two radicals can occur, but does not necessarily lead to repair since about 4 eV (approx 100 kcal/mole) must be dissipated without changing the configuration of the protein M is usually stable at cryogenic temperatures but can change into a different radical on raising the temperature[9,10,23]

Rowlands and Whiffen have collected from the literature the structures assigned to 61 radicals obtained by irradiation of organic compounds[24] In 43 cases the radical appears to have been formed by breakage of the C—H bond and the removal of H It seems therefore that in many cases the radical R_i will be atomic hydrogen

$\dot{R}_i$ is very potent chemically, especially if it is in the excited state[25] For H to react with other side-chains an activation energy of about 5–9 kcal/mole[26,27] is needed and this energy will often be available If only molecules of the MR_n type are present $\dot{R}_i$ can react with MR_n

$$R_i + MR_n \rightarrow M + R_iR_n \qquad (2a)$$

In the special case that R_i is atomic hydrogen the reaction is

$$H + MR_n \rightarrow M + R_nH \qquad (2b)$$

The reactions (2a) and (2b) could proceed also in a backward direction as proposed by Varshavskii *et al* [22] to explain the chemical migration of free radical sites through semi-crystalline polyethylene The forward reactions result in the formation of another protein radical, and a small molecule which could either react with a protein radical or escape as gas They will proceed fast when R_i carries enough energy to overcome the activation barrier After $\dot{R}_i$ has become thermal (at room temperature) the reactions will be much slowed down but can still proceed at a measurable rate, especially when the entropy of activation is low

In polymers with a small number of possible side-groups R_i, only a few different types of gas molecules will be found In polyethylene most of the gas consists of molecular hydrogen, while in polydimethyl–siloxane hydrogen, methane and small amounts of ethanol were found[21] In proteins the situation could be more complex, but apparently reactions (1) and (2) occur with preference at the site of the glycine residue In experiments designed to detect the evolution of molecular hydrogen the production of gas (partly consisting of H_2) could be observed during the irradiation of amino-acids and proteins[28]

The thermal annihilation of radicals in irradiated amino-acids and proteins at about 100° C suggests that M can react with its environment It has been observed[29] that during thermal annealing of radicals in alanine and in protein, gas is produced This leads to the suggestion that reactions of the following type can occur

$$M + MR_i \rightarrow MM + R_i \qquad (3a)$$

$$M + MR_i \rightarrow M_1 + M_2 + \dot{R}_i \qquad (3b)$$

Reaction (3a) represents a cross-linking between 2 macromolecules, whereas reaction (3b) leads to two changed macromolecules M_1 and M_2 Both reactions produce a small radical that undergoes further reaction and eventually appears as gas

If SH compounds are present in the dry material the radicals $\dot{R}_i$ can abstract an H-atom from RSH

$$\dot{R}_i + RSH \rightarrow RS + R_iH \qquad (4)$$

If sufficient RSH is present and when the affinity of R_i to RSH is high, reaction (4) will compete with (2) and might even be favoured This results in protection in the dry state as has been observed by several authors[16,18,30] When reaction (4) completely dominates reaction (2) the radiation sensitivity will appear reduced to about 50 per cent of the sensitivity of the protein in the absence of protective compounds This is the order of magnitude actually found for enzymes[16-18,30,31] and for bacteriophage[32] when irradiated in the dry state in vacuum

Oxygen, when present, will compete with the protein for reaction with $\dot{R}_i$ to form peroxy radicals[31,33] that will peroxidize the protein to similar damage as with irradiation in vacuum and subsequent exposure to oxygen[20] If, however, protective compounds are present, the oxygen will compete with the protective agent for $\dot{R}_i$ to form peroxy radicals, reducing the protective effect of SH compounds in the presence of oxygen, as has been observed by Butler and Robins[30,31] for trypsin

Several observed experimental facts appear to be in agreement with the proposed model

(1) The inactivation curves of enzymes irradiated in the dry state are exponential. The number of interactions N leading to reaction (1) will be proportional ($N = a D$) with the radiation dose D. The average number of enzyme molecules affected by one interaction through reactions (1–4) will be q. This number will depend on the environmental conditions. The probability that an affected molecule will be inactivated is p, p depending on conditions before or during assay of the enzyme activity. The average number of inactivated molecules per interaction will then be pq. The activity remaining after a dose D can now be given by

$$A(D) = A\,e^{-pqaD}$$

(2) The slope of the inactivation curves plotted on semi-log paper is dependent on temperature. When irradiations are carried out at different temperatures, the inactivation curves are still exponential but with different slopes. If reaction (1) is dependent on temperature the probability q will also be dependent on temperature. From the available data[34], it appears that q is an exponential function of the reciprocal value of T in the temperature range 70°–300° K.

(3) In the presence of SH compounds the radiation sensitivity of enzymes is reduced to about half the sensitivity without protecting agents present. At high concentrations of the protective agent the protection factor[17,31] can become slightly higher than two. This occurs when, after interaction with ionizing particles, the SH compounds themselves are contributing hydrogen atoms for reaction with protein radicals. Such a reaction could lead to restoration of the original chemical composition of the protein molecules. The probability that such a restoration occurs without changes in the native configuration of the protein must be considered small since the protection factor in the dry state is usually in the neighbourhood of 2–3.

(4) The frequently observed transfer of spins to SH compounds in mixtures of proteins or amino-acids with SH compounds is diffusion controlled[8].

(5) The protection with SH compounds is also achieved when irradiations are carried out at liquid nitrogen temperature[35] since reactions with $\dot{R}_i$ can start when the temperature of the sample is brought back to room temperature. The fact that the protection factor for irradiations at low temperature is nearly the same as for radiation at room temperature seems to indicate that excitation of R_i is not a necessary condition for reaction with protein.

(6) When oxygen is present during irradiation both the protein radicals and $\dot{R}_i$ will react with oxygen. The effect to the protein could be slightly different from the changes caused by irradiation in vacuum and subsequent exposure to oxygen before dissolution. Hunt *et al.*[20] find that for RNase the radiation sensitivity for exposure to oxygen during irradiation is about 1·3 times the sensitivity for irradiation in vacuum with subsequent exposure to oxygen.

(7) When protective compounds are present during an irradiation in oxygen, the protective effect cannot be exerted and an oxygen effect becomes apparent[30,31].

(8) At very high LET values, the rise in temperature in the centre of the track can be several thousands of degrees. Atomic hydrogen and other small radicals that have been formed will not easily combine or recombine in the region of high temperature. Because of the high pressure and temperature they will diffuse farther away from the central core of the track at low LET. The mechanism of energy transfer needed to explain the enlarged radiation cross-sections for enzymes irradiated with fully ionized carbon or oxygen nuclei[36] then simply is the diffusion of hot small radicals.

(9) Since the processes in the region of the track of particles with very high LET will be governed by the transient high temperature with values of thousands of degrees, the effects will not be influenced much by a change in the ambient temperature of about 200° C. For these particles the radiation sensitivity of enzymes should not depend much on the temperature at which radiation is carried out. Brustad[36] has actually observed this for trypsin, DNase and lysozyme.

(10) In the region of high temperature around the track of a high LET particle, scavenging of the small radicals $\dot{R}_i$ by SH compounds will not be effective, and the radicals will be able to diffuse away from the central core. In this core therefore no protection can be expected. Outside the core around the δ-ray tracks interaction with SH presumably still could occur. The overall effect of the presence of SH compounds thus results in a small protective effect. Brustad[36] has observed that trypsin is not protected by cysteine when irradiated with 115-MeV carbon nuclei. It appears that better agreement between theory and experiment can only be expected after the theory has been more refined and experimental data have become more abundant.

(11) When protein is surrounded by an excess of chemically different material the radiation effects in the protein fraction will be influenced by the radical production in the surrounding material and by the nature of these radicals. Sensitization can be expected to occur when the surrounding substances produce more, and more aggressive, radicals than proteins. Sensitization of enzymes in the presence of excess foreign material has actually been observed for invertase and RNase in acetate and in sucrose for 4-MeV deuterons[16], for trypsin in dextran or in ribose after irradiation with 115-MeV carbon-ions[36] and for trypsin exposed to fast electrons in the presence of magnesium salts[31].

(12) The fraction of molecules which show thermolability[37] is independent of the presence of protective compounds[36], indicating that in the presence of protective agents only the number of events damaging to the protein-molecules are reduced but that the nature of the damage has not been changed.

(13) The fraction of molecules which show thermolability is the same for irradiations performed at room temperature or at liquid nitrogen temperature[38], indicating again that by decreasing the temperature only the number of damaging events (the formation of a protein radical) is reduced but not their character.

(14) The presence of SH does not prevent the formation of free radicals in protein, a fact observed and explicitly stated by Henriksen *et al.*[10]

(15) A strongly reducing radical such as H could be expected to react with the disulphide bridge frequently present in proteins. Gordy *et al.*[39] have observed that in proteins containing –SS– linkages a cystine-like radical pattern is often found. If the reaction proceeds in the following way

$$\mathrm{H} + R_1-\mathrm{S}-\mathrm{S}-R_2 \rightarrow R_1-\mathrm{S}-\mathrm{H} + R_2-\mathrm{S}$$

breakage of the disulphide link would occur. This has been observed by Satlow and Zahn[40] in irradiated wool. After oxygen is admitted to the R–S radical this would react and oxidize the sulphur containing residue. After hydrolysis of irradiated wool an increase in the cysteic acid concentration has been found by Fritze *et al.*[41]

The model proposed here leads to a somewhat similar overall reaction as the hypothesis recently forwarded by Alexander and Ormerod[42,43]. The essential differences are:

(1) Real protection is possible through a mechanism of radical scavenging, similar to the mechanism now widely accepted for protection against the indirect effect. In the hypothesis by Alexander and Ormerod the important effect is a repair process.

(2) The protection is limited to a factor 2–3 in agreement with experimental observations, both on proteins and on bacteriophage. In the model by Alexander and Ormerod there are no reasons given why repair should not take place to a much higher degree than is actually observed.

(3) A simple chemical mechanism is given to explain the apparent interaction between the protein radical and the protective substance

The proposed model will have to be tested for quantitative agreement and for its capacity to produce valid predictions Especially the chemical reactions and their reaction kinetic constants will have to be specified in greater detail Some of the required experiments are being carried out and have yielded preliminary results which tend to support the hypotheses involved

If the model can be confirmed by more quantitative evidence, the original concepts of direct and indirect action will have to be re-defined the scavenging of small diffusible radicals not being restricted to the indirect action on compounds dissolved or suspended in water It appears, further, that with slight modifications the model can be extended to solutions and so provide a general theory for radiation effects in the condensed state

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RADIOLYSIS OF ISOPRENOIDS

FROM U.S NAVAL RADIOLOGICAL DEFENSE LABORATORY, SAN FRANCISCO

2,6-Dimethyloctane

RADIOLYTIC yields of hydrogen and hydrocarbons through C_5 have been determined for 2,6-dimethyloctane, since this paraffin serves as a model for hydrogenated rubber

The 2,6-dimethyloctane was synthesized by a new route (The 2,6-dimethyloctane was synthesized by Chemicals Procurement Laboratories, 130th Street, College Point, New York) 2,6-Dimethyloctanol-8 (ref 1) was converted to the bromide, which was converted to the Grignard reagent, which was converted to the paraffin by treatment with water Specimens for radiolysis were distilled with a 20 *T P* Stedman column b p 157 0°–157 1° C (763 mm), n_D^{20} 1·4112, d^{20} 0 7291, M_D(calc) 48 38, M_D (found) 48 49 $C_{10}H_{22}$ requires C, 84 39, H, 15 61, found C, 84 18, H, 15 83

Specimens of approximately 30 ml were irradiated They were degassed by conventional vacuum techniques of freezing, pumping and trap-to-trap distillation, and then distilled into the irradiation cell which was sealed to one of the traps through a seal-off tube The cell was a 50-ml , quartz, round-bottomed flask fitted with a horizonal 'Pyrex' break-seal on the neck and a seal-off tube The cell was baked-out for 12 h with pumping while the degassed specimen remained frozen in a trap Since 2,6-dimethyloctane frequently ruptured cylindrical vessels during freezing–thawing operations, bulbous traps and irradiation cells were used

Gamma-irradiations were made at 22°–25° C with cobalt-60 Dosimetry was carried out with the ferrous sulphate dosimeter ($G(Fe^{3+}) = 15\ 5$) which duplicated the specimen in volume and geometry In calculating radiation yields (G = molecules/100 eV), the small correction due to the difference in electron density between specimen and dosimeter was made

Vapour-phase products in the cell were analysed with a Perkin–Elmer gas chromatograph, model 154 Irradiation cells were attached through the break-seal to a Toepler pump (300-ml capacity) joined to a 3 5-ml. loop suitably fitted with stopcocks to serve as the introductory unit of the gas chromatograph The cell gases were transferred to the loop with five operations of the Toepler pump and without freezing the solvent 50-ft dimethylsulpholane and diphenyl ether columns (20 wt per cent on 60–80 mesh firebrick) at 0° C were used with nitrogen as the carrier gas A thermistor detector and a flame ionization detector were used in series to measure hydrogen and hydrocarbons, respectively The chromatographic unit was calibrated for response to each of the radiolytic products found In some experiments for measuring hydrogen and methane only, it was convenient to use a 50-ft Linde molecular sieve column at 30° C, helium carrier gas and a thermistor detector In this case, the solvent was frozen with liquid nitrogen during each operation of the Toepler pump, and was thawed and re-frozen between each operation

Radiation yields were based on the analysis of the vapour-phase products in the cell and are averages of at least three irradiations Experiments showed that the Toepler pump removed at least 98 per cent of the hydrogen and methane from the liquid phase within the cell Undoubtedly, other hydrocarbons were not removed as efficiently because of solubility in the solvent, and their radiation yields are considered to be semi-quantitative

Table 1 gives the product yields and Fig 1 shows the linearity of the hydrogen gas and methane yields

Hardwick has proposed that the radiolysis of liquid paraffins proceeds via hydrogen atom and organic radical intermediates[2,3] It has not been necessary to invoke ion-molecule reactions to explain the products 'Hot' and thermal hydrogen atoms are produced by C—H bond scission The former abstract hydrogen on their first collision with a neighbour molecule to produce hydrogen gas The latter diffuse, react competitively with solvent and solute, and produce hydrogen gas by abstraction or

Table 1 RADIATION YIELDS (G) FOR 2,6-DIMETHYLOCTANE

Product	Molecules/100 eV
Hydrogen	3 32
Methane	0 28
Ethane	0 04
Ethylene	0 14
Acetylene	0 004
Propane	0 04
Propylene	0 10
Normal butane	0 08
Butene-1	0 04
Cis-Butene-2	0 01
Trans Butene-2	0 03
Butadiene-1,3	0 001
Isobutane	0 04
Isobutylene	0 02
Normal pentane	0 004
Pentene-1	0 002
Cis-Pentene-2	0 007
Trans Pentene-2	0 0008
Isopentane	0 03
2-Methylbutene-2	0 002
2-Methylbutene-1	0 007

self-combination In some cases, an activated hydrocarbon molecule may detach a hydrogen molecule, leaving a bi-radical or olefine Caged alkyl radicals from C—C bond scission recombine or disproportionate to a paraffin plus an olefine Freely diffusing alkyl radicals combine to produce paraffins or disproportionate According to Buckley and Szwarc[4], alkyl radicals may abstract hydrogen from hydrocarbons to produce paraffins

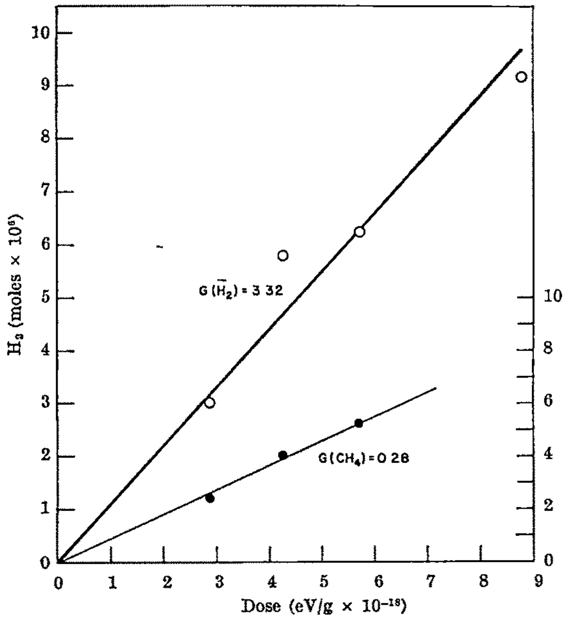

Fig 1 Yields of hydrogen and methane from 20 g of 2,6-dimethyloctane

On the basis of these views, direct routes are readily perceived to the following radiolytic products listed in Table 1 hydrogen, all paraffins except n-pentane, the olefines, ethylene, propylene, butene-1, butene-2, isobutylene and 2-methylbutene-1 For example, disproportionation accounts for the olefines On the other hand, the formation of the following trace products would seem to require some sequence of reactions n-pentane, pentene-1, pentene-2, 2-methylbutene-2, butadiene-1,3 and acetylene

WESLEY E SHELBERG
JAMES F PESTANER

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2,6-Dimethyloctadiene-2,6 and Squalene

RADIOLYTIC yields of hydrogen and hydrocarbons through C_5 have been determined for 2,6-dimethyloctadiene-2,6 and squalene, since these olefines serve as models for natural rubber

2,6-Dimethyloctadiene-2,6 was prepared by the reduction of geraniol (purified through the calcium chloride complex[1]) with sodium in liquid ammonia (Geraniol and squalene were obtained from Distillation Products Industries, Rochester, New York) 1,500 ml of liquid ammonia was added to 462 g of geraniol in a 4-l Erlenmeyer flask fitted with a strong, efficient stirrer No precautions were taken to prevent condensation of moisture within the flask 54 g of sodium was added in small pieces to the stirred mixture for 1 h, during which time liquid ammonia was added occasionally to maintain the volume Additional sodium would not react The mixture was left for 0 5 h after which 1 5 l of ethanol was added in small amounts while the flask was kept in an ice bath Ethanol and ice were then added alternately and cautiously until any remaining sodium had reacted Finally, an excess of water was added, and the organic layer was extracted with ether The ether solution was washed with water until it was no longer alkaline, washed with saturated salt solution and dried with anhydrous sodium sulphate The ether was distilled away in a 3-l flask with a large distilling head, the flask was immersed in an oil bath ultimately raised to 100° C The residual mixture of 2,6-dimethyloctadiene-2,6 and geraniol was distilled *in vacuo* using a 3-l round-bottomed flask with a Claissen head, using an oil bath at 90°–110° C, and using slow distillation to avoid superheating A yield of 154 g (74 per cent) of crude 2,6-dimethyloctadiene-2,6 was obtained, b p 58°–60° C (13 5–14 mm)

Pure geraniol was recovered afterwards at 3–4 mm (b p 94° C) The diene gradually develops an acrid odour on exposure to air, but this may be prevented by storing it in the degassed state Specimens were distilled immediately before radiolysis with a 20 *T P* Stedman column, b p 166 0° C (774 mm), n_D^{20} 1 4491; d^{20} 0 7734, M_D (calc) 47 45, M_D (found) 47 49 $C_{10}H_{18}$ requires C, 86 85, H, 13 15, found C, 86 90, H, 13 15 The infrared spectrum of the purified diene agreed with that of Sheppard and Sutherland[2] It showed the absence of hydroxyl groups and the presence of less than 2 per cent of isopropenyl groups, $CH_2 = C(CH_3)$— The isopropenyl unsaturation occurs in the geraniol and cannot be removed therefrom by reported purification procedures A hypothetical specimen free from isopropenyl unsaturation would be expected to acquire the properties of isopropenyl unsaturation on irradiation due to the allylic resonance associated with one of the scission radicals $\dot{C}H_2C(CH_3) = CHCH_2CH_2C(CH_3) = CHCH_3 \longleftrightarrow CH_2 = C(CH_3)\dot{C}HCH_2CH_2C(CH_3) = CHCH_3$

Squalene (98 per cent) was purified immediately before radiolysis by passage through a silica-gel column, as described by Sax and Stross[3], and vacuum distillation $C_{30}H_{50}$ requires C, 87 73, H, 12 27, found C, 87 74, H, 12 25

As shown in the previous communication, specimens (20 g) were degassed in quartz cells, they were irradiated with γ-radiation from cobalt-60, and radiation yields

Table 2 RADIATION YIELDS (G) FOR 2,6-DIMETHYLOCTADIENE-2,6 AND SQUALENE

Product	Molecules/100 eV 2,6-Dimethyloctadiene-2,6	 Squalene
Hydrogen	$7\ 7 \times 10^{-1}$	$5\ 8 \times 10^{-1}$
Methane	$5\ 2 \times 10^{-2}$	$1\ 8 \times 10^{-3}$
Ethane	1×10^{-2}	1×10^{-4}
Ethylene	6×10^{-3}	4×10^{-4}
Acetylene	none	2×10^{-5}
Propane	3×10^{-3}	7×10^{-6}
Propylene	2×10^{-2}	4×10^{-4}
Normal butane	4×10^{-3}	none
Butene-1	2×10^{-2}	none
Cis-Butene-2	5×10^{-3}	8×10^{-7}
Trans Butene-2	9×10^{-3}	none
Butadiene-1,3	9×10^{-4}	4×10^{-4}
Isobutane	2×10^{-3}	none
Isobutylene	1×10^{-2}	1×10^{-3}
Normal pentane	3×10^{-3}	none
Pentene-1	2×10^{-3}	none
Isopentane	2×10^{-3}	none
3-Methylbutene-1	1×10^{-2}	none
2-Methylbutene-2	5×10^{-3}	3×10^{-4}
2-Methylbutene-1	1×10^{-2}	none

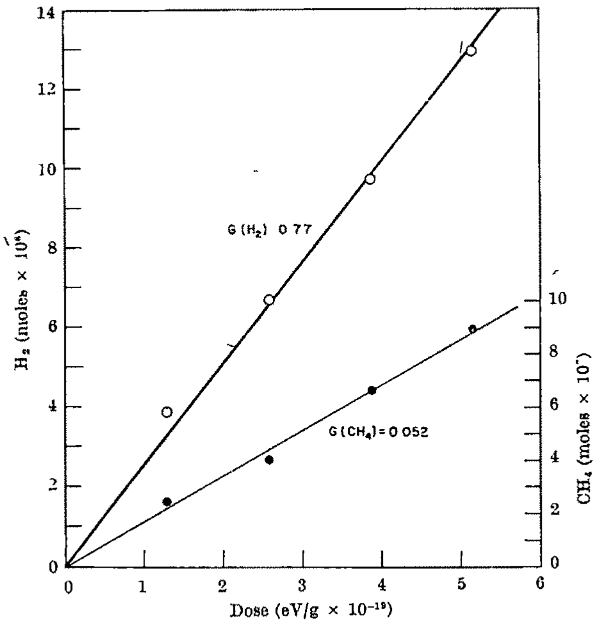

Fig 2 Yields of hydrogen and methane from 20 g of 2,6-dimethyloctadiene-2,6

were based on the gas chromatographic analysis of the vapour phase products in the cell This resulted in quantitative yields for hydrogen and methane and semi-quantitative yields for other hydrocarbons

Table 2 gives the product yields and Figs 2 and 3 show the linearity of the hydrogen gas and methane yields The product yields from squalene are generally less than those from 2,6-dimethyloctadiene-2,6

It is postulated that molecules of 2,6-dimethyloctadiene-2,6 and squalene undergo C—H and C—C bond scission on radiolysis to produce hydrogen, methyl and olefinic radicals These radicals are not expected to diffuse beyond a few molecules because of the great scavenging ability of the solvent, but are expected to react with adjacent and nearby molecules to produce stable products The reactions undoubtedly include addition of radicals to double bonds, abstraction of hydrogen, radical coupling, disproportionation, and rearrangement of unsaturation due to allylic resonance It does not seem reasonable to speculate on specific routes to observed products because of the complexity possible in the reactions

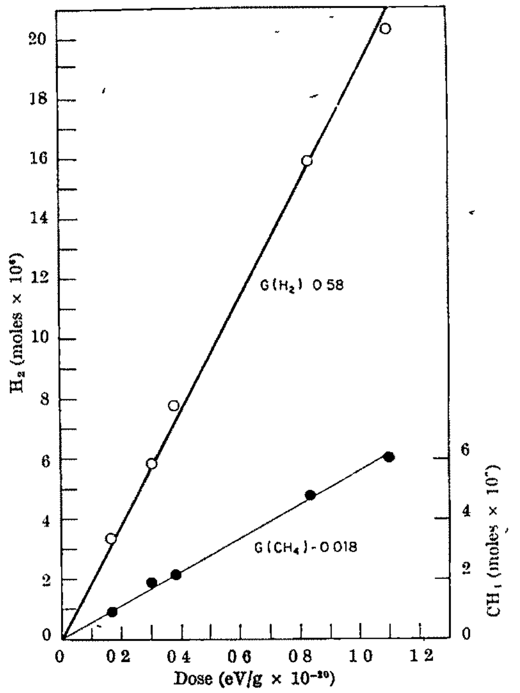

Fig 3 Yields of hydrogen and methane from 20 g of squalene

Wesley E Shelberg
James F Pestaner
Richard Y Yahiku

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EFFECT OF X-IRRADIATION ON SUSCEPTIBILITY OF CELLS TO TRANSFORMATION BY POLYOMA VIRUS

By Prof MICHAEL STOKER

Medical Research Council Experimental Virus Research Unit, Institute of Virology, University of Glasgow

THE ability of polyoma virus to alter the hereditary properties of cells (polyoma transformation)[1,2] can be estimated quantitatively *in vitro* using the *BHK*21 line of hamster fibroblasts[3,4], and investigations can now be made on the conditions which affect the frequency of transformation following virus cell interaction

Since it is believed that X-irradiation predisposes to neoplasia, and some radiation-induced leukæmias in experimental animals are found to be transmissible[5], it was decided to examine the effect of X-rays on the sensitivity of *BHK*21 cells to transformation by polyoma virus

The tissue culture fluids and media, and the feeder layer cultures[6], the source and culture of *BHK*21 cells[3], the preparation of high titre virus stocks of a small plaque variant of polyoma virus[7] and the standard conditions for infection of *BHK*21 cells in suspension[4], have been described previously

The standard medium (designated *ETC*) consists of modified Eagle's medium with twice the normal content of amino-acids and vitamins. 10 per cent tryptose and 10 per cent unheated calf serum In all experiments, clone 13 of *BHK*21 cells was used after not more than 2 weeks' growth from a frozen stock at the thirtieth generation

Hamster cells, as well as mouse cells used for feeder layers, were irradiated as suspensions in *tris* buffer[8] with 2 5 per cent calf serum (afterwards called 'buffer') or as confluent monolayer cultures in medium A Watson-type Mobilix X-ray apparatus was used at 81 kV and 3 m amp delivering 4 1 r /sec in air The radiation was unfiltered and the target to cell distance was 12 7 cm The maximum depth of buffer or medium during the irradiation was 5 mm Following irradiation the suspended cells were centrifuged at 250*g* for 5 min and re-suspended in fresh buffer the intact monolayer cultures were washed with buffer

X-ray sensitivity of uninfected BHK21 *cells* The X-ray sensitivity of uninfected *BHK*21 cells was first determined Cells were exposed in suspension to various doses of

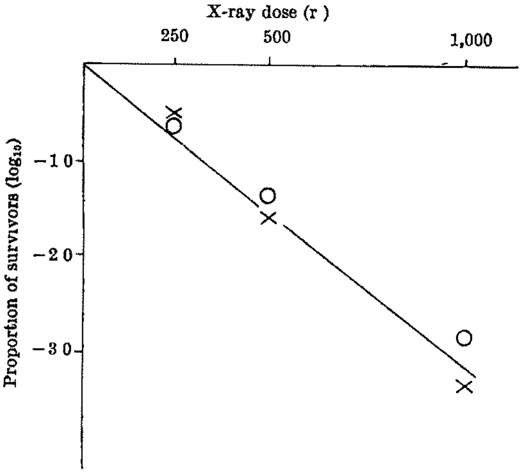

Fig 1 Sensitivity of normal and transformed *BHK*21 cells to X-irradiation Shows proportion of colony-forming cells surviving after irradiation compared with unirradiated controls
O, Normal, ×, transformed

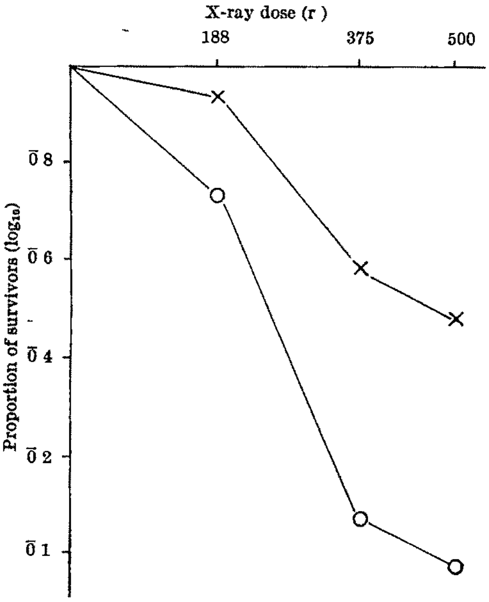

Fig 2 Effect of polyoma virus on irradiated normal *BHK*21 cells Cells were irradiated for various times and then exposed to virus Shows proportion of colony-forming cells compared to unirradiated controls
O, Normal, ×, transformed

irradiation and the numbers of surviving cells were determined by colony counts after plating on feeder layers of irradiated mouse cells For comparison a cloned, virus-free, line of polyoma transformed *BHK*21 cells was examined in the same way The results which are shown in Fig 1 do not reveal any significant difference in sensitivity between the normal and transformed cells No colonies with transformed or otherwise abnormal morphology were seen among about 10^4 survivors of normal cells after X-irradiation and conversely no normal colonies

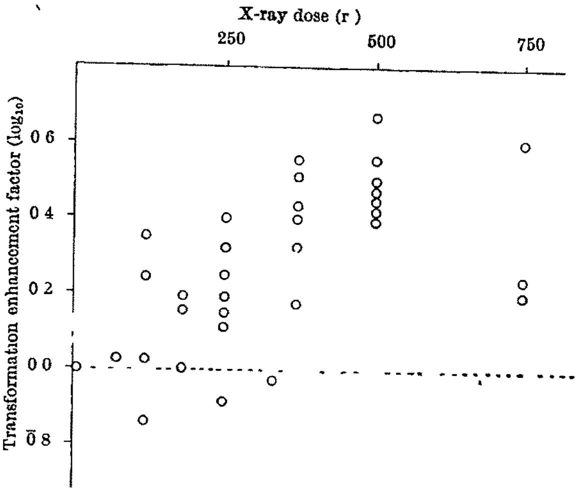

Fig 3 Effect of polyoma virus on irradiated normal *BHK*21 cells Pooled results of eight experiments showing enhancement in transformation frequency found after irradiation

$$\text{Enhancement factor} = \frac{\text{Proportion of transformed colonies from irradiated cells}}{\text{Proportion of transformed colonies from unirradiated cells}}$$

have been found among the surviving colonies of transformed cells

Effect of irradiation on frequency of transformation To investigate the effect of irradiation on subsequent polyoma transformation, batches of 1–2 million *BHK*21 cells were exposed in suspension to various doses of irradiation Equal numbers of cells (about one million) from each batch were infected in suspension with 10^8 plaque-forming units of polyoma virus and assayed for transformation by the standard method Table 1 gives the numbers of normal and transformed colonies which developed, and also the frequency of transformed colonies among total colonies (column 6) Fig 2 shows graphically the proportion of survivors which gave either normal or transformed colonies

It will be seen from Table 1 and Fig 2 that the irradiation reduced the number of viable cells giving normal colonies more rapidly than the number giving transformed colonies After 500 r and subsequent infection the proportion of normal viable cells had fallen from 46 to 4 4 per cent whereas the transformed viable cells only fell from 2 5 to 0 78 per cent This difference leads to an increase in the transformed colony frequency (column 6)

The increment in transformation produced by irradiation of the cells may be shown by the ratio of the transformed colony frequency after irradiation compared to the frequency in the unirradiated controls This will be called the enhancement factor, and it is shown in column 7 of Table 1 Fig 3 shows the enhancement factor for increasing irradiation obtained from the pooled results of eight experiments There is a good deal of variation between experiments, but it appears from the figures that the increase in transformation frequency is related to dose of X-rays at least up to 500 r At higher doses the number of surviving cells is too few for satisfactory estimates

From these results it may be concluded that prior irradiation of *BHK*21 cells followed by polyoma virus

Table 1 INCREASE IN FREQUENCY OF TRANSFORMED COLONIES ARISING FROM *BHK*21 CELLS EXPOSED TO POLYOMA VIRUS AFTER X-IRRADIATION OF THE CELLS

Dose of X-ray (r)	Normal colonies / Cells plated	(%)	Transformed colonies / Cells plated	(%)	Transformed colonies / Total colonies (*M*) (%)	Enhancement factor*
0	548/1,200	46	30/1,200	2 5	5 2	—
188	812/3,200	25	71/3,200	2 2	8 0	1 5
375	509/9,000	5 6	115/12,000	0 97	12 8	2 5
500	545/12,000	4 4	94/12,000	0 78	14 8	2 8

* Enhancement factor is ratio of transformed colony frequency (column 6) with irradiated cells compared with unirradiated cells

Table 2. INCREASE IN FREQUENCY OF TRANSFORMED COLONIES ARISING FROM IRRADIATED CELLS INFECTED IN MONOLAYERS TO AVOID CELL AGGREGATION

Dose of X-rays (r)	Unclumped cells (%)	Normal colonies / Cells plated	(%)	Transformed colonies / Cells plated	(%)	Transformed colonies / Total colonies (%)	Enhancement factor*
0	95	446/1,125	40	25/4,000	0.62	1.5	—
125	90	330/1,125	29	34/4,000	0.85	2.8	1.8
250	94	433/2,250	19	44/8,000	0.55	2.8	1.8
375	99	728/5,625	13	89/20,000	0.45	3.4	2.2
500	96	602/11,250	5.4	89/40,000	0.22	4.0	2.6

* See Table 1

infection increases the probability that the survivors will yield transformed clones.

Possible effect of cell aggregation. Both unirradiated and irradiated *BHK*21 cells in suspension are markedly aggregated by polyoma virus. For example, the cell suspension used for the assay shown in Table 1 contained about half the cells in clumps of two or more. Earlier experiments have shown that clumping does not affect the number or growth of transformed colonies, by comparison with colonies arising from single cells[4,9]. This is probably due to dispersal of the clumps after dilution of the infected suspension. It was still important to show that cell aggregation had no effect on the enhancement of the transformation after irradiation. For example, it was possible that a transformed cell in an aggregate with an untransformed cell might be more likely to form a transformed colony if the normal cell were previously damaged by irradiation.

Attempts to obtain single cell suspensions by sedimentation of aggregates after infection in suspension were unsuccessful. It was therefore decided to adsorb the virus to cells immobilized in monolayer cultures, and then to suspend the cells and assay for transformed colonies in the usual way. Preliminary experiments showed that cells suspended from monolayers immediately after adsorption of virus were still aggregated, perhaps because virus was still attached to the cell surface. However, if after adsorption the infected monolayers were left intact under medium for 3 h before suspension, it was possible to obtain predominantly single cell suspensions.

In terms of virus dose, transformation occurred less frequently when cells were infected as monolayers. In the technique finally adopted $1 \cdot 3 \times 10^9$ plaque-forming units in 0.2 ml of buffer were allowed to adsorb for 1 h at 37°, but this produced a lower transformation frequency than the standard exposure of cells in suspension to 10^9 plaque-forming units in 1 ml.

To determine the effect of irradiation on transformation using well-dispersed cells, separate monolayer cultures were exposed to increasing doses of X-irradiation, washed with buffer, and infected as described. The cells were then incubated in 5 ml of *ETC* medium for 3 h at 37°, suspended, and counted. The proportion of single cells ranged from 90 to 99 per cent and only two clumps with more than 2 cells were found. Table 2 gives the results of the assay. It will be seen that the increased transformation frequency was still observed with irradiated cells, even though the majority of cells were not aggregated.

*Effect of irradiated uninfected BHK*21 *cells on colony formation by transformed cells.* The increase in relative numbers of transformed colonies arising from irradiated cells did not necessarily mean that there was a real increase in the proportion of cells transformed. There might simply be a greater chance that transformed cells would form colonies when plated under these conditions after prior irradiation. Despite the presence of a large excess of irradiated mouse feeder cells in all assay plates, it was possible that addition of irradiated *BHK*21 cells might in some way enhance the growth of transformed cells.

To investigate this possibility, a standard transformation assay was carried out with 10^6 unirradiated *BHK*21 cells exposed to 10^9 plaque-forming units per ml of polyoma virus. 10^3 infected cells were then plated on irradiated mouse feeder layers, with and without the addition of five times the number of uninfected *BHK*21 cells irradiated with 750 r. Table 3 shows the results. About 5 per cent of the added irradiated cells survived, and this contributed to the increased total colonies counted in the assay with irradiated cells. It is clear that the presence of homologous irradiated cells did not alter the growth and colony formation by freshly transformed cells.

Table 3. EFFECT OF EXCESS IRRADIATED HAMSTER CELLS ON STANDARD POLYOMA TRANSFORMATION ASSAY USING UNIRRADIATED CELLS

No. of uninfected hamster cells irradiated (750 r.)	No. of infected hamster cells unirradiated	No. of colonies Total	No. of colonies Transformed	Transformed colonies / Total colonies (%)
Nil	1,000	407	24	5.9
5,000	1,000	458	22	4.8
10,000	nil	224	0	—

Conclusions. It will be seen from these results that the apparent frequency of transformation by polyoma virus is increased among cells which have survived X-irradiation. This effect might be due to an increase in the colony-forming efficiency of transformed cells rather than to an increase in the number of initial transformations. There was no evidence, however, that the experimental conditions following irradiation favoured the growth of transformed cells in the assay system, nor was the effect influenced by cell aggregation. It therefore seems likely that relatively more cells were actually transformed among the survivors after irradiation.

There is no direct evidence of polyoma virus multiplication in *BHK*21 cells, and even if new virus was produced by irradiated cells, the additional contribution would be unlikely to affect the transformation frequency by fresh infections. A satisfactory explanation of the phenomenon is yet to be found, but there are two main possibilities.

First, the population may be heterogeneous, and contain cells which are selected because they are genetically, or for temporary physiological reasons, more resistant to irradiation. These resistant cells may for some unknown reason also be more sensitive to polyoma transformation.

Secondly, it may be supposed that damage by X-irradiation directly causes an increase in sensitivity to the virus transformation. Such damage would have to be sub-lethal for the transformation to become manifest, unless one supposes that the virus can rescue otherwise lethally irradiated cells.

Whatever the explanation, if X-irradiation increases the susceptibility of cell populations to transformation by polyoma virus, it may also increase the sensitivity to other tumour viruses. If so, one factor in the carcinogenic effect of X-irradiation may be the increased probability that virus-induced tumours will arise in irradiated tissue. It will be desirable to test the effect of other carcinogens on cell susceptibility to tumour viruses.

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ADENOSINE TRIPHOSPHATE-DEPENDENT REDUCTION OF NICOTINAMIDE ADENINE DINUCLEOTIDE BY FERRO-CYTOCHROME *C* IN CHEMOAUTOTROPHIC BACTERIA

By Dr M. I. H. ALEEM and Dr H. LEES
Department of Microbiology, University of Manitoba, Winnipeg

AND

Dr D. J. D. NICHOLAS
Department of Chemical Microbiology, Long Ashton Research Station, University of Bristol

RECENT biochemical investigations with chemoautotrophic bacteria have been concerned with the mechanism of electron transfer[1-4], phosphorylation systems coupled to the oxidation of inorganic compounds[5-8] and with the fixation of carbon dioxide[9,10]. One of the metabolic problems remaining to be resolved is the source of reducing power required to drive the endergonic processes associated with the fixation of carbon dioxide. Since chemoautotrophic bacteria do not utilize sugars or organic acids as primary energy sources and do not use light energy as do photosynthetic organisms, there has been much speculation about the source of reducing power and how it is generated in these organisms. It is well known that reduced nicotinamide adenine nucleotides are required for fixation of carbon dioxide in photosynthesis, and recently adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH) were found to stimulate the incorporation of carbon dioxide into cell-free extracts of the nitrifying bacterium *Nitrobacter*[9]. Since the potentials of the compounds oxidized by chemoautotrophic bacteria are much more positive than the NAD/NADH couple, NO_2^-/NO_3^-, +0·421, $NH_4OH/NH_2OH.H$, +0·562, NH_2OH^+/NO_2^- +0·45; Fe^{2+}/Fe^{3+}, +0·77, NAD/NADH, −0·32; a mechanism which involves the reversal of electron transfer may be involved in reducing the nicotinamide adenine nucleotides although Gibbs and Schiff[11], in a recent review, do not favour this possibility. In this communication we show that NAD is reduced by ferro-cytochrome *c* in cell-free extracts of *Nitrobacter*, *Nitrosomonas* and *Ferrobacillus* provided ATP is added. The concept of an energy-dependent electron flow from succinate to NAD promoted by ATP or energy-rich precursors of ATP in animal mitochondria was first proposed by Chance and Hollunger[12]. Chance and Olson and Chance and Nishimura[13] suggested that a similar process would account for the light-stimulated NAD reduction in intact cells of purple bacteria. Bose and Gest[14] showed that the 'photoreduction' of NAD or fumarate by succinate and photo-evolution of hydrogen in *Rhodospirillum rubrum* was promoted by 'dark' oxidation-reduction reactions which were dependent on energy-rich intermediates produced by the interaction of light with the photo-chemical apparatus.

Cells of *Nitrosomonas europaea*, *Nitrobacter agilis* and *Ferrobacillus ferrooxidans* grown in batch cultures were collected in a Servall continuous flow centrifuge. Cells of *Nitrosomonas* or *Nitrobacter* were washed in 0·05 M

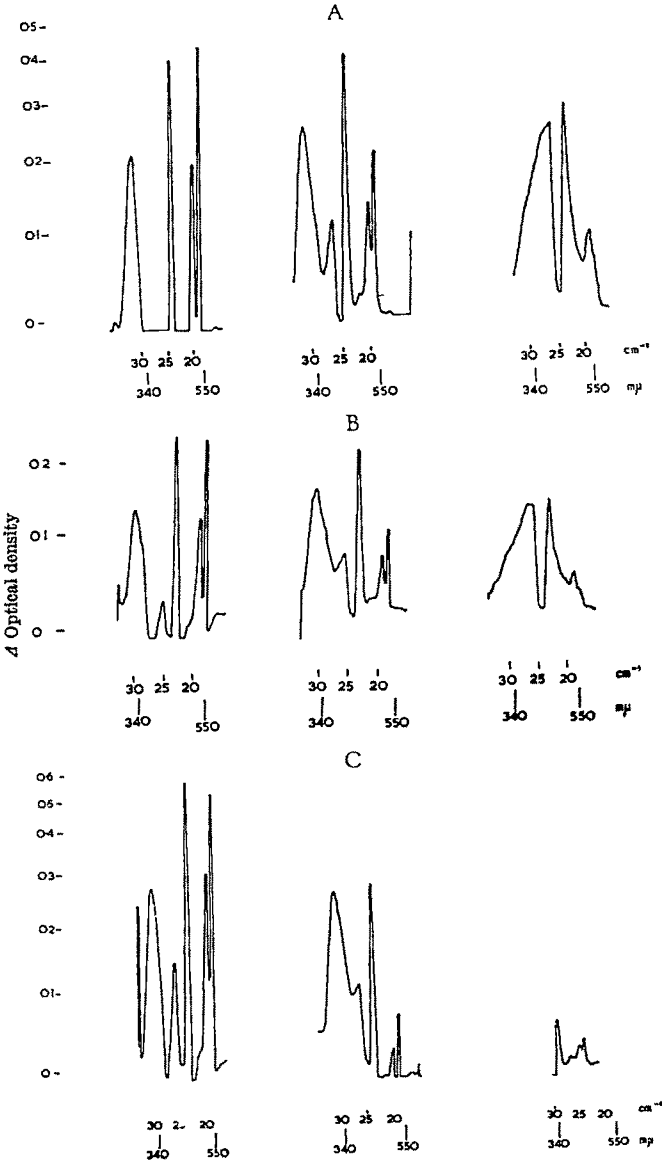


Fig. 1. Effect of adenosine triphosphate on the reduction of nicotinamide adenine dinucleotide by mammalian ferro-cytochrome *c* by cell-free extracts of *Nitrobacter* (fraction *a*). The results illustrate rapid scans, each taking 30 sec, of a difference spectrum over the range 30–25 cm⁻¹ or 300–600 mμ made in a Unicam *SP* 700 recording spectrophotometer. The reaction was followed under anaerobic conditions in special silica cuvettes (1 cm light path) fitted with Thunberg attachments. The reaction mixture contained 2 μmoles NAD, 0·05–0·1 ml. enzyme (preparation *a* or *b*) containing 0·5 mg protein/ml., in a final volume of 3 ml. 0·1 M *tris*–hydrochloric acid buffer *p*H 7·8. Adenosine triphosphate (40 μmoles in 0·2 ml. of the buffer) was added to the test cuvette only. Mammalian cytochrome *c* (horse heart, Sigma type III) was reduced with palladized asbestos and hydrogen, and 0·3 μmoles in 0·2 ml. *tris*–hydrochloric acid buffer was put in the sidearm of the Thunberg cuvette. After evacuating and gassing the cuvette with oxygen-free nitrogen (repeated three times), the reduced cytochrome *c* was tipped into the cuvette to start the reaction, which was then followed over a period of 10 min by the rapid scanning technique. The following enzymatic traps for NADH were also included in some of the reaction mixtures: alcohol dehydrogenase (0·1 mg crystalline preparation from yeast) and 0·1 ml. 1 per cent acetaldehyde (Fig. 1*B*) and lactic dehydrogenase (50 μg crystalline Sigma type I from rabbit muscle) and 5 μmoles sodium pyruvate (Fig. 1*C*). Time differences between the scans (left to right) are 1 and 2 min.

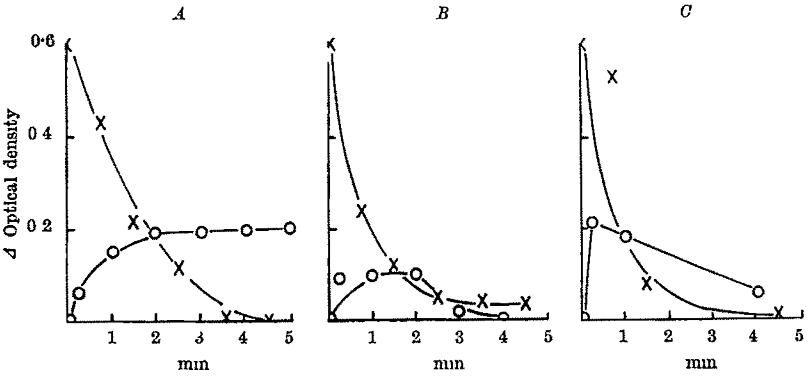


Fig. 2. Reduction of nicotinamide adenine dinucleotide (NAD at 340 mμ, O—O) by mammalian ferro cytochrome *c* (550 mμ, ×—×) in the presence of adenosine triphosphate (ATP) in cell-free extracts of *Nitrobacter* (fraction *a*). The results plotted from rapid scans, each taking 30 sec of a difference spectrum over the range 300–600 mμ in a Unicam *SP* 700 recording spectrophotometer. Details of reaction mixtures given in Fig. 1. Reaction mixtures in Fig. 2*A* as for Fig. 1*A*; in Fig. 2*B* as for Fig. 1*B* (containing the alcohol dehydrogenase trap), in Fig. 2*C* as for Fig. 1*C* (containing the lactic dehydrogenase trap)

tris-hydrochloric acid buffer (*p*H 7·5) and *Ferrobacillus* in de-ionized water adjusted to *p*H 2·5 with dilute sulphuric acid. Cell-free extracts were prepared by disrupting cells with a Mullard ultrasonic probe (20 kc/s) for 20 min at 0° and centrifuging at 10,000*g* for 30 min to remove cell debris. The supernatant fraction (*a*) was re-centrifuged at 144,000*g* for 1 h, resulting in a particle-free supernatant solution (*b*). Both fractions *a* and *b* were used as enzyme source.

The reduction of NAD (340 mμ) and the oxidation of reduced cytochrome (550 mμ) were followed by making a series of rapid scans, each taking about 30 sec, of a difference spectrum over the range 300–600 mμ using a Unicam *SP* 700 recording spectrophotometer. The reaction mixtures in 3 ml of 0·05 M *tris*-hydrochloric acid buffer (*p*H 7·8) contained 2 μmoles NAD, 0·1 ml 4 per cent mammalian cytochrome *c* (horse heart Sigma type III) reduced with palladized asbestos and hydrogen, in an apparatus designed by Walker and Nicholas[15], and 0·05–0·1 ml enzyme containing 0·5 mg protein/ml. Adenosine triphosphate (40 μmoles) was included in the test cuvette only. The reaction was started by adding reduced cytochrome *c* to both cells. The experiments were performed under anaerobic and aerobic conditions. Since the addition of natural substrates to the assay mixtures, for example, nitrite for *Nitrobacter*, hydroxylamine for *Nitrosomonas* and ferrous iron for *Ferrobacillus*, would result in the reduction of the cytochrome components by the respective cytochrome *c* reductases, measurement of any ATP dependent oxidation of ferro-cytochrome *c* would not be feasible. Moreover, these substrates also have an appreciable absorption in the 340 mμ region.

Since oxidized cytochrome *c* has a shoulder near 340 mμ which could be confused with the NADH peak, it was essential to obtain unequivocal evidence for NADH formation by other methods. This was achieved by using two independent trapping systems involving the enzymatic action of alcohol dehydrogenase and acetaldehyde and that of lactic dehydrogenase and pyruvate, both of which specifically oxidize NADH.

In this communication we illustrate results for extracts of *Nitrobacter* only since they are similar to those obtained with *Nitrosomonas* and *Ferrobacillus*.

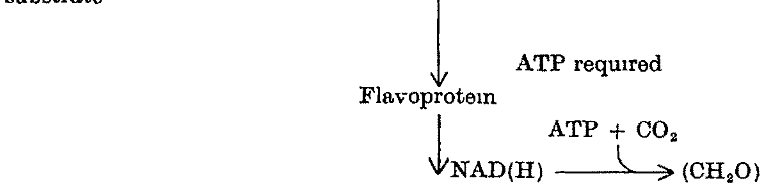
The results in Figs. 1 and 2 show that the reduction of NAD which is concomitant with the oxidation of ferro-cytochrome *c* is dependent on ATP. Within 1 min of starting the reaction there was a rapid formation of a compound absorbing at 340 mμ with a simultaneous oxidation of ferro-cytochrome *c* (Figs. 1*A* and 2*A*). That the compound absorbing at 340 mμ was NADH was confirmed by using the alcohol dehydrogenase (ADH) and the lactic dehydrogenase (LDH) traps (Figs. 1*B* and 2*B*, and 1*C* and 2*C*). The faster initial rate of production of NADH (maximum within 15 sec) and its subsequent rapid disappearance in the presence of the LDH trap is probably due to the differential behaviour of the ADH and the LDH traps. Whereas without a trap NADH formed and cytochrome *c* oxidized reached a steady state after 2 min, both were fully oxidized in the presence of the ADH or LDH systems. Inorganic phosphate was liberated from ATP during these reactions. NADP was a poor substitute for NAD in this system.

In the absence of either ATP or reduced cytochrome *c* no reduction of NAD could be demonstrated. Although in whole cells the theoretical ATP requirement for the reduction of NAD approximates to 4 : 1, in the cell-free systems, however, it is between 10 and 20 : 1. There was little enzymatic reduction of NAD when less than 5 μmoles ATP was used. The reduction of NAD by ferro-cytochrome *c* may involve flavin systems since atebrin (10^{-4} M) inhibited NADH formation and the addition of equimolar amounts of flavin mononucleotide (FMN) completely reversed this effect. Further evidence that flavins are involved in the electron flow was deduced from pronounced negative spectral changes in the region 375–385 mμ. Similar results were obtained with aged particles where presumably the link between flavin and NAD had been lost on storage or with fresh preparations using small amounts of ATP (< 10 μmoles).

It is concluded that the observed effects cannot be due to the differential behaviour of cytochrome oxidase in the two cuvettes since similar results were obtained under aerobic or anaerobic conditions. These effects were also observed in fraction *b* containing little of the oxidase, and it was established that ATP even at 40 μmoles did not depress cytochrome oxidase activity.

It has also been possible to demonstrate conclusively that the primary oxidation of an inorganic substrate catalysed by a chemoautotroph generates both the energy and the reducing power. Cell-free extracts from *Nitrobacter*, for example, when provided with nitrite, ADP and NAD, exhibited a marked and rapid stimulation in fixation of carbon dioxide. The carbon dioxide fixation rate dropped considerably on omission of any of the components, indicating thereby the dependence of the chemosynthetic carbon dioxide assimilating system on phosphorylation and the reduction of the pyridine nucleotide.

The following general scheme is proposed for the overall metabolism of chemoautotrophic bacteria, for example, *Nitrobacter*, *Nitrosomonas* and *Ferrobacillus*.



The reducing power required for the endergonic reduction of carbon dioxide in these organisms is generated by the reversal of electron transfer by ATP from ferro-cytochrome *c* to NAD. The unique ability of these bacteria to utilize ATP to reverse electron flow from electropositive to electronegative respiratory carriers is a physiologically important mechanism which is vital to their chemosynthetic mode of life

Experiments now in progress may elucidate whether the reduction of NAD results from a true reversal of electron transfer by the normal respiratory chain or whether other carriers are involved

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IN VIVO DESTRUCTION OF EXOGENOUSLY APPLIED INDOLYL-3-ACETIC ACID AS INFLUENCED BY NATURALLY OCCURRING PHENOLIC ACIDS

By Dr. M. H. ZENK and G. MÜLLER

Botanical Institute, University of Munich

THERE is quite a controversy in the literature as to the growth activity of certain naturally occurring phenolic acids While Vendrig and Buffel[1,2] have stated that caffeic acid may be a very important growth substance, there is another possibility, that the phenols may act *in vivo* as inhibitors or activators of the indolyl-3-acetic acid (IAA) oxidizing system[3,4], thus influencing growth This influence of phenols was clearly established by enzymatic tests *in vitro* The significance of the influence of caffeic acid and other phenolic acids *in vivo* is, however, not yet clearly established[2]

Since the *in vivo* action of the IAA-oxidizing system has been questioned, with regard to its being an artefact produced by cut-surfaces[5], we tried first to demonstrate the *in vivo* existence of this enzyme system Carboxyl labelled IAA (IAA*) (synthesized via a modified procedure of Thesing and Schulde[6] by lowering the concentration of ^{14}NaCN gramine derivative = 1 · 1 5 spec act 3 4 μc /μmole) was used as a substrate As a result of the action of the IAA-oxidase on the auxin, IAA is decarboxylated[7] and the resulting $^{14}CO_2$ is flushed, together with the respiratory carbon dioxide, out of the reaction vessel by means of carbon dioxide free air and then trapped in potassium hydroxide using a Vigreux-column Refixation of the carbon dioxide by the tissue did not occur Thus, kinetic measurements of the IAA-oxidase were possible Dark-grown *Avena* coleoptiles were used as plant material In order to obtain undamaged tissue, the coleoptiles were cut 2 mm below the node in the mesocotyl part of the seedling The cut surface was closed by dipping the remaining mesocotyl part into liquid collodium After drying, the collodium formed a film on the tissue which was impermeable to any aqueous solution

Fig 1 shows that if the tissue is placed in an IAA solution, decarboxylation occurs readily The increase in the rate of decarboxylation is linear after a lag-period of 3 h. Since there was no cut surface, the coleoptiles had to absorb the IAA by cuticular absorption Part of the IAA entering the tissue was destroyed by a naturally occurring IAA-oxidizing system The destruction of IAA is independent of a wound-effect

Thus having demonstrated the *in vivo* existence of an IAA oxidase system, we determined the influence of certain phenolic acids on this system If the interpretation of the auxin-sparing action of some of the cinnamic acids is correct, then we would expect after the addition of the synergistic phenolic acids[8] an inhibition of the decarboxylation reaction On the other hand, growth antagonists should result in an enhancement of the IAA oxidation

In order to obtain more consistent and comparable results, the apical 3 mm of the coleoptiles were discarded and three successive 3 3-mm sections were prepared without the primary leaf 1 g ± 2 mg of those segments were used for an experiment The tissue was shaken gently at constant temperature (21° C), and carbon dioxide was collected at 2-h intervals for 12 h Fig 2 shows the

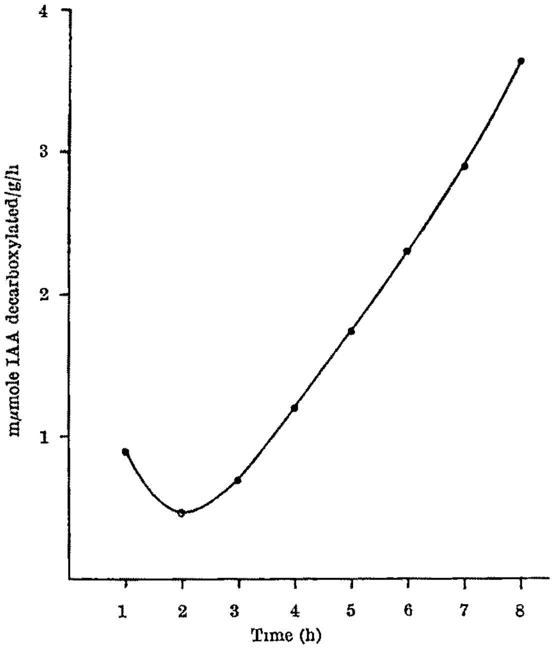

Fig 1 Kinetics of the decarboxylation of IAA* by intact *Avena* coleoptiles (var Victory), 2 cm long, with their cut ends in the mesocotyl part closed by the collodium film Incubation medium 12 5 ml 10^{-5} M IAA* (125 mμM) 10^{-3} M citrate phosphate buffer *p*H 5 0/g of coleoptiles Carbon dioxide was collected at hourly intervals and the radioactivity measured after correction for self-absorption and computed with the specific activity of the IAA used

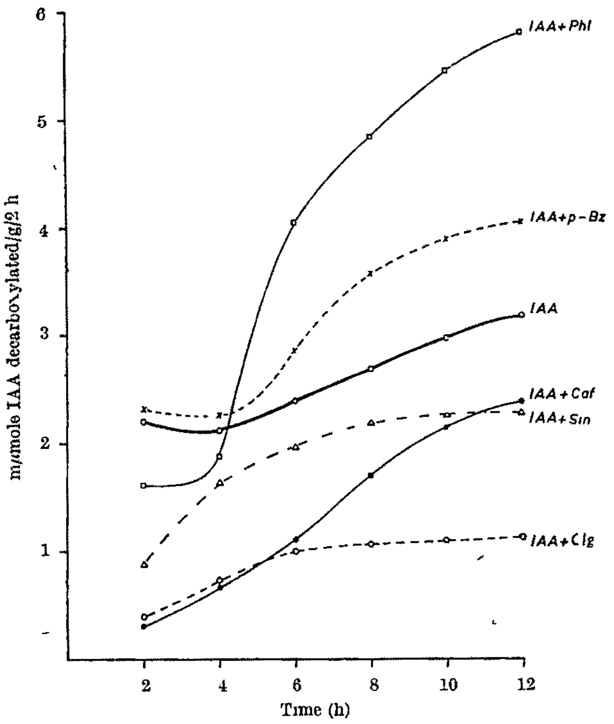

Fig 2 Kinetics of the decarboxylation of IAA* by 3-mm *Avena* coleoptile sections *in vivo* as influenced by phenolic acids Incubation medium 12·5 ml 10^{-5} M IAA (125 mμM), 10^{-3} M citrate phosphate buffer *p*H 5·0/g ± 2 mg coleoptile tissue and as indicated 10^{-4} M phenolic acids chlorogenic (Clg), sinapic (Sin), caffeic (Caf), *p*-hydroxybenzoic (*p*-Bz) and phloretic (Phl) acid The $^{14}CO_2$ was collected in 2-h intervals

influence of certain phenolic acids on the decarboxylation reaction. While the auxin synergists[6], caffeic, sinapic and chlorogenic acids actually inhibited the decarboxylation and therefore inhibited the inactivation of IAA, a slight enhancement of the *in vivo* destruction of IAA by the addition of *p*-hydroxybenzoic and phloretic acids was noticed

There are also reports of *p*-coumaric acid being a very effective cofactor of the IAA-oxidase *in vitro*[9], although *m*-coumaric and *o*-coumaric acid showed only negligible influence A drastic inhibiting influence of *p*-coumaric acid on IAA-induced growth could also be shown[4,8] Fig 3 demonstrates the influence of the isomeric coumaric acids on the decarboxylation of IAA by *Avena* coleoptile sections While *m*- and *o*-coumaric acid are only slightly active in promoting the decarboxylation, *p*-coumaric acid has an extremely stimulating effect on this reaction There is, starting with the fifth hour, a linear increase in the rate of decarboxylation of IAA The total amount of IAA decarboxylated per 2-h intervals increases logarithmically over the measured period of 12 h With IAA alone in the incubation fluid no such drastic increase of the rate of IAA destruction is observed *p*-Coumaric acid is known to stimulate *in vitro* the oxidation of IAA very markedly, we, however, do not feel that the action of *p*-coumaric acid is a matter of adapting the enzyme system which is responsible for IAA destruction *in vivo*[10] Thus, after the addition of Mn^{++} ions in a final concentration of 10^{-3} M to the incubation medium containing IAA and the coleoptile sections, the decarboxylation rate of IAA increases immediately 300 per cent over the IAA control without showing a lag period This suggests that the enzymes for IAA decarboxylation are already present, but not functioning at such a high turnover rate The increase in the decarboxylation rate induced by *p*-coumaric acid is as yet not clear

The influence of the concentration of *p*-coumaric acid on the decarboxylation during an 8-h period is shown in Fig 4 There is a maximum increase in the auxin destruction rate at 10^{-4} M Similar to the *in vitro* experiments, 0·1 μM *p*-coumaric acid/ml seems to be optimal also for auxin destruction *in vivo*

Finally, the influence of different phenolic acids on the uptake and decarboxylation of IAA during a 12-h period was investigated *in vivo* and compared with the effect

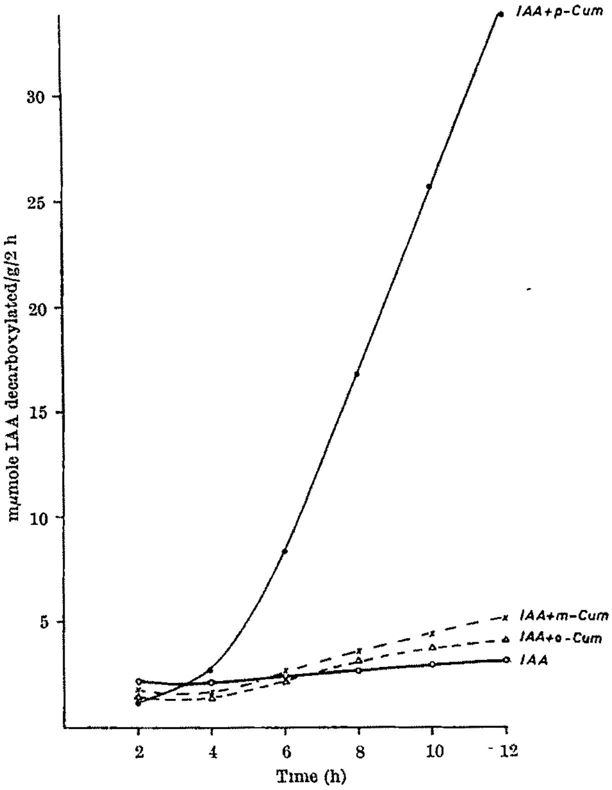

Fig 3 Kinetics of the decarboxylation of IAA* by 3-mm *Avena* coleoptile sections *in vivo* as influenced by 10^{-4} M coumaric acids Incubation medium as in Fig 2 *p*-Coumaric (*p*-Cum), *m*-coumaric (*m*-Cum) *o*-coumaric (*o*-Cum) acid

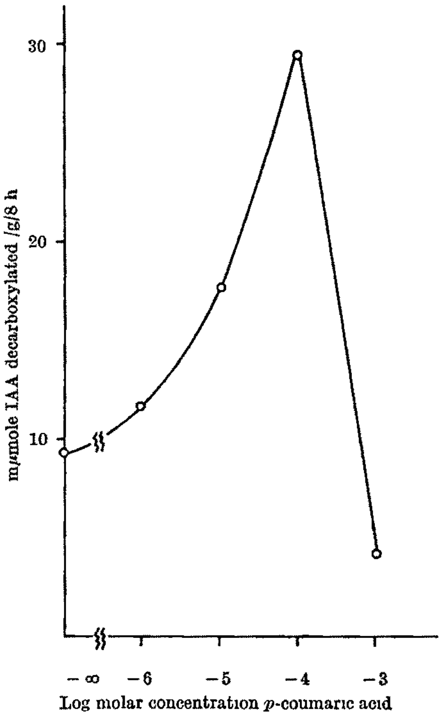

Fig 4. Decarboxylation of IAA* by 3-mm *Avena* coleoptile sections *in vivo* in the presence of various molar concentrations of *p*-coumaric acid Incubation medium 12·5 ml 10^{-5} M IAA (125 mμM), 10^{-3} M citrate phosphate buffer *p*H 5·0/g of coleoptile tissue and the indicated amount of *p*-coumaric acid

Table 1

IAA 10^{-5} M + various phenolic acids 10^{-4} M	Uptake mμmoles IAA/g/12 h	IAA control (%)	Decarbox mμmoles IAA/g/12 h	IAA control (%)	Enzymatic destruction of IAA in control (%)
IAA control	46.0	100	14.9	100	100
+ Chlorogenic acid	52.0	113	5.4	36	73
+ Caffeic acid	46.0	100	7.9	53	79
+ Dihydrocaffeic acid	41.5	90	10.4	69	88
+ Sinapic acid	46.2	100	11.2	75	83
+ *p*-Coumaric acid	32.0	69	90.6	608	181
+ *o*-Coumaric acid	39.6	87	16.4	109	104
+ *m*-Coumaric acid	45.3	98	19.5	131	117
+ Phloretic acid	36.8	80	23.2	155	188
+ *p*-OH Benzoic acid	37.0	80	18.9	127	113
+ Ferulic acid	45.6	99	21.0	141	126
+ Isoferulic acid	36.7	80	20.4	137	101
+ Cinnamic acid	39.9	86	14.1	94	100
+ Tyrosine	40.2	87	14.1	94	100

of the same acids on the IAA oxidase from peas[11]. The amount of IAA taken up by the tissue was measured after the end of the 12-h incubation by extracting the coleoptiles with hot ethanol and determining the radioactivity in an aliquot of the alcoholic solution[12].

According to Table 1 the tested phenols can be divided into three groups. The synergistic acids (chlorogenic, caffeic, dihydro-caffeic, and sinapic) which inhibited the decarboxylation of IAA, thus clearly having an auxin-sparing influence. Secondly, the growth-inhibiting phenols (*p*-coumaric, phloretic, ferulic, isoferulic, *m*-coumaric, *p*-hydroxybenzoic, *o*-coumaric acids). Those acids increase the IAA decarboxylation, depriving the tissue of auxin, and thus retarding growth. Thirdly, acids which influence neither growth nor decarboxylation (cinnamic acid and tyrosine). Gibberellic acid is in contrast to *in vitro* experiments[13] without any influence on IAA decarboxylation *in vivo*. The auxin-sparing action of the synergistic phenols is also reflected in the higher amount of IAA taken up by the coleoptiles. Tissue in the presence of *p*-coumaric acid, on the other hand, shows the least uptake of IAA.

The influence of the various phenolic acids on the *in vivo* decarboxylation is closely resembled by the *in vitro* destruction of IAA by an enzyme system from peas. There is, however, an interesting exception. We are able to confirm the report[9] that phloretic acid enhances the enzymatic oxidation of IAA drastically *in vitro*; however, there was only a slight effect on the *in vivo* system compared with *p*-coumaric acid. In the case of caffeic acid, the dihydro form is about 20 per cent less effective in inhibiting the decarboxylation of IAA than caffeic acid itself. We have observed that the phenolic acids taken up by the coleoptiles are converted in the corresponding glucose esters, which occur widely in Nature[14,15]. Those esters are still effective as modifiers of the IAA oxidase. It could be possible that the dihydrocinnamic acids are partly metabolized in a different way, thus showing less influence on the oxidation of IAA *in vivo*, or reaching only a lower effective concentration in the tissue than the corresponding cinnamic acids. Generally, however, the same structural requirements for the cinnamic acids to influence growth[4,8] and the IAA oxidase *in vitro*[9] and *in vivo* hold true. We feel, therefore, that it is now clearly established by means of *in vivo* experiments that the phenolic synergists and phenolic inhibitors of growth act through inhibition or activation of the IAA-oxidizing system.

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SOME EFFECTS OF ADRENALINE AND ANTI-ADRENALINE COMPOUNDS ON PLATELETS *IN VITRO* AND *IN VIVO*

By DR. J. R. O'BRIEN

Portsmouth and Isle of Wight Area Pathological Service, Central Laboratory, Portsmouth

It has often been suggested that 'stress' may release noradrenaline into the blood stream and that 'stress' may precipitate arterial thrombosis. Platelet-to-platelet adhesion, that is platelet aggregation, plays a part in thrombus formation and in hæmostasis of small blood vessels as, for example, in the Borchgrevink bleeding time[1]. In 1941 Derouaux[2] showed that in some circumstances the catecholamines may shorten the bleeding time, and recently Mitchell and Sharp[3,4] have shown that noradrenaline causes platelet aggregation *in vitro*, while Mustard[5] has shown that adrenaline shortens the half-life of platelets in the pig. These observations may be inter-related and suggested a detailed examination of the effect of the catecholamines on platelets.

Fresh citrated human platelet-rich plasma is placed in a magnetically stirred cuvette in a colorimeter at 37° C and the light transmission is continuously recorded. An increase in transmission occurs when the platelets aggregate and a decrease occurs if the platelet clumps come apart—disaggregation[6]. The addition of adrenaline acid tartrate to give a final concentration of M × 10^{-6} causes immediate partial aggregation followed by a lag phase when slight disaggregation may occur. This is followed by rapid and total aggregation. Inspection of a family of curves obtained when different doses of adrenaline were added (Fig. 1) shows that the slope of the initial aggregation is linearly related to the log of the dose. The time after the addition of adrenaline to the beginning of the lag phase, that is to the first inflexion in the curve, decreases slightly with higher concentrations. The length of the lag phase is also dependent on the dose, but the final aggregation is independent of the initiating concentration and is always rapid and complete. Exactly similar curves are obtained by adding noradrenaline, which is ten-fold less active. Isoprenaline is inactive.

Adenosine and adenosine monophosphate (AMP) have been shown to inhibit aggregation caused by adenosine diphosphate (ADP), thrombin, 5-hydroxytryptamine (5-HT) and they delay the effects of tri-ethyl tin[6,7]. These two inhibitory compounds at a final strength of

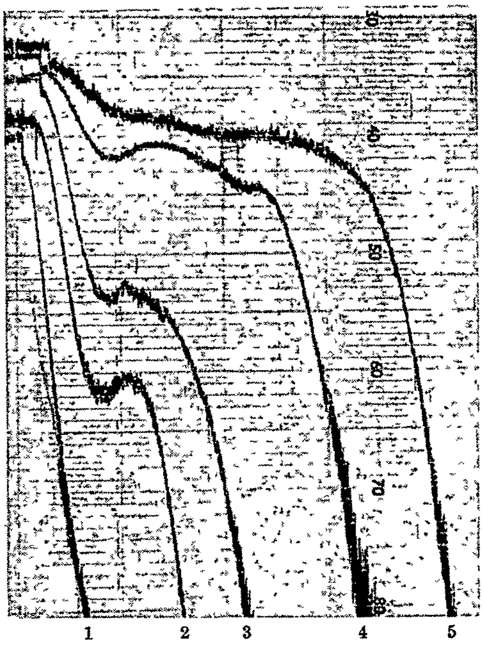

Fig 1 Light transmission is recorded on the ordinate, a decrease indicating aggregation The abscissa records time, one major division represents 2 min Adrenaline in 0 1 ml saline was added after the control (horizontal) period to give the following final concentrations curve 1, 5 M × 10^{-6}, curve 2, M × 10^{-6}, curve 3, 5 M × 10^{-7}, curve 4, 2 M × 10^{-7}, curve 5, M × 10^{-7}

5 M × 10^{-5} added before adrenaline 2 M × 10^{-6} inhibit aggregation added during the lag phase they will still inhibit the second phase of aggregation Adenosine triphosphate (ATP) inhibits ADP-induced aggregation but has no effect or enhances aggregation caused by thrombin or 5-HT or by tri-ethyl tin[7] ATP M × 10^{-6} added before a small dose of adrenaline or noradrenaline causes an increase in the degree of aggregation in the first phase but has little effect on the subsequent stages A much higher concentration of ATP added before the adrenaline may modify or block the second phase of aggregation

If phentolamine ('Rogitine'), which is an inhibitor of other pharmacological actions of adrenaline, is added in equimolar concentrations before the addition of adrenaline it completely inhibits aggregation and this inhibition is apparently competitive since a ten-fold increase in adrenaline requires a ten-fold increase in phentolamine to cause an equal degree of inhibition Another anti-adrenaline compound, dibenyline and the anti-serotonins '*Ro* 3/0837' and, '*UML* 491' have no effect on adrenaline-induced aggregation except at a relatively high concentration (10 µg/ml)

The interpretation of these findings is difficult particularly because we do not know the nature of the alteration in the platelet surface when it becomes more 'sticky' and because more than one kind of alteration may result in a similar degree of stickiness Adrenaline and noradrenaline initiate two waves of aggregation which can be separated and have other differences, yet both waves are inhibited by the same compounds All these findings are compatible with the hypothesis that adrenaline causes platelet ADP probably derived from ATP to be liberated and that this release is concerned with the 'stickiness'. Indeed, Spaet[8] has recently shown spectroscopically that nucleotides are in fact liberated It is remarkable that noradrenaline, 5-HT and the adenosine phosphates are all normally present in the platelets and the addition to the plasma of each of these substances causes aggregation, although these reactions when examined in detail show important differences On the other hand, thrombin and tri-ethyl tin which also cause aggregation are not present in the platelet Many problems of kinetics biochemistry and physical chemistry are raised but not answered by these observations

Table 1 COMPOUNDS INDUCING PLATELET AGGREGATION AND THEIR INHIBITORS

Aggregating compound	Concentration	Specific inhibitor
Adenosine diphosphate	M × 10^{-8}	Adenosine*
5-Hydroxytryptamine	5 M × 10^{-6}	'*UML* 491' ('Deseryl')
Adrenaline	M × 10^{-7}	Phentolamine ('Rogitine')
Noradrenaline	5 M × 10^{-7}	
Thrombin	0 06 U/ml	Heparin

* Adenosine inhibits aggregation produced by all these aggregating compounds

Any of the physiological substances shown *in vitro* to produce aggregation might be involved *in vivo* This problem could be investigated since each aggregating compound has *in vitro* a specific competitive inhibitory compound(s), which has virtually no effect on aggregation induced by other compounds (Table 1) Accordingly, these specific inhibitors and some other compounds dissolved in 0 1 ml of saline were injected intradermally into the human forearm and the Borchgrevink bleeding time was estimated from cuts made through the injected areas From Table 2 it will be seen that only phentolamine caused prolongation of the bleeding time Erythema and local œdema were produced by phentolamine and to an even greater extent by histamine and hyaluronidase, but neither of the last two injections prolonged the bleeding time Accordingly, the prolongation of the bleeding time by phentolamine suggests that it may specifically inhibit adrenaline or noradrenaline, and, therefore, that these catecholamines may normally be responsible for platelet adhesion or aggregation in these circumstances The other compounds studied did not prolong the bleeding time, nor does heparin unless it is present at a very high concentration[9] Thus these experiments also suggest that ADP (other than that liberated from adrenaline) and thrombin and 5-HT do not play a major part in capillary hæmostasis

Table 2 EFFECT OF INHIBITORS, ETC, ON THE BLEEDING TIME

Material injected	Amount injected	No of observations	Mean bleeding time (min)
Saline		8	3 5
Adenosine	0 25 mg	4	4 5
'*UML* 491'	0 1 mg	4	3 95
Phentolamine	0 5 mg	8	>12 5
Phentolamine	0 05 mg	4	5 5
Dibenyline	0 3 mg	2	4 2
Histamine	0 05 mg	12	3 75
Hyaluronidase	30 I U	2	2 7

Table 3 SOME EFFECTS OF CATECHOLAMINES AND THEIR INHIBITORS

	Smooth muscle stimulated	Platelet aggregation *in vitro*	Bleeding time prolongation
Adrenaline	Yes	Yes	—
Noradrenaline	Weaker	Weaker	—
Isoprenaline	No	No	—
Adrenaline effect inhibited by			
Phentolamine	Yes	Yes	Yes
Dibenyline	No	No*	No

* Except at a high concentration

The results presented have no direct bearing on thrombosis circumstantially and indirectly they suggest that the local release of adrenaline or noradrenaline may activate the hæmostatic mechanism perhaps by liberating ADP The remarkable parallelism of the effects of the catecholamines and their inhibitors in three very different situations, namely, the nictitating membrane of the cat *in vivo*, platelet aggregation *in vitro* and the bleeding time (Table 3) might suggest that some basic common process is involved

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LETTERS TO THE EDITOR

RADIOASTRONOMY

Faraday Rotation Effect in Extragalactic Radio Sources

Cooper and Price[1] have shown that the polarization of radio emission measured at three different points in the radio source Centaurus *A* rotated progressively with increasing wave-length They found the rate of rotation to be proportional to the square of the wave-length This fact suggested to Cooper and Price a direct explanation in terms of Faraday rotation in a magneto-ionic medium. Recently, Gardner and Whiteoak[2] measured the polarization of 18 radio sources at different wave-lengths in order to investigate whether Faraday rotation was present All the sources showed the quadratic dependence of polarization position-angle on wave-length characteristic of Faraday effect In the discussion of their results, Gardner and Whiteoak suggested the possibility that some of the Faraday rotation occurs in our Galaxy (Fig 1 in ref 2)

The Faraday rotation θ of the plane of polarization of radio radiation from an extragalactic source at a wave-length λ can be expressed as a sum of four different terms

$$\text{const}\,\frac{\theta}{\lambda^2}=\underbrace{\int N_{e1}H_1\mathrm{d}r}_{\text{ionosphere}}+\underbrace{\int N_{e2}H_2\mathrm{d}r}_{\text{galaxy}}+\underbrace{\int N_{e3}H_3\mathrm{d}r}_{\text{intergalactic space}}+\underbrace{\int N_{e4}H_4\mathrm{d}r}_{\text{radio source}} \quad (1)$$

each of which contributes to the integral effect observed The symbols N_{ek} and H_k stand for electron density and longitudinal magnetic field When θ is measured in radians, λ in metres, N_e in particles/c c , H in gauss and r in parsecs, the constant has the value 0 124 × 10^{-5} According to results obtained by Evans[3], the first integral is nearly two orders of magnitude smaller than the observed value of the left-hand term in equation (1), therefore the ionospheric contribution to the Faraday rotation effect in radio source radiation is negligible Also, the third term in equation (1) appears to be negligible, because although the path of radiation through intergalactic space is very long there is no evidence of sufficiently high magnetic fields and electron densities This point of view seems to be supported by the lack of correlation between the Faraday rotation measure and the distance of the radio sources, although such a correlation might not occur if the intergalactic magnetic fields were not sufficiently regular Hence the majority of the rotation probably occurs either within our own Galaxy or within the radio source itself

Gardner and Whiteoak[2] discussed the possibility of a galactic origin for the main part of the Faraday rotation Although they showed that sources with the smallest rotations are at high galactic latitudes, the considerable scatter in both magnitude and sign of the rotation at intermediate latitudes makes the galactic origin of the main part of Faraday rotation less probable

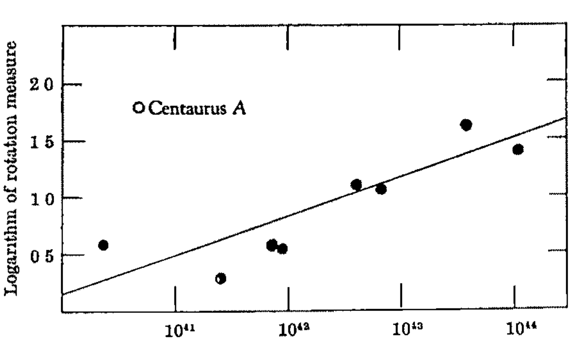

Fig 1 Total luminosity versus Faraday rotation measure for extragalactic radio sources

Let us examine in more detail the hypothesis that Faraday rotation is produced mainly within the source itself Under the condition of minimum total energy of a radio source radiating by means of an acceleration-radiation mechanism, the prevailing magnetic field is

$$H\propto\psi^{-2/7}L^{2/7}r^{-6/7} \quad (2)$$

where L is the total radio luminosity, ψ is a 'filling factor' determining the portion of the source's volume occupied by filaments and knots of the magnetic field and relativistic particles trapped in it, and r is the radius of the emitting region[4]

The Faraday rotation measure depends on r and H through the relation

$$\lambda^{-2}\theta\propto N_eHr \quad (3)$$

For the purpose of correlation testing, it is reasonable to assume that the magnetic field in expression (2) and the longitudinal magnetic field in expression (3) are comparable Therefore, on the basis of the order-of-magnitude relation

$$L\propto\psi H^{\frac{1}{2}}N_e^{-3}\,(\lambda^{-2}\theta)^3 \quad (4)$$

we can expect a correlation between radio luminosity L and rotation measure $\lambda^{-2}\theta$, if the rotation is produced mainly in a radio source itself

The correlation between total radio luminosity L and rotation measure $\lambda^{-2}\theta$ for extragalactic radio sources is shown in double logarithmic scale in Fig 1 The polarization data were given by Gardner and Whiteoak[2] and the values of total luminosities by Maltby, Matthews and Moffet[4] The points in Fig 1 represent those extragalactic radio sources from Gardner and Whiteoak's list for which the distance is known The full line represents the expected relation between total luminosity and rotation measure given by (4), provided the factor $\psi H^{\frac{1}{2}}N^{-3}$ does not change considerably The line has a slope equal to 0 33

The open circle in Fig 1 corresponds to the values of L and $\lambda^{-2}\theta$ for the central source of Centaurus *A* As is well known, the central source of Centaurus *A* is immersed in a large radiation halo of complex structure It is very probable that polarized radiation from the central source, passing through the extended halo region, will be subject to additional Faraday rotation, the amount of which may be very large because of the size of the extended source and the magnitude of possible magnetic fields (6 × 10^{-6} gauss (ref 4)) This could explain the deviation of the open circle in Fig 1

In so far as it is possible to draw conclusions on the basis of only eight points, the correlation expressed in Fig 1 strongly suggests that the Faraday effect occurs mainly within radio sources, not in the Galaxy or in intergalactic space Furthermore, the rather small scatter of points in Fig 1 indicates that the factor $\psi H^{\frac{1}{2}}N^{-3}$ does not vary greatly from one radio source to another

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A G PACHOLCZYK *

Harvard College Observatory,
Cambridge, Mass

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* On leave of absence from the Warsaw University Observatory and the Astronomical Institute of the Polish Academy of Sciences

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Occultation of the Crab Nebula by the Solar Corona in June 1963

In a recent communication, Basu and Castelli[1] described observations at wave-lengths of 10 cm and 25 cm of the occultation of the radio source in Taurus, the Crab Nebula, by the solar corona in June 1962. The results were interpreted as indicating a progressive increase in the angular diameter of the source with decreasing angular distance from the Sun, due to scattering by irregularities of electron density in a non-uniform corona.

Several observers have demonstrated that such an effect occurs at metre wave-lengths, for example, Hewish[2,3] and Vitkevitch[4] have shown that, over the range of wave-lengths 1·9–7·9 m, and for radial distances less than 12 solar radii, the results are in accord with a simple scattering theory in which the angular extent of the scattered distribution (defined as the brightness distribution produced when a point source is viewed through the corona) is proportional to the square of the wave-length. Assuming this dependence on wave-length to hold down to centimetre wave-lengths, and extrapolating from the metre observations of Hewish, values ~2″ at 10 cm and ~10″ at 25 cm are derived for the expected angular diameter (between e^{-1} points) of the Gaussian scattered distribution at distances ~5 solar radii from the Sun. From their observations Basu and Castelli inferred angular diameters of 20′ at 10 cm and 25′ at 25 cm, which therefore cannot be accounted for by the simple theory.

In view of this discrepancy, observations of the Crab Nebula were undertaken in June 1963 using the twin 90-ft. dishes of the California Institute of Technology's Owens Valley Radio Observatory. The two antennæ were used as an east–west interferometer of spacing 680λ operating at a frequency of 1,660 Mc/s (18 cm wave-length). Any increase in angular diameter of the source, observed with the interferometer, results in a decrease in the recorded source response which may be interpreted directly in terms of the angular extent of the scattered distribution. With the arrangement described here, the quiet Sun was largely resolved and gave a contribution to the total response which was only a fraction of the response due to the source itself. In the absence of an appreciable solar contribution it was possible to detect scattering angles as small as 0·7′. The source was observed daily at several different hour angles during the period June 11–19, conjunction occurring on June 14–15 (Fig. 1). In order to allow for the solar contribution, additional observations of the response were made each day with the antennæ directed towards the positions which the source took relative to the Sun on each of the occulting days. The contribution was at all times less than 15 per cent except on June 15 when the solar response was 25 per cent of that due to the source. As the angular separation of source and Sun decreased, the gain was decreased by measured amounts (always less than 0·4 db) to preserve the same detector levels in the presence of solar noise. The response of the source 3C 123 was observed each day and used to correct for small changes in receiver sensitivity.

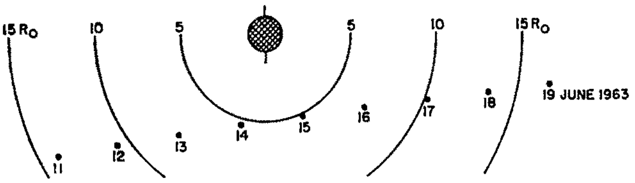

Fig. 1. Position of the Crab Nebula relative to the Sun at 12 noon P.S.T. $R_{\odot}$, Solar radius

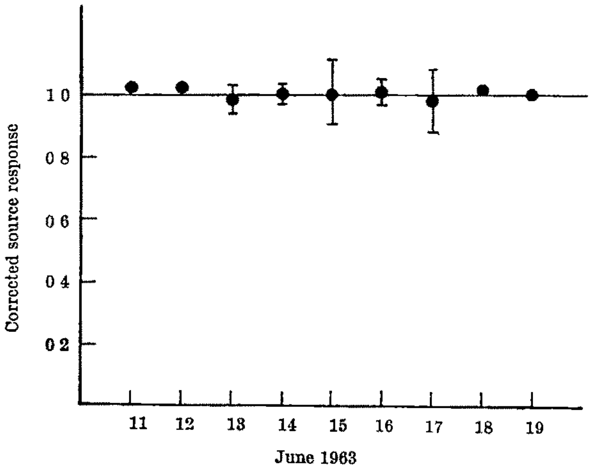

Fig. 2. Corrected response of the Crab Nebula. The error limits indicate uncertainties in removing the solar contribution. Results for hour angles ± 1 h have been combined in this figure

When the foregoing factors were taken into account in the analysis, no positive evidence for a variation in the source response, corresponding to an increase in angular diameter, was found (Fig. 2). The corrected source response remained constant to within 12 per cent, enabling an upper limit of 1·2′ to be set to the angular diameter of the scattered distribution in the east–west direction. The observations recorded here, therefore, give no evidence for the existence of enhanced scattering at centimetre wave-lengths which cannot be accounted for by the simple theory applicable at metre wave-lengths.

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J. D. WYNDHAM
B. G. CLARK

Owens Valley Radio Observatory,
California Institute of Technology,
Pasadena

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PHYSICS

Charge Transfer and Excimer Formation

The general properties of excited dimers, or excimers, have been well established[1-4]. In concentrated solutions of some polycyclic aromatic hydrocarbons, a singlet excited molecule can interact with an unexcited neighbour to produce the excited dimer, or excimer. This excimer can then break up into de-excited monomer molecules with the emission of a characteristic dimer fluorescence. The forces binding the dimer have been ascribed by Förster[5] to resonance dipole interaction. The criterion has been given for excimer formation that the molecule should possess a weak ^1L_b level lying beneath a strong ^1L_a level. Most of the excimer-forming molecules obey this criterion, a notable exception being anthanthrene.

It has been alternatively suggested[6] that the binding of the dimer may arise from charge transfer interaction in which one molecule donates charge to its partner or shares charge with its partner. In this picture the dimers behave like charge-transfer complexes and the excimer emission band is a charge-transfer band. It has been well established[7-9] that the frequency ν_{CT} of the charge transfer band is given to a first approximation by the relation:

$$h\nu_{CT} = I - E_A - \Delta \qquad (1)$$

when I is the ionization potential of the electron donor, E_A is the electron affinity of the electron acceptor and Δ represents the various other interaction terms, including resonance dipole interaction, which are assumed small. If the excimer emission is to be explained in terms of charge transfer one would expect a similar relationship between the frequency ν_D of the excimer emission, taken here as the frequency of strongest emission, and other molecular parameters, thus

$$h\nu_D = (I - S_1) - E_A - \Delta$$

The ionization potential of the excited molecule is $I - S_1$. S_1 is taken as ^1L_b.

In Table 1 are presented the ionization potentials, first singlet excited states (^1L_b) and electron affinities of 20 polycyclic aromatic hydrocarbons. Also presented in Table 1 are the energies of the excimer emission bands of those hydrocarbons forming dimers. The ionization potentials and electron affinities in Table 1 are those of the molecules in the vapour state. In solution they will be greatly reduced and increased respectively due to the polarization forces of the solvent. To a first approximation, the solvent can be assumed to have a similar effect on the energy-levels of all the molecules.

Table 1. ENERGIES OF POLYCYCLIC AROMATIC HYDROCARBON (ENERGIES IN EV)

	I*	^1L_b†	$I-S_1$	E_A*	$h\nu_D$	
Benzene	9.21	4.71	4.50	−0.43	3.68	‡
Naphthalene	8.10	3.88	4.22	0.68	3.10	§
Anthracene	7.37	3.32	4.05	1.41	—	ref. 3
Tetracene	7.00	2.63	4.37	1.78	—	ref. 3
Phenanthrene	8.09	3.58	4.51	0.69	—	ref. 3
1 2 Benzanthracene	7.45	3.22	4.23	1.33	2.43	ref. 2
Chrysene	7.80	3.44	4.38	0.98	—	ref. 3
Pyrene	7.55	3.34	4.21	1.23	2.57	ref. 2
Triphenylene	8.09	3.54	4.55	0.69	—	ref. 3
1 2 5 6 Dibenzanthracene	7.80	3.14	4.66	0.98	—	ref. 3
3 4 Benzpyrene	7.19	3.08	4.11	1.59	2.41	ref. 2
1 2 7 8 Dibenzpyrene	7.68	3.14	4.54	1.10	—	ref. 3
1 2 Benzpyrene	7.56	3.22	4.34	1.22	2.65	ref. 2
1 2 3 4 Dibenzanthracene	7.61	3.30	4.31	1.17	2.55	ref. 3
Picene	7.80	3.30	4.50	0.98	—	ref. 3
Perylene	7.03	2.86	4.18	1.75	2.00	ref. 2
1 2 4 5 Dibenzpyrene	7.27	3.08	4.29	1.51	2.54	ref. 3
Coronene	7.44	2.88	4.56	1.34	—	ref. 3
Anthanthrene	7.00	2.86	4.14	1.78	2.08	ref. 2
1 12 Benzperylene	7.35	3.04	4.31	1.43	2.46	ref. 3

* Slifkin, M. A., *Nature* (in the press).

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In Table 1 it is seen that those molecules which form dimers in solution at room temperature have $I - S_1$ less than 4.36 eV. All molecules having $I - S_1$ greater than 4.36 eV do not form dimers at room temperature. Anthracene, which forms a very stable dimer, has the very low $I - S_1$ value of 4.05 eV.

In Fig. 1 is shown a plot of $h\nu_D$ against $I - S_1 - E_A$. This is seen to give a good straight line with the exception of 3 4 benzpyrene, whose $I - S_1 - E_A$ value is 0.3 eV too low. Δ has been neglected and it is suggested that Δ does not vary greatly in this class of compounds. This correlation strongly suggests that the excimer emission band can be interpreted as a charge transfer band.

The meaning of the $I - S_1$ correlation is none too clear. The correlation would tend to suggest that possible charge transfer occurs as a two-stage process, the first stage involving ionization with perhaps the solvent acting as an intermediate.

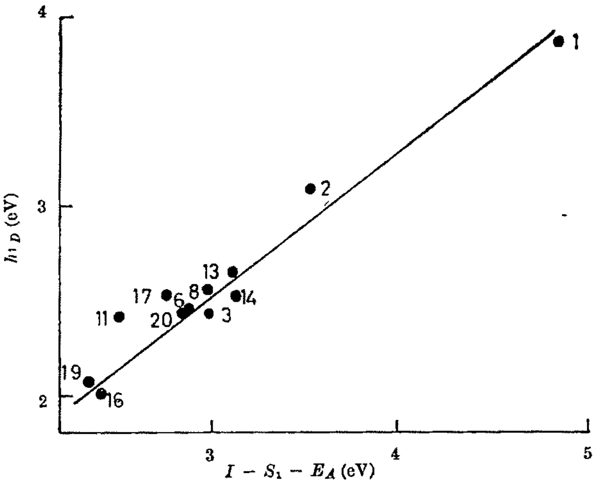

Fig. 1. Plot of $h\nu_D$ versus $I - S_1 - E_A$

I thank Dr. J. B. Birks and Dr. F. H. Read for useful discussions. I also thank Dr. J. B. Birks and Mr. J. Aladekomo for the naphthalene result.

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M. A. SLIFKIN*

Physical Laboratories
University of Manchester

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* Present address: Department of Physics, Rutherford College of Technology, Newcastle upon Tyne 1.

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Measurement of Ion Diffusion Coefficient in Liquid *n*-Hexane

By a method elaborated by me the diffusion coefficients of both negative and positive ions have been measured in liquid *n*-hexane at 25° C (ref. 1). This method is based on that of diffusion from a body of limited dimensions with boundary conditions. One-sign ions were introduced to the measuring chamber designed as a parallel plate condenser and then time decrease of ion number caused by diffusion was examined. Experimental conditions were chosen so that the influence of Coulomb repulsion of one-sign ions could be neglected.

The diffusion x coefficient was evaluated from the formula

$$D = \frac{(\ln Q_1 - \ln Q_2)\, l^2}{\pi^2 (t_2 - t_1)} \qquad (1)$$

where Q_1, Q_2 = electric charge measured for two measuring times t_1 and t_2, l = electrode distance.

Figs. 1 and 2 show the dependence $\ln Q = f\,t$ for both positive and negative ions in *n*-hexane. The coefficients of diffusion calculated from this dependence were $D_- = (2.75 \pm 0.22) \times 10^{-5}$ cm²/sec., $D_+ = (1.43 \pm 0.11) \times 10^{-5}$ cm²/sec. Hitherto there was no experimental method of direct measurement of diffusion coefficient of ions of one sign in dielectric liquids. The diffusion coefficient was calculated from Nernst–Einstein formula

$$\frac{u}{D} = \frac{e}{kT} \qquad (2)$$

where u = ion mobility, D = ion diffusion coefficient; k = Boltzmann constant; T = absolute temperature.

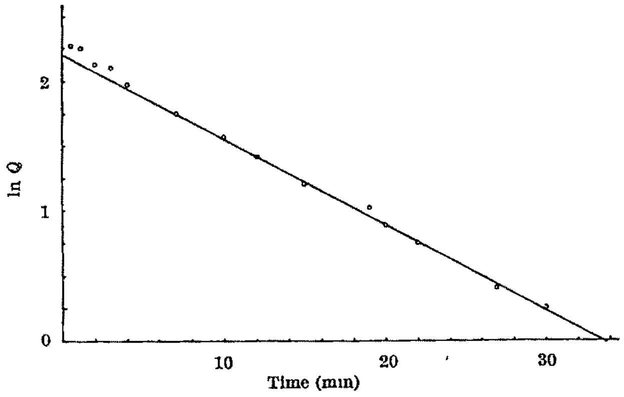

Fig. 1. Time decrease of the positive ions No. Electrode distance l = 3.6 mm

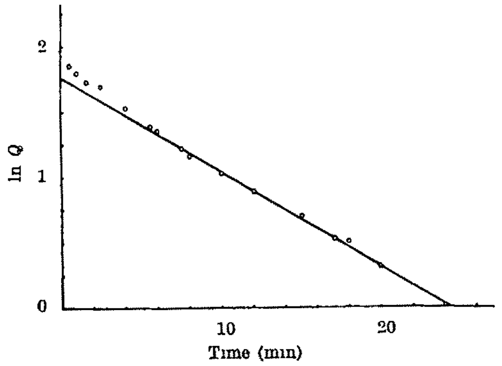

Fig 2. Time decrease of the negative ions No Electrode distance $l = 4\ 8$ mm

At 25° C the ratio $e/kT = 39$ V^{-1} and for results obtained by me $u_-/D_- = 34\ 5$ V^{-1}, $u_+/D_+ = 42\ 5$ V^{-1}

This ratio was calculated by the use of ion mobility values obtained by O Gzowski[2] I have also carried out the measurements of ion mobility using the method described in ref 3, and obtained the same values as in work in ref 2 Thus, within experimental error, the law of Nernst–Einstein is well confirmed for both positive and negative ions in *n*-hexane That conclusion is important, because it shows the difference between the thermal motion of molecules having uncompensated electric charge and the motion of neutral molecules

The measurements of self-diffusion coefficient carried out with different methods give[4] for it $D_0 = 4\ 2 \times 10^{-5}$ cm^2/sec In the work of Davis, Rice *et al* [5,6] the problem of such difference is discussed The ratio of self-diffusion to diffusion coefficient of positive ions evaluated by these authors was 3 – 5 for different liquids Ar$^+_2$, Kr$^+_2$, Xe$^+_2$

I thank Prof I Adamczewski for advice

E. GAZDA

Department of Physics,
Technological Institute,
Gdańsk,
Poland

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Faraday Effect Ellipsometer

T P Murray[1] described the measurement by ellipsometry of the thickness of oil films present on commercial tinplate In his arrangement the elliptically polarized light reflected from the specimen surface was brought into a plane by means of a quarter-wave plate and the extinction position of the analyser found After de-oiling the specimen a new extinction position was found by adjustment of the analyser only, the difference being proportional to oil film thickness Angular shifts of a few tenths of a degree are common whereas the light intensity varies only slowly for several degrees about the minimum Murray overcame this difficulty by reading the analyser for equal photoelectric responses either side of the minimum, but it occurred to me that a more elegant technique, leading to an easier to construct and possibly more accurate instrument, would be to use Faraday cells as described by E J Gillham[2] for an automatic polarimeter

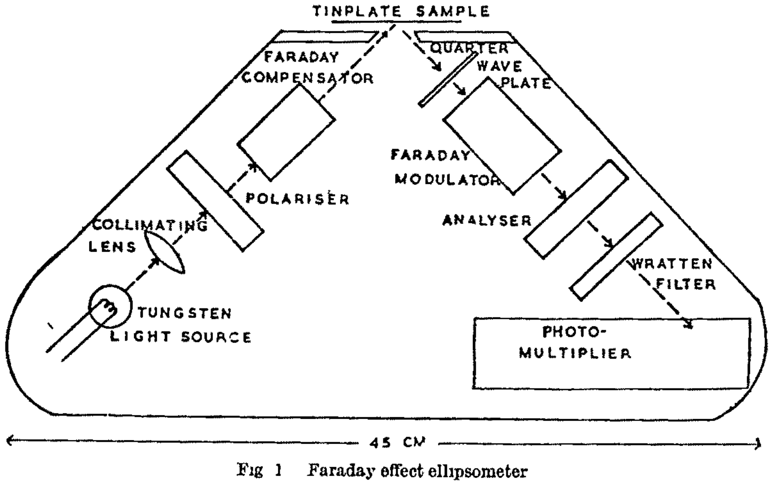

Fig 1 Faraday effect ellipsometer

Accordingly, a Faraday effect ellipsometer has been built primarily for measurements of thickness of oil on commercial tinplate but applicable to any metal substrate of reasonable brightness and flatness and bearing a removable transparent film of the order of a few molecules thick A diagram of the instrument is shown in Fig 1 The function of the Faraday cells is essentially the same as described by Gillham, one serving to oscillate the plane of polarization and thus modulate the light intensity while the other introduces a compensating shift equivalent to a small analyser rotation Strictly speaking, both Faraday cells should be placed between the quarter-wave plate and the analyser, but in order to achieve a compact instrument the compensator has been transposed to the incident beam This reduces the compensator sensitivity referred to the analyser somewhat and would be unacceptable if compensation of more than about a degree is required

In use the instrument is first adjusted by setting the plane of polarization of the incident light to be at 45° to the plane of the incident and reflected rays With a typical specimen in position the analyser and the quarter-wave plate are adjusted in turn for the best minimum photo-cell current For all further measurements with this type of specimen, the quarter-wave plate is left fixed. Measurements of film thickness are now made by first adjusting the analyser for an accurate null using the Faraday modulator and the usual electronic circuits for phase-sensitive de-modulation of the resulting photomultiplier signal After removing the film the specimen is replaced in exactly the same position and the null is re-established by direct current in the Faraday compensator This current is proportional to thickness for a given film and substrate for films thin enough for the approximate Drude equations to apply, say to at least 100 Å

Table 1 FARADAY COMPENSATOR READINGS IN MILLI-DEGREES

Commercial oiled tinplate	De-oiled by Soxhlet extraction	Oiled by dipping in dioctyl sebacate solutions Oil concentration in volume (%)				Shift due to oil film
		0 02	0 04	0 06	0 08	
–15	200					215
	195	45				150
	210	70				140
	205		–75			280
	220		–55			275
	225			–250		475
	205			–285		490
	225				–395	620
	215				–405	620

In this instrument modulation is carried out at 50 c/s and the modulator produces an angular swing of about ± 2° Compensation is possible over a range of about 0 75° referred to the analyser, and the noise-level using a tinplate specimen and about 0 1 c/s equivalent band-width in the de-modulator filter is equivalent to about 3 millidegrees, and this is the limiting factor in setting the instrument This is about an order of magnitude worse than that reported by Gillham for his polarimeter and is mainly due to the poor quality, optically speaking, of commercial tinplate surfaces None the less, it is very adequate for measuring films, such as oil on tinplate, which typically produce a shift of 0 3° and is nearly an order of magnitude better than the repeatability of setting claimed by Murray

To illustrate that the instrument is responsive to the thin films of oil on tinplate (of the order of 30 Å) a piece of commercial tinplate was de-oiled and oiled a number of times, compensator readings on the ellipsometer being taken at each stage

De-oiling was by Soxhlet extraction in benzene and oiling was by dipping in and controlled slow removal from dilute solutions of oil (dioctyl sebacate) in benzene The amount of oil so deposited can be varied in an approximate manner by changing the oil concentration The results are shown in Table 1 in terms of milli-degrees of compensation Ten milli-degrees is equivalent to about one angstrom unit of oil film thickness The changes in the de-oiled state represent a slight lack of reproducibility of the surface rather than instrumental error

I thank Prof A B Winterbottom for his advice and the Metal Box Co , Ltd , for permission to publish this communication

M J RICHARD

Research Department, Metal Box Co , Ltd ,
Kendal Avenue, Acton, London, W 3

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METEOROLOGY

Measurement of Conducted Flow of Heat in a Sea Ice Cover

In any micro-meteorological study or heat economy survey, the measurement of the conducted flux of heat at, or below, the Earth's surface is important The two methods normally used to achieve this either involve measuring the temperature gradient if the thermal conductivity can be accurately established, or insertion of a heat flux meter, the basis of which is the measurement of temperature gradient across a thin slab of material of known conductivity The accuracy of heat flux meters is limited unless the effective conductivity of the device used matches that of the medium in which the flow of conducted heat is being measured For example, in using a flux meter with square horizontal surfaces with a width to thickness ratio of 12 1, a 20 per cent mismatch in conductivities results in an approximately 5 per cent error in indication

For surface media which are subject to changes in thermal conductivity or are transparent to radiation, one or other of the foregoing methods of heat flow measurement become inapplicable The first difficulty is met when rainfall alters the effective conductivity of soils or porous rocks so that measuring techniques are restricted to use of a flux meter Snow and ice covers, on the other hand, present problems due to the penetrating visible radiation heating the sensor surfaces unequally In the case of fresh-water ice, the properties of which remain essentially constant, the problem is solved by measuring the temperature gradient with small thermocouples having reflecting surfaces

Sea ice presents an example of the most complex type of situation where both problems of variability in thermal conductivity and transparency to radiation have to be considered I was able to overcome both these difficulties during a heat budget study on annual sea ice in Hudson Bay This was achieved by excavating a pit of about 1 5 m across and 25 cm deep and allowing cold fresh-water to freeze, forming a reference block Frozen into this block were a vertical sequence of four small thermocouples with reflecting surfaces electrically insulated by thin tubular polythene envelopes and spaced 5 cm apart by a transparent 'Perspex' frame The relatively thick slab of prepared ice was used to gain sufficient temperature difference to avoid the necessity for thermopiles, the increased area of which would have resulted in more serious local absorption of radiation The four thermocouples enabled a uniform temperature gradient to be detected in the reference ice, under which conditions the most widely separated thermojunctions could be used to determine the heat flux most accurately

Some optical and conductive matching between the reference block and the surrounding sea ice was accomplished during the preparation of the former By insulating the prepared excavation from above, the reference ice was made to grow from below and form a relatively dense and transparent medium On the other hand, permitting the ice to form from above, progressively in layers, resulted in a bubbly mass of small crystal structure An alternative method which proved satisfactory in controlling the density of the block was to freeze varying mixtures of snow and water Ice of a given density appeared to be more opaque when prepared by the latter method Although there was some degree of independent control over thermal conductivity and transparency, relatively accurate matching was attempted only for the former, and was checked by a determination of the conductivity of the surrounding sea ice Transparency matching was judged by appearance only, but if necessary could be checked by means of a simple light meter. Usually, a light snow cover results in radiation penetration being of reduced importance

Measurement of the thermal conductivity of the reference ice is necessary It will be shown elsewhere[1] that a simple measurement of density is sufficient to fix the conductivity of non-saline ice and that the additional determination of salinity enables the thermal conductivity of sea ice to be specified as a function of temperature The device proved to be satisfactorily sensitive and accurate and its properties, in marked contrast to those of the sea ice, remained essentially constant over a period of three months, in particular no significant diffusion of sea ice brine into the reference ice occurred The sensitivity of the 'ice heat flux meter' was demonstrated by the fact that the indicated values of surface heat flux were reflected in corresponding rates of sea ice growth after a calculable time delay[2]

PETER SCHWERDTFEGER

Department of Meteorology,
University of Melbourne

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GEOLOGY

Age of the Great Dyke of Southern Rhodesia

The isotopic composition of strontium has been determined for two whole-rock specimens of gabbro from the Wedza Complex of the Great Dyke of Southern Rhodesia In addition, concentrations of rubidium and strontium were measured for these rocks by the method of isotope dilution These results led to an estimate of the time since differentiation of this intrusive

The rock specimens were obtained through the courtesy of Dr B G Worst, who has made a detailed study of the structure and petrogenesis of the Great Dyke[1] He concluded that the 'Dyke' is in reality a downfaulted remnant of an original sheet-like mafic intrusive composed of four complexes and may be related to the igneous cycle of the Bushveld Igneous Complex

The isotope ratio determinations for strontium were made in duplicate on a mass spectrometer featuring a 6-in radius of curvature, 60° sector analyser tube The details of the analytical procedure have been discussed by Faure and Hurley[2] and will not be repeated here The measured $^{87}Sr/^{86}Sr$ ratios were normalized to a standard value for $^{86}Sr/^{88}Sr = 0\ 1194$ by a procedure outlined by Faure and Hurley[2,3] In addition, a correction of $-0\ 003$ was applied to the $^{87}Sr/^{86}Sr$ ratios in order to eliminate a consistent bias from the results as indicated by replicate analyses of a secondary strontium isotope standard ($SrCO_3$, Eimer and Amend, lot 49232, Table 1).

The rubidium determinations were made in duplicate by isotope dilution while the strontium determinations were confirmed by X-ray fluorescence analysis The analytical results are summarized in Table 1

Table 1 ANALYTICAL DATA FOR THE GREAT DYKE, SOUTHERN RHODESIA

M I T sample	rock type	$\frac{^{87}Sr}{^{86}Sr}$	$\frac{^{86}Sr}{^{88}Sr}$	$\left[\frac{^{87}Sr}{^{86}Sr}\right]_{corr}$	Rb (p p m)	Sr (p p m)	$\frac{^{87}Rb}{^{86}Sr}$
4937	Quartz gabbro/ Wedza complex	0 7212	0 1199	0 7227	34 6	153 9*	
		0 7232	0 1189	0 7217	31 0		
	Average	0 7222 ± 0 0010	0 1194 ± 0 0005	0 7222 ± 0 0005	32 8 ± 5 5%	153 9 ± 5%	0 617 ± 0 046
4936	Norite/ Wedza complex	0 7059	0 1193	0 7056	6 38	245 0*	
		0 7048	0 1192	0 7042	5 16		
	Average	0 7054 ± 0 0005	0 1193 ± 0 0001	0 7049 ± 0 0007	5 77 ± 10 7%	245 0 ± 5%	0 068 ± 0 008

The measured $^{87}Sr/^{86}Sr$ ratios were corrected for isotope fractionation assuming that $^{86}Sr/^{88}Sr = 0\ 1194$ (see ref 2)

Blank corrections of 0 234 μg Sr and 0 10 μg Rb were made to all isotope dilution analyses

* Confirmed by X-ray fluorescence analysis using a norite of known Sr content (by isotope dilution) as comparison standard Sample 4937, Sr = 155 p p m , sample 4936, Sr = 241 p p m

$SrCO_3$, Eimer and Amend, lot 492327, average of nine analyses on one instrument $^{87}Sr/^{86}Sr = 0\ 7084 \pm 0\ 0007(\sigma)$, corrected relative to $^{86}Sr/^{88}Sr = 0\ 1194$

The time elapsed since differentiation of the two rock specimens was calculated by solving two simultaneous equations of the form

$$\left[\frac{^{87}Sr}{^{86}Sr}\right]_p = \left[\frac{^{87}Sr}{^{86}Sr}\right]_0 + \frac{^{87}Rb}{^{86}Sr}(e^{\lambda t}-1)$$

where

$\left[\frac{^{87}Sr}{^{86}Sr}\right]_p$ = atomic ratio of ^{87}Sr to ^{86}Sr at the present time

$$\left[\frac{^{87}Sr}{^{86}Sr}\right]_0 = \text{same at } t = 0$$

$$\lambda = 1\ 47 \times 10^{-11}\ yr^{-1}$$

The solution of the two equations yields $t = 2{,}110 \pm 350$ m y and $\left[\frac{^{87}Sr}{^{86}Sr}\right]_0 = 0\ 7028 \pm 0\ 0013$ The errors assigned are uncertainties and include maximum and minimum values compatible with the analytical results The large uncertainty of ± 17 per cent assigned to the age is partly due to the fact that the radiogenic ^{87}Sr enrichment of sample No 4937 is only about 2 5 per cent relative to the initial abundance of this isotope This work is the first attempt, to the best of our knowledge, to determine the time since differentiation of the Great Dyke of Southern Rhodesia by radioactive dating techniques

Nicolaysen *et al* [4] reported a rubidium–strontium age of 1,940 ± 50 million years (recalculated to $\lambda = 1\ 47 \times 10^{-11}\ yr^{-1}$) for the mafic rocks of the Bushveld Complex of the Republic of South Africa This determination was made on a biotite concentrate from a biotite bearing pyroxenite of the Rustenburg Platinum Mine Nicolaysen *et al* [4] concluded from their data that " new age measurements on micas, monazites and zircons from the Bushveld Complex are consistent with an age of 1,950 ± 150 million years " Schreiner[5] was the first to report age determinations by the whole-rock rubidium–strontium method His age for the red granite which intrudes the mafic rocks of the Bushveld Complex is 1,800 m y (recalculated to $\lambda = 1\ 47 \times 10^{-11}\ yr^{-1}$)

The age of the Great Dyke of Southern Rhodesia is 2,110 ± 350 million years and is therefore identical within the limits of the errors to the age of the Bushveld Igneous Complex This conclusion supports the earlier suggestion by Worst[1] that the Great Dyke and the Bushveld Complex were emplaced during the same magmatic cycle

We thank Dr B G Worst for sending us the whole-rock specimens of the Great Dyke This work was supported by the U S Atomic Energy Commission contract *AT*(30–1)–1381

G FAURE *
P M HURLEY
H. W FAIRBAIRN
W H PINSON, JUN

Department of Geology and Geophysics,
Massachusetts Institute of Technology,
Cambridge, Mass

---

* Present address Department of Geology, The Ohio State University, Columbus 10, Ohio

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Geology of the East Rutts (South Devon)

AN expedition by the Oxford Underwater Research Group was made during July 1963 to examine the submerged pinnacle of rock known as the East Rutts (50° 13 5′ N , 3° 59′ W)

The rock is situated five miles south-west of the Bolt Tail promontory and 5 6 miles south of St Anchorites Rock in Bigbury Bay

It is approximately elliptical in plan, being about 530 yd in length in a north-east–south-west direction and 220 yd in a north-west–south-east direction Echo-sounding traverses indicated that the uppermost part of the pinnacle lay about 30 ft below the surface whereas the sea-floor in the vicinity of the rock lay at 120 ft

Extensive gullies were seen to cut through the rock formation in a north-east–south-west direction, and an almost sheer face was observed at the south-west end of the pinnacle

Samples of the rock *in situ* were obtained by aqualung divers of the Group and were subjected to petrological examination, X-ray and chemical analysis

In thin-section the rock shows a mainly anhedral mosaic of dolomite grains with some calcite enclosing lenses and stringers of strained quartz grains Clustering of the dolomite grains at the edge of the quartz and the presence of dolomite rhombs in the quartz patches suggest diagenetic replacement Specimens from a depth of 50 ft are finer-grained (average grain size diameter 0 1 mm) than those from 100 ft (average grain size diameter 0 5 mm), rare small flakes of mica are present in the former and more extensively in the latter, together with a little anhedral iron ore

An X-ray powder photograph showed the presence of quartz, dolomite, calcite, mica and ore.

A chemical analysis of the rock from a depth of 100 ft is given It compares closely with the analysis of the Knox dolomite from Morrisville, Alabama, U S A , quoted by Pettijohn (1956, Table 81, Analysis *D*) except in its somewhat higher silica content

Analysis

	%
SiO_2	8 47
R_2O_3	0 59
MgO	18 07
CaO	28 32
CO_2	42 39
Na_2O	1 07
K_2O	0 97
H_2O (110° C)	0 31
Total	100 19

Neutron activation analysis of the rock indicated 19 7 ± 0 68 per cent for its magnesia content

The presence of a large mass of dolomite in the East Rutts area is surprising, considering the present stratigraphic and structural ideas on that part of the English Channel Hill and King[1] indicate the presence of metamorphic rocks in the locality, with New Red Sandstone deposits to the north and west, the latter giving place to the metamorphic rocks comprising the Eddystone reef Apart from presenting the evidence of the existence of the dolomite we have as yet no explanation to offer for its position other than the very tentative suggestion that it may be a very localized facies of the Devonian or of New Red Sandstone lying within the Eddystone–Start Point metamorphic thrust zone It is hoped that further investigations now planned will provide more evidence

We thank Mr A Stone, of the Neutron Generator Laboratory, Wantage Research Laboratories, for checking the magnesia content of the rock by neutron activation analysis We also thank Dr K Sandford, of the Department of Geology and Mineralogy, University of Oxford, for helpful discussions, and Mr A Hancock and Mr D Gill for the collection of *in situ* samples of rock under arduous diving conditions

D S Rawson
Oxford Underwater Research Group,
114 High Street,
Oxford

J D Bell
Department of Geology and Mineralogy,
Oxford

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Local Distribution of Elements in the Tests of Foraminifera

Electron probe X-ray microanalysis permits the determination on a micro-scale of local distribution and concentration of elements in a sample In a preliminary enquiry, the tests of calcareous Foraminifera have been investigated

The analysis was preceded by optical emission spectrographic analysis of the tests of approximately 12 specimens of each species investigated These analyses determined the elemental composition of the tests plus included sedimentary particles which cannot (with certainty) be cleaned out of the innermost chambers The results of the spectrographic analyses were used to eliminate the necessity of preliminary searching on the electron probe, thus saving much time

Foraminifera for probe analysis were cleaned in dilute hydrogen peroxide After drying, they were embedded (under partial vacuum) in polystyrene The method of embedding is described in detail by me (submitted for publication) A median section was cut and polished, and a thin metal layer was deposited on it by vacuum evaporation, to ensure electrical continuity

Generally, interpretation of results was complicated by topographical and microstructural effects The calcium and silicon concentration variation in Recent *Elphidium excavatum* (Terquem) and *E bartletti* (Cushman), calcium concentration variation in Recent *Quinqueloculina seminulum* (L), calcium, iron, silicon, manganese, and magnesium concentration variation in the Tertiary *Operculina victoriensis* (Chapman and Parr), and calcium and magnesium concentration variation in Recent *Operculina complanata* (Defrance) have been recorded

Improved magnesium detection is desirable, because this element has been shown to be of classificatory and ecological significance in the Foraminifera

Kenneth Hooper
Department of Geology,
Carleton University, Ottawa

METALLURGY

Effect of Impurities on the Electrical Resistivity Increase during Elongation of Cadmium at 71° K

Observations of electrical resistivity increases in metals during plastic deformation[1] have been attributed to changes in the concentration of point, line and planar defects Further experiments have demonstrated that prior deformation and recovery[2] as well as impurity concentration[3,4] affect the slope of the resistivity–elongation curves It is the purpose of this communication to present some results obtained on cadmium containing impurities The influence of lead, magnesium and copper, in particular, is discussed

Polycrystalline cadmium wire, which for most experiments was 1 mm in diameter, was obtained in various degrees of purity as indicated in Table 1 Specimens, about 14 cm long, were elongated in liquid nitrogen and the resistance change was measured potentiometrically as a function of elongation A more detailed description of the experiment is available in ref 5

Table 1 Emission Spectrograph Analysis of Cadmium Impurities (p p m)

Sample impurity	69-Cd + 1,000 p p m Pb	69-Cd + 1,000 p p m Mg	69-Cd + 10 p p m Cu	Commercial Cd	49-Cd	59-Cd
Pb	1,000	<1	<1	100	3	<1
Mg	<1	1,000	<1	<1	10	<1
Si	<1	<1	<1	<1	10	<1
Fe	<1	<1	<1	<1	10	<1
Cu	10	10	10	10	3	<1
Ag	<1	<1	<1	3	10	<1
Al					10	
Total impurities (nominal)	1,000 p p m Pb 10 p p m Cu	1,000 p p m Mg 10 p p m Cu	10 p p m Cu	110 p p m	56 p p m	1 p p m
Slope (approx)	<3 4	3 4	3 4	1 8	1 2	3 4

The results of the deformation experiments at liquid nitrogen are shown in Fig 1 The closed and open squares represented by curves *A* and *B* are data on the same 59-cadmium specimen measured while the load was on and after the load was removed, respectively Part of the difference between *A* and *B* can be attributed to the elastic recovery, which is estimated to be less than 0 1 per cent However, the greater part of the difference is undoubtedly due to the presence of the stress and its subsequent effect on the scattering of conduction electrons It can be seen that both *A* and *B* show a linear dependence on strain The results for cadmium doped with copper, lead and magnesium also fall on curve *B* although the cadmium containing lead shows some deviation Curve *C*

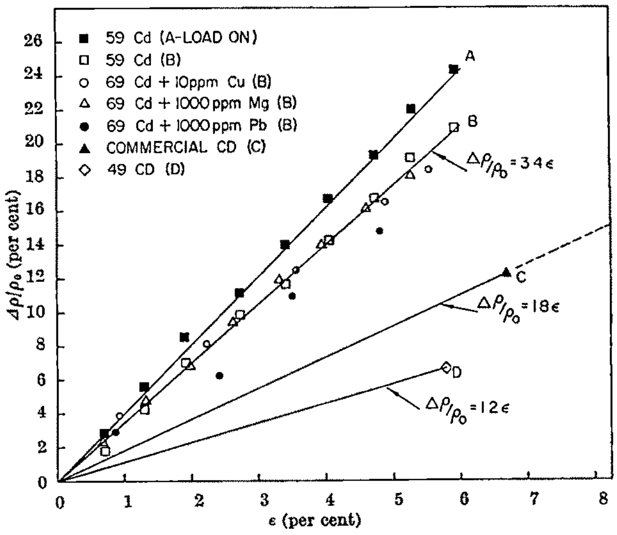

Fig 1 Percentage resistivity increase for cadmium specimens of various purities as a function of elongation strain at 78° K

shows some results obtained earlier in this laboratory for cadmium of commercial purity These latter specimens were 5 mm in diameter and their resistance was measured using a Kelvin double bridge Only one point of this data is shown on Fig 1 Other results obtained from this experiment indicate a linear behaviour to approximately 13 per cent elongation The point on curve *D* represents the average data of eighteen 49-cadmium specimens The deviation from the average value noted on the plot is insignificant

It is interesting to note that the addition of slight amounts of copper has very little influence on the ρ versus ε curves over what is observed for high-purity cadmium Additional impurities such as magnesium, at least at a concentration of 1,000 p p m , shows no effect Lead however, does affect the slope of these curves As the lead concentration increases, the slope decreases from that observed for high-purity cadmium and then increases toward the high-purity slope as the lead concentration approaches 1,000 p p m

The effect of other impurities such as silicon, iron, silver and aluminium is not very clear Nevertheless, the 49-cadmium contains all these elements and does have the lowest ρ versus ε slope Whether it is primarily the lead or these other elements which is influencing this behaviour must await further investigation

It is recognized that impurities in copper and other metals influence defect formation such as stacking faults It can be speculated that the behaviour noted in cadmium is associated with twin formation, stacking faults, dislocation multiplication, influence of impurities on preferred orientation, etc Obviously, a more complete investigation is necessary to elucidate this phenomenon

F R STEVENSON*
H R PEIFFER

Rias,
7212 Bellona Avenue,
Baltimore 12, Maryland.

* Now at Lewis Research Center, National Aeronautics and Space Administration, Cleveland, Ohio

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Elevated Temperature Embrittlement induced in a 20 per cent Chromium : 25 per cent Nickel : Niobium Stabilized Austenitic Steel by Irradiation with Thermal Neutrons

IT has been known for some time that neutron irradiation at ambient temperatures produces increases in the proof and ultimate tensile stresses and reductions in ductility of austenitic steels in room temperature tensile tests[1] These changes in properties result from the interaction of dislocations with the vacant lattice sites and interstitial atoms produced by fast neutron-atom collisions and are recovered on testing at temperatures of about 500° C due to annihilation of the point defects[2]

More recently it has been established that irradiation in a nuclear reactor also reduces the ductility of austenitic steels and austenitic type alloys at test temperatures above about 600° C. This high-temperature brittleness is manifested as decreases in the elongation and reduction of area values in post-irradiation uniaxial tensile tests[3], reduced strengths and elongations in post-irradiation stress rupture tests[4] and reductions in the circumferential elongations to fracture in 'in-pile' tube bursting tests[5]

The mechanism responsible for this type of embrittlement is being investigated in a double-vacuum-melted 20 per cent chromium 25 per cent nickel niobium stabilized steel, the analysis of which is given in Table 1

Table 1 ANALYSIS OF STEEL

Element	C	Cr	Ni	Mo	Nb	Ti	Cu
Wt %	0 025	20 7	24 85	0 013	0 7	0 008	0 003
Element	Sn	Mn	As	P	S	B	Pb
Wt %	0 0025	0 67	0 001	0 003	0 01	0 0005	0 0015
Element	Ta	N					
Wt %	0 03	0 008					

Tensile specimens with a parallel gauge-length of 0 835 in were die-stamped from a strip 1 in wide and 0 0215 in thick and heat-treated for 0 5 h at 1,050° C in pure argon followed by rapid cooling The specimens were electropolished and individual batches were irradiated as follows (1) at 40° C in the core of the *Herald* light-water moderated and cooled reactor; (2) at 135° in the 4VGR hole, (3) at 50° C in the 2V hole in the *Dido* heavy-water moderated and cooled reactor Cobalt and nickel monitors were included with each batch of specimens to measure the thermal and fission neutron doses respectively The specimens were afterwards tested at room temperature, 750° or 850° C at a strain rate of 2×10^{-4} sec^{-1} in a hard beam autographically recording dynamometer tensile machine operated remotely within a 4-ft thick concrete cell, the elevated temperature tests were made in purified argon and the temperatures were stabilized for 0 5 h before commencing the tests At least two specimens were tested in each condition and the mean results of the 750° and 850° C tests are given in Table 2

Table 2 ELEVATED TEMPERATURE TENSILE TEST RESULTS ON UNIRRADIATED AND IRRADIATED 20 PER CENT CHROMIUM 25 PER CENT NICKEL NIOBIUM STABILIZED AUSTENITIC STEEL

Irradiation facility	Neutron dose (n cm^{-2})		Test temperature (° C)	0 2% Proof stress (lb /in ^{2})	Ultimate tensile stress (lb /in ^{2})	% Elongation
	Thermal	Fission				
—	—	—	750	18,200	27,600	55 5
Herald	$7\,6 \times 10^{18}$	$3\,8 \times 10^{18}$	750	18,500	26,400	48 3
Dido 4VGR	$2\,2 \times 10^{19}$	$1\,9 \times 10^{16}$	750	16,400	27,200	29 1
Dido 2V	$1\,5 \times 10^{20}$	$7\,4 \times 10^{17}$	750	14,600	27,800	17 9
—	—	—	850	13,200	14,200	56 4
Herald	$7\,6 \times 10^{18}$	$3\,8 \times 10^{18}$	850	13,100	14,400	41 1
Dido 4VGR	$2\,2 \times 10^{19}$	$1\,9 \times 10^{16}$	850	14,600	15,800	38 6
Dido 2V	$1\,5 \times 10^{20}$	$7\,4 \times 10^{17}$	850	13,100	15,300	20 8

With the possible exception of the 0 2 per cent proof stresses of the samples tested at 750° C, the stress values are not significantly affected by the irradiation; however, the elongations to fracture are markedly reduced at both test temperatures This elevated temperature embrittlement is a function of the thermal neutron dose and cannot be correlated with the fission (or fast) neutron dose This is in contrast to the room temperature point defect hardening and embrittlement which is dependent on the fast neutron dose as shown in Table 3

Table 3 ROOM TEMPERATURE TENSILE TEST RESULTS ON UNIRRADIATED AND IRRADIATED 20 PER CENT CHROMIUM 25 PER CENT NICKEL NIOBIUM STABILIZED AUSTENITIC STEEL

Irradiation facility	Irradiation dose (n cm^{-2})		0 2% Proof stress (lb /in ^{2})	Ultimate tensile stress (lb /in ^{2})	% Elongation
	Thermal	Fission			
—	—	—	30,700	76,800	48 2
Dido 4VGR	$2\,2 \times 10^{19}$	$1\,9 \times 10^{16}$	37,500	84,600	42 2
Dido 2V	$1\,9 \times 10^{20}$	$1\,7 \times 10^{18}$	48,100	84,700	30 2
Herald	$7\,6 \times 10^{18}$	$3\,8 \times 10^{18}$	54,500	89,000	31 6

Additional experiments are in progress to confirm these observations and to investigate the mechanism whereby the thermal neutrons are effective in causing the elevated temperature embrittlement of austenitic steels

We thank Mr J Fennell for assistance with the testing of the specimens

A C ROBERTS
D R HARRIES

Metallurgy Division,
Atomic Energy Research Establishment,
Harwell, Didcot

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Equilibrium Diagram for the Nickel-Cadmium System

To study the effect of size factor in alloying behaviour it was decided to determine the ternary system Cu–Ni–Cd, on which no data have been published The binary systems Cu–Ni and Cu–Cd have been fully investigated, the results of the various investigations being summarized by Hansen[1] Of the Ni–Cd system only cadmium-rich alloys have been studied in detail a further investigation of this system was therefore carried out in conjunction with the work on the ternary diagram

Alloys were prepared from analytical reagent quality nickel powder and stick cadmium, the powder was given a reducing treatment in hydrogen to remove any oxide present Weighed amounts of the pure metals were sealed in evacuated silica capsules for melting, which was carried out by heating for about 1 h at 1,200°–1,250° C, conditions which had been shown to be adequate for the nickel to be taken into solution The capsules were then rapidly cooled to give a fine-grained ingot and annealed for a minimum period of three weeks to homogenize the alloy At the end of the annealing period the alloys were water-quenched and then weighed to check whether any loss had occurred in melting The nominal compositions of ingots which were satisfactory in this respect were taken as the actual compositions

The liquidus surface for the Ni–Cd systems was examined by equilibrating nickel in the form of wire with cadmium at constant temperature At the end of the soaking period the capsule containing the sample was quenched and broken open The residuum of the wire was separated from the melt and the latter analysed for its nickel content The results obtained from this study are indicated in Fig 1 Cooling curves on alloys close to 50 atom per cent nickel failed to show any arrest between the liquidus and 502° C, micro-examination of these cooling curve ingots showed the formation of peritectic walls of β Ni–Cd surrounding dendrites of the α-phase The peritectic temperature was therefore determined from heating curves on homogenized alloys and gave a value of 695° C for the decomposition temperature of the β phase The formation of the γ′ phase was studied by combined dilatometry and thermal analysis The volume change at 405° reported by Voss[2] was not observed, an alloy of γ′ together with a small amount of γ showed an arrest point accompanied by partial collapse of the specimen at 490° C and a second arrest and complete collapse at 502° C It is significant that Voss, who reported a peritectic temperature of 502°, obtained this value on higher nickel content alloys (~10 wt per cent nickel) than Swartz and Phillips[3] (6–8 wt per cent nickel), and it is probable the former detected the peritectic involving γ′ and the latter that of γ, which would be more prominent in the lower nickel alloys they studied The nickel-rich solubility limit is confirmed by an alloy of 21 2 atom per cent nickel, which showed the presence of a trace amount of γ′ at 430° C compared to a reported solubility of 19 5 atom per cent at this temperature Other than the β-phase (Ni–Cd) no other compounds were observed in the system

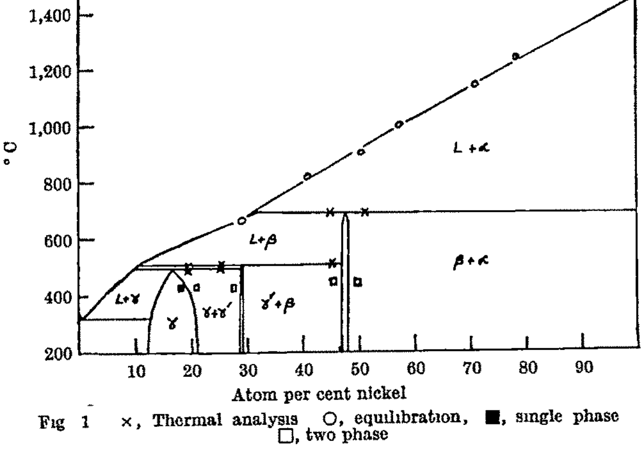

Fig 1 ×, Thermal analysis ○, equilibration, ■, single phase □, two phase

The β-phase structure has been studied by X-ray diffraction using the powder method The compound possesses the structure $Fd3m$ with a cell edge of 11 40 kX units and contains approximately 48 formula units in the unit cell

R J Lott
J K Critchley

Department of Chemistry,
Brunel College,
London, W 3

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CHEMISTRY

Spin-orbit Coupling and Intermolecular Energy Transfer

Recently[1,2] we reported on the intermolecular energy transfer in chemiluminescent solutions, an excited product of the reaction being a donor and anthracene derivatives being acceptors (activators) The activated chemiluminescence spectra were identical with the fluorescence spectra of the activators, indicating that the singlet state of the activator is the excited one[2] A dependence of the chemiluminescence intensity on the activator concentration was used for estimating the ratio k_{PA}/f_P (k_{PA}, transfer constant, f_P, emission probability of the excited product, P' (Fig 1)) The relative values of the k_{PA}'s were found to be greater the more halogens were introduced in the activator molecule and the greater was the atomic weight of the substituent In this communication these features of the phenomenon are regarded as evidence for the spin-orbit coupling in the activator molecule

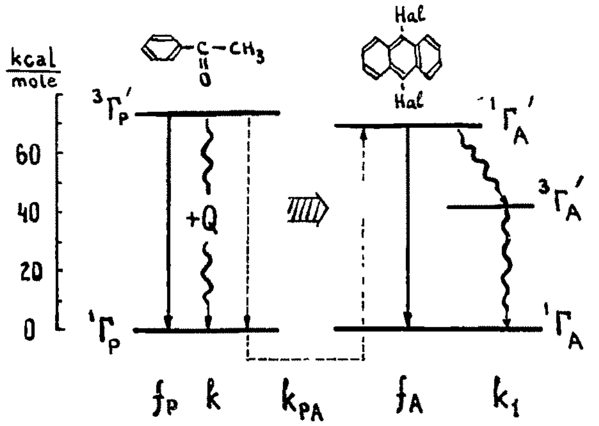

Fig 1 Energy-levels diagram for the chemiluminescence in ethylbenzene oxidation and for the energy transfer on the anthracene derivatives The solid, waved and dashed lines represent, respectively, radiative, radiationless transitions and energy transfer Deactivation by a quencher, Q, is discussed in ref 3

The excited product P' formed in the hydrocarbon oxidation was identified as a ketone in its triplet state[3] Therefore, the energy transfer may be represented as a coupled transition $|{}^3\Gamma'_P \rightarrow {}^1\Gamma_P| \rightarrow |{}^1\Gamma_A \rightarrow {}^1\Gamma'_A|$, as shown in Fig 1 There were reasons for considering it as an exchange-resonance transfer—its features strongly differed[2] from those required by the theories of the inductive-resonance or radiative transfer, and the maximum value of k_{PA} (for 9,10-di-bromoanthracene) was only 20–30 times less than the number of collisions So we believe that the

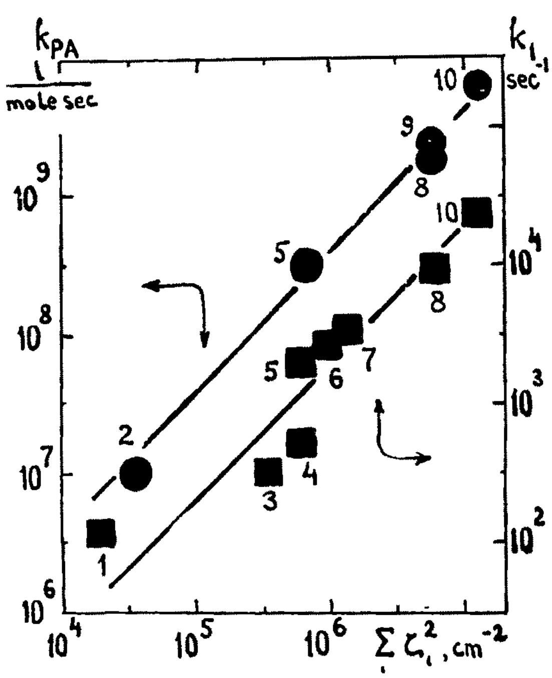

Fig. 2. Substituents influence on probabilities *T-S* intra- and inter-molecular transitions. k_1—from ref. 6, k_{PA}—from ref. 2—assuming f_P for *cyclo*hexanone equal to 10^3 sec^{-1}. *Cyclo*hexanone is formed in *cyclo*hexane oxidation at 50° C. The straight lines correspond to direct proportionality between the abscissas and ordinates. Values of ζ_i from ref. 5. 1, Anthracene (*A*), 2, 9,10-diphenyl-*A*, 3, 1-chloro-*A*, 4, 1,5-dichloro-*A*, 5, 9,10-dichloro-*A*, 6, 2,9,10-trichloro-*A*, 7, 1,5,9,10-tetrachloro-*A*, 8, 9-bromo-*A*, 9, 9-bromo,10-phenyl-*A*, 10, 9,10-dibromo-*A*

energy transfer is brought about by the exchange of electrons between the donor and acceptor in the state of a diffusion-controlled collision. Then its probability should be proportional to the matrix element squared of the operator of the exchange interaction[4]

$$k_{PA} \sim |<H_1>|^2 = |\int \psi'_P{}^*(1)\psi_A{}^*(2)H_1\psi'_A(1)\psi_P(2)d\tau|^2 \quad (1)$$

$$H_1 = e^2/r_{12}$$

$<H_1>$ is not equal to zero except when the excited donor and acceptor have the same multiplicity and their normal states have the same multiplicity also[4]. This selection rule is obviously not valid in our case since the excitation is transferred from the triplet state to the singlet one, that is

$$\psi'_P = {}^3\psi'_P \text{ and } \psi'_A = {}^1\psi_A$$

For that reason $<H_1>$ and k_{PA} are close to zero and the transfer is 'forbidden'.

Introduction of halogens strengthens an interaction of the spin and orbital momenta. That leads to the 'mixing' of states of different multiplicity

$$\psi'_A = {}^1\psi'_A + \lambda\,{}^3\psi'_A \quad (2)$$

Combination of equations (1) and (2) leads to the following expression

$$k_{PA} \sim \lambda^2|<H_1>|^2_{3,3} \quad (3)$$

where $|<H_1>|_{3,3}$ is the part of the $|<H_1>|$ corresponding to the 'pure' triplet–triplet transition

$$|<H_1>|_{3,3} = \int {}^3\psi'_P{}^*(1)\,{}^1\psi_A{}^*(2)H_1\,{}^3\psi'_A(1)\,{}^1\psi_P(2)d\tau$$

Now the coupled transition becomes permissible and hence the greater becomes factor λ. The latter may be calculated in principle by the methods of the perturbation theory of quantum mechanics

$$\lambda = \frac{<H_{so}>}{\Delta E} \quad (4)$$

where $<H_{so}>$ is the matrix element of the spin-orbit operator and ΔE, energy difference for levels ${}^1\Gamma'_A$ and ${}^3\Gamma'_A$. A precise calculation of λ is difficult and I considered a simplified model of the activator molecule assuming that it consists of 'isolated' atoms, each of them independently influencing the excited electron. In this approximation, a contribution of each atom into k_{PA} may be represented by ζ_i^2, where ζ_i is a radial part of the matrix element $<H_{so}>$ for an isolated atom—a quantity well known from atomic spectra[5]. Hence k_{PA} should be proportional to $\sum_i \zeta_i^2$. In fact this correlation takes place for a number of anthracene derivatives, as is shown in Fig. 2.

It is remarkable that, according to Hoffman and Porter, halogen-induced spin-orbit coupling increases the probability of the intramolecular radiationless transition ${}^3\Gamma'_A \rightarrow {}^1\Gamma_A$ in the anthracene derivatives[6]. The probability of this triplet–singlet conversion may be written in the form

$$k_1 \sim |<H>|^2 = |\int {}^3\psi'_A{}^* H\,{}^1\psi_A d\tau|^2 \quad (5)$$

where H is a certain operator which depends on transition mechanism (for example, for dipole–dipole radiative transition H is the dipole momentum operator). $<H>$ is equal to zero in the absence of the spin-orbit coupling. On the analogy with the aforementioned energy transfer quantities, $|<H>|^2$ and k_1 should be proportional to the same quantities $\sum_i \zeta_i^2$ for halogen derivatives of anthracene. This correlation is also satisfactorily carried out as can be seen from Fig. 2. (At the same time, a dependence of k_1 on the position of a substituent is seen from Fig. 2 which indicates the limitations of the simple model used.) That verifies my hypothesis of the identical mechanism of halogen influence on both intra- and inter-molecular processes.

Considering the possibilities of chemiluminescence as a method of studying excited states I should like to emphasize some positive characteristics of this way of excitation. If I had used photo-excitation it would be very difficult to study the energy transfer from a poorly luminescent donor (ketone) on to a well luminescent activator because of the unavoidable direct excitation of the activator which is strongly absorbing in the spectral region common with the donor. In the case of chemical luminescence, excited states are being formed by 'dark' way and the strongly luminescent components can be excited only through energy transfer, the latter being a subject of the present investigation.

R. F. VASSIL'EV

Institute of Chemical Physics,
Academy of Sciences of the U.S.S.R.,
Moscow

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Vaporization of Uranium Monophosphide

IN the search for new oxygen-free refractory materials, we have found that the subphosphide of thorium, $ThP_{0.7}$, has a high thermodynamic stability[1] which is comparable with that of uranium monosulphide[2]. We have included the uranium monophosphide, UP, and its mixtures with uranium dioxide in our investigations[3]. Since recent reports[4,5] indicate the potential usefulness of uranium monophosphide for high-temperature reactor application, we present here our preliminary results concerning the thermodynamics of vaporization of uranium monophosphide.

The uranium monophosphide was prepared by reaction of the elements using the Faraday method and thermally degrading the resulting triuranium tetraphosphide, U_3P_4, to uranium monophosphide at 1,400° C. Analysis showed that it contained 0.38 per cent oxygen. An X-ray diffraction pattern showed the presence of uranium monophosphide and traces of uranium dioxide. The mass spectrometric investigation was done with an instrument at the National Bureau of Standards similar to that described by Chupka and Inghram[6]. A tantalum Knudsen effusion cell with a tungsten liner was used. Temperatures were measured with a calibrated optical pyrometer under black-body conditions.

The vaporization of uranium monophosphide was investigated over the temperature range from 1,750°–2,250° K. During its vaporization the major primary ion species observed and measured were U^+, P^+, P_2^+, and UO^+. In a uranium monophosphide–uranium dioxide mixture, the UO_2^+ ion was also a major species. In no case was gaseous uranium monophosphide observed. X-ray analysis of the residue left in the effusion cell from the uranium monophosphide samples showed only lines of uranium monophosphide. Lines of uranium dioxide could no longer be detected. On the basis of these observations, we suppose that uranium monophosphide vaporizes congruently over the temperature-range measured according to the following reaction:

$$UP_{(s)} = U_{(g)} + \frac{1}{x} P_{x(g)} \; (x = 1 \text{ or } 2) \qquad (1)$$

Oxygen impurities in the uranium monophosphide sample are believed to react according to

$$UP_{(s)} + UO_{2(s)} = 2UO_{(g)} + \frac{1}{x} P_x \; (x = 1 \text{ or } 2) \qquad (2)$$

The observations that no uranium dioxide could be detected in the residue and that the UO^+ ion intensity decreased in relation to the U^+ ion intensity as the vaporization progressed indicate that reaction (2) is preferred to reaction (1). If the uranium dioxide content becomes large, reaction (2) becomes the predominant reaction. The appearance of the UO_2^+ ion resulting from the vaporization of uranium dioxide according to

$$UO_{2(s)} = UO_{2(g)} \qquad (3)$$

is also observed.

The ion intensities are correlated to vapour pressures through silver calibration[6,7]. The difference in electron multiplier efficiencies of Ag^+ and the ionic species observed above uranium monophosphide was determined experimentally. Corrections for fragmentation and double ionization were considered. The intensity of U^+ ion was corrected for the effect of oxygen content according to equation (2). For the ionization cross-section of the phosphorus monomer the value of 13.8 as given by Otvos and Stevenson[8] was used. For the phosphorus dimer the value 21.8 was estimated. A value of 37.5 was derived for the cross-section of uranium on the basis of congruency of vaporization according to equation (1) using the cross-section of the phosphorus monomer as a standard.

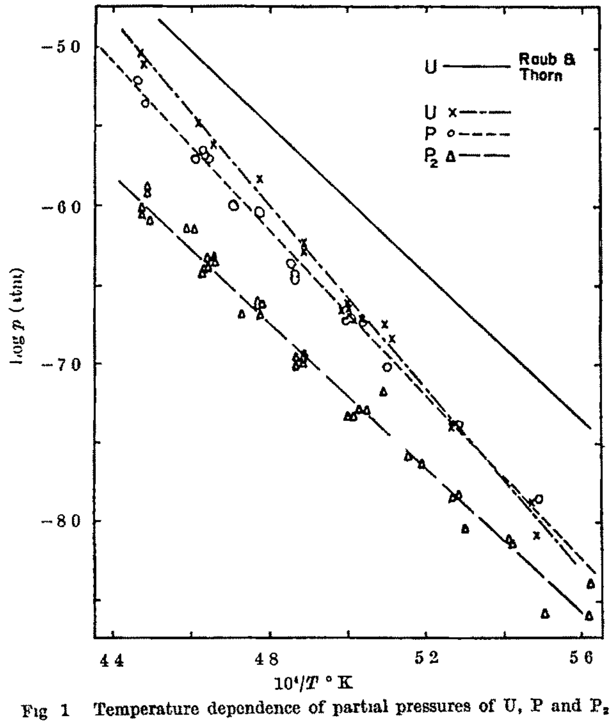

Fig. 1. Temperature dependence of partial pressures of U, P and P_2

In Fig. 1 the logarithm of the vapour pressure of each species over uranium monophosphide is presented as a function of the reciprocal of absolute temperature. The derived average values for partial molal heats of vaporization in kilocalories per mole, at 2,000° K, are as follows: $\Delta\bar{H}$ (U) = 131.69 ± 2.25, $\Delta\bar{H}$ (P) = 119.94 ± 2.63; $\Delta\bar{H}$ (P_2) = 105.46 ± 1.66, where the uncertainties represent standard deviations. From these values a heat of vaporization, ΔH° vap., in kilocalories per mole of uranium monophosphide, is obtained as 184.42 ± 3.08 for the reaction (1) when $x = 2$ and 251.63 ± 4.88 when $x = 1$. The temperature dependence of the partial pressures over the temperature range of investigation is linear within the limits of experimental errors and can be expressed by the following equations:

$$\log p_{(\mathrm{atm})}\,[P] = \frac{-26{,}211}{T} + 6.4107 \pm 0.0716$$

$$\log p_{(\mathrm{atm})}\,[P_2] = \frac{-23{,}046}{T} + 4.2550 \pm 0.1036$$

$$\log p_{(\mathrm{atm})}\,[U] = \frac{-28{,}778}{T} + 7.7950 \pm 0.0687$$

Also included in Fig. 1 is the calculated vapour pressure of uranium[9]. Due to the different temperature dependences of the uranium pressures of solid uranium monophosphide and liquid elemental uranium, the uranium dissociation pressure of uranium monophosphide will become equal to that of elementary uranium at 2,600° K. This implies that above 2,600° K the vaporization of uranium monophosphide becomes incongruent and can be expressed by equation (1) and equations (4) and (5):

$$UP_{(s)} = U_{(l)} + \frac{1}{x} P_x \; (x = 1 \text{ or } 2) \qquad (4)$$

$$U_{(l)} = U_{(g)} \qquad (5)$$

The congruency of the vaporization below 2,600° K may need to be modified to account for a deviation from stoichiometry that increases at high temperatures[4]. Indications of this in our work are supported by the decrease in lattice parameter from 5.589 ± 0.001 to 5.584 ± 0.001 Å of uranium monophosphide during vaporization and by the observation that there is a slight decrease in total vapour pressure and a decrease in the partial pressure ratio of phosphorus monomer and uranium as the vaporization proceeds. Possible secondary reactions with the container materials have not been considered in the evaluation. From the extrapolation of the total pressure to one atmosphere, a boiling point of uranium monophosphide of 3,890° K can be predicted. For uranium mononitride the decomposition temperature is approximately 3,110° K (ref. 10). As compared with uranium monosulphide[2], the total vapour pressure of uranium monophosphide in our temperature range is lower by a factor between three and ten. Uranium monophosphide thus appears to be a refractory material of high stability. This stability against vaporization and the apparent congruency of the vaporization process add to its potential usefulness as a nuclear fuel material at high temperatures. The similarity in vaporization behaviour and density, and the expected similarity in thermal expansion may make it a promising additive to uranium dioxide base nuclear fuel elements

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K A GINGERICH
P K LEE

Department of Chemistry,
The Pennsylvania State University,
University Park, Pennsylvania

J. EFIMENKO

U S National Bureau of Standards,
Washington, D C

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Effect of the Compressibility of the Carrier Gas on the Experimentally Determined Number of Plates of a Chromatographic Column

THE height-equivalent to a theoretical plate of a chromatographic column, either packed or tubular, can be represented by the general equation

$$h = A + B\frac{D_g}{v} + C_g\frac{v}{D_g} + C_l\frac{v}{D_l} \qquad (1)$$

where v designates the carrier gas velocity, D_g and D_l the diffusion coefficients of the particular component considered in the carrier gas and in the fixed phase, respectively, and where A, B, C_g and C_l are appropriate constants ($A = 0$ in tubular columns)

Experimentally, when the drop in pressure in a column is small compared with the gas pressure, the number of plates in a column, operated at constant gas flow and temperature, that is, the length of the column divided by h, can be determined from the relation

$$\frac{l}{h} \cong n^* = 8\ln 2\left(\frac{t}{\Delta t}\right)^2 = 5\ 54\left(\frac{t}{\Delta t}\right)^2 \qquad (2)$$

where t and Δt designate the elution time after injection and the width of the eluted band at mid-height, respectively

When the drop in pressure is not negligibly small, the fourth term in the right-hand side of equation (1), which varies with v, that is, inversely with the pressure, will cause h to vary with the pressure If, however, this last term is negligibly small, h is independent of the pressure, since both v and D_g vary inversely with the pressure In this case, the number of plates in the column as defined here remains a well-defined quantity, but relation (2) must be modified to take care of the various effects due to the gas compressibility The calculations are as follows The velocity v at any point in the column is determined by the relation

$$v = \frac{\mathrm{d}p}{\mathrm{d}x}\frac{1}{r} = v_0 \times \frac{p_0}{p} \qquad (3)$$

where $\frac{\mathrm{d}p}{\mathrm{d}x}$ = pressure gradient, r = pneumatic resistance per unit length, v_0 = gas velocity near column exit, p_0 = pressure at exit (usually atmospheric pressure) Since r is proportional to the carrier gas viscosity, which is substantially independent of pressure, r may be regarded as constant throughout a uniform column

Integration of equation (3) gives p as a function of inlet and exit pressures, and distance from the inlet of a column of length l

$$p^2 = p_1^2 + \frac{x}{l}(p_0^2 - p_1^2)$$

The average pressure and average squared pressure in the column can be obtained immediately from the relation just written by appropriate integrations

$$\bar{p} = \frac{2}{3}\frac{p_1^3 - p_0^3}{p_1^2 - p_0^2}, \quad \overline{p^2} = \frac{p_1^2 + p_0^2}{2}$$

The time of travel of a sample for which the column has a capacity ratio k is

$$t = (1 + k)\int_0^l \frac{\mathrm{d}x}{v} = (1 + k)\frac{1}{v_0p_0}\int_0^l p\mathrm{d}x =$$
$$\frac{2}{3}\frac{1 + k}{v_0p_0}\frac{p_1^3 - p_0^3}{p_1^2 - p_0^2}\,l \qquad (4)$$

As a sample packet is carried along the column from higher to lower pressures, its second moment (variance) is increased by the combined effects of diffusions and isothermal expansion This latter effect can be equated out of the mathematical treatment by considering the specific second moment of mass, w, an increment of which is given by

$$\mathrm{d}w = p^2\ \ \mathrm{d}u = p^2h\mathrm{d}x$$

since a sudden isothermal expansion of a gas packet leaves w invariant Integration over the entire column length gives

$$w = h\int p^2\mathrm{d}x = h\,\frac{p_1^2 + p_0^2}{2}\,l$$

At the column exit, w is related to the observed bandwidth at mid-height by

$$\Delta t^2 = 5\ 54\ (1 + k)^2\,\frac{w}{p_0^2v_0^2} =$$
$$5\ 54\ (1 + k)^2\,\frac{p_1^2 + p_0^2}{2p_0^2v_0^2}\,lh \qquad (5)$$

Equations (2), (4) and (5) give for n^* as a function of the actual number of plates, $n = \frac{l}{h}$

$$n^* = \frac{8}{9}\,\frac{(p_1^3 - p_0^3)^2}{(p_1^2 - p_0^2)^2(p_1^2 + p_0^2)}\,n \qquad (7)$$

As defined by equation (2), n^* is directly related to the square of the resolution, and the coefficient of n in the right-hand side of (7) corresponds to the slight loss of resolution which is incurred when operating a column for which C_l is negligible with a large drop in pressure This coefficient is unity when $p_1 - p_0$ is negligibly small if compared to p_0, and approaches $\frac{8}{9}$ monotonously as p_1 increases indefinitely

MARCEL J E GOLAY

Technische Hogeschool,
Eindhoven

Hydrogen-bond Energies in the Crystalline State

MUCH interest and significance pertain to the energy of the hydrogen-bond molecular interaction present in many gaseous, liquid and solid systems[1] There are very few reliable estimates for its magnitude in the crystalline state, where its role is often a predominant feature of the lattice structure

Systematic measurements of the molar sublimation heats[2] which are very close to, if not identical with, the lattice energies per mole, allow more direct estimation of these hydrogen-bond energies than has been possible heretofore For the even-carbon members of the straight-chain monohydric alcohol series (from C_8 to C_{22}) all in the same isostructural polymorphic form, we find in kcal/mole

$$\Delta H_{\text{subl}} = 10\ 0 + 1\ 80\ n$$

where n is the 'carbon number' $H(CH_2)_n\,OH$ Putting $n = 0$ gives the total head-group contributions to the lattice energy, that is, the sum of the contribution due to the (OH) group and that arising from the difference between a terminal methyl (CH_3) and a chain methylene (CH_2) group The (CH_2) contribution is clearly 1 80 kcal/mole

In the heats of evaporation of the normal paraffins[3] the difference in the (CH_3) and (CH_2) contributions is 0 6 kcal/mole This difference can be expected to increase for the process of sublimation, and other considerations suggest that then ΔH_{subl} (CH_3) − ΔH_{subl} (CH_2) = 1 0 ± 0 2 kcal/mole This leads to ΔH_{subl} (OH) = 9 0 ± 0 2 kcal/mole

To deduce the energy increment due to the hydrogen-bonding condition of the (OH) group in these compounds, we still require an estimate of its lattice energy when not involved in hydrogen-bonding, but otherwise similarly placed to the (OH) group in the alcohols A definite minimum value for this 'free (OH) group' lattice energy has been deduced as 1 4 kcal/mole from the difference of the sublimation heats of 2,4 6-tri-*tert* butyl phenol and 2,4,6-tri-*tert* butyl benzene In this highly sterically hindered phenol the (OH) group is completely protected from hydrogen-bonding even in the crystalline state[4] A maximum of 2 8 kcal/mole is indicated for the same factor, for example, by ΔH_{subl} (CH_3), and a most probable value is 2 2 ± 0 4 kcal/mole Accordingly, the hydrogen-bond energy is (9 0 ± 0 2 − 2 2 ± 0 4), that is, 6 8 ± 0 6 kcal/mole

Again, for the straight-chain even-carbon monocarboxylic acids[5] and their methyl esters, we find, in kcal/mole

$$C_nH_{2n+1}COOH \quad \Delta H_{\text{subl}} = 14\ 2 + 1\ 50\ n$$

$$C_nH_{2n+1}COOCH_3 \quad \Delta H_{\text{subl}} = 7\ 7 + 1\ 95\ n$$

Both these series of compounds crystallize in a similar head-to-head layer lattice structure and so it is reasonable to suggest

$$\Delta H_{\text{subl}}\,(COOH) - \Delta H_{\text{subl}}\,(COOCH_3) = 14\ 2 - 7\ 7 = 6\ 5 \text{ kcal/mole}$$

In the absence of hydrogen-bonding in the acids, the difference (in the reverse direction) would arise from the replacement of (O—H) by (O—CH_3) This difference (essentially the lattice energy of the CH_3 group) is estimated to be 2 4 ± 0 4 kcal/mole, and this leads to 6 5 + 2 4 = 8 9 ± 0 4 kcal/mole for the carboxylic acid hydrogen-bond energy in the crystal

The energy increase relative to the alcohols (8 9 and 6 8 kcal/mole) is expected thus, in crystalline hexadecanol[6] the oxygen–oxygen distance (O–H O) is 2 71 ± 0 02 Å, in crystalline formic acid it is 2 58 Å and in dodecanoic acid[7] it is 2 56 Å Likewise, in the vapour of formic acid (HCOOH) the hydrogen-bond energy is 7 06 kcal/mole[8] that this becomes 8 9 kcal/mole in the crystalline state for the higher acids conforms to the shortening of the (O–H O) distance between the vapour state of the dimer and the crystal[9] by 0 15 Å A similar shortening with change of phase occurs for acetic acid

It may be mentioned that the difference[10] ΔH_{subl} (C_6H_5OH) − ΔH_{subl} (C_6H_6) is 16 1 − 10 5 = 5 6 kcal/mole correcting this for the 'free (OH) group' contribution by the figure already quoted for the difference between 2,4,6-tri-*tert* butyl phenol and 2,4,6-tri-*tert* butyl benzene (1 4) suggests 4 2 kcal/mole as the hydrogen-bond energy in crystalline phenol This is essentially the same as the value (4 3 kcal/mole) for hydrogen-bonded phenol in carbon tetrachloride

Further confirmation of the foregoing 'solid state' hydrogen-bond energies comes from measurements of the activation enthalpies ΔH^* involved in the kinetic process of their rupture, and so necessarily higher than their equilibrium ΔH values In crystalline *cyclo*pentanol and *cyclo*hexanol, dielectric studies[11] have given ΔH^* = 9 6 and 10 4 ± 0 4 kcal/mole for the bond in the alcohols, while ultrasonic data[12] give ΔH^* = 10 5 kcal/mole for the benzoic acid dimer

MANSEL DAVIES
BRIAN KYBETT

Edward Davies Chemical Laboratories,
University College of Wales,
Aberystwyth

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Quantitative Gas-liquid Chromatography of the Higher Fatty Acids

THE efficient separation of the higher fatty acids on polyester substrates[1–3], particularly with the addition of phosphoric acid, has been established for some time The quantitative aspects of such analyses have not, however, been stressed, since in addition to association affecting the response of thermal conductivity detectors, adsorption of various types in the column may occur[4]

The direct analysis of volatile fatty acids in dilute aqueous solutions on a quantitative basis has recently been shown to be possible through addition of formic acid vapour to the carrier gas, thus effectively suppressing adsorption of the weaker acids on the support and possibly in the substrate as well[5] This procedure can only be carried out with a flame ionization detector, since in this detector formic acid gives no response[6], but the presence of the formic acid does not affect the response characteristics of other carbon compounds

The extension of this quantitative technique to the higher fatty acids, using an efficient polyester substrate to give separations of the unsaturated acids equivalent to those obtained with their methyl esters, has been found to be practical

A flame ionization detector and simple device for adding formic acid vapour to the carrier gas were utilized as previously described[5], excepting that a pipping-pen integrator was used Although a low percentage of polyester would speed analyses[2,4], an available column of 20 per cent ethylene-glycol succinate (EGS) on 80–100 mesh 'Chromosorb *W*' was operated at 212° C with helium as the carrier gas at 20 lb /in ^{2} input for fatty acid analyses and at reduced flow (7 lb /in ^{2}) for certain ester analyses

Freshly prepared fatty acids from linseed oil, and their methyl esters prepared by the boron trifluoride procedure[7], were used in the present study Iodine values (Wijs) were determined by the Official Methods of the American

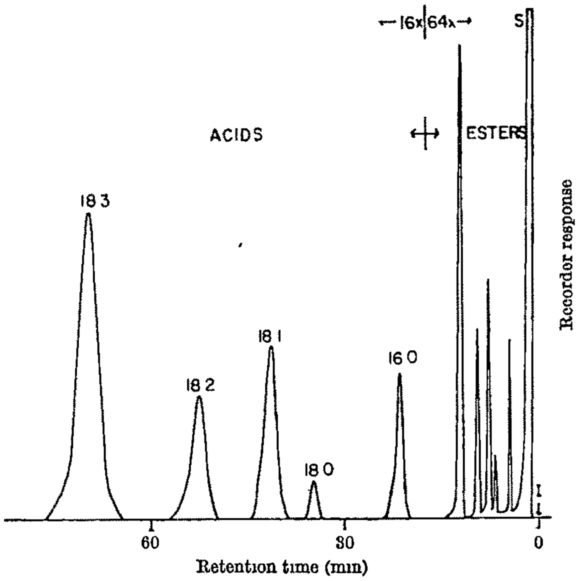

Fig 1 Simultaneous analysis of fatty acids from linseed oil and their methyl esters Column (6 ft × 1/8 in outer diam stainless steel, 20 per cent EGS on 80–100 mesh 'Chromosorb *W*') at 212° C utilizing helium carrier gas at 20 lb /in ² with added formic acid vapour Ester peaks are in the same order as acid peaks

Oil Chemists' Society All procedures were carried out in inert atmosphere. The esters and acids were injected in carbon disulphide solution (2 5 per cent w/v), samples of 0 005 ml being commonly used

With plain carrier gas repeated injections of the fatty acids failed to give any response on the recorder, indicating complete adsorption on the column When formic acid was added to the carrier gas reproducible analyses of acids were immediately obtained (Fig 1), although the previously adsorbed acids were not displaced by the formic acid in any obvious manner[5].

Since the acids and esters examined did not overlap (Fig 1, Table 1) it was feasible to carry out a simultaneous analysis of both materials For quantitative studies, however, the esters were also analysed (in the presence of formic acid vapour) at the reduced flow rate in order to give larger peaks The compositions were then calculated (Table 2) and from these were computed the iodine values, in reasonable agreement with the Wijs iodine values The ratios of the peak heights for the acids and esters when analysed simultaneously (Fig 1) were identical within ± 2 per cent, indicating that proportionality was maintained The individual counts for these ester peaks, although subject to high error owing to the few counts available, indicated on the average approximately equal weight response for the acids and esters Although the response should be slightly higher for the esters these observations all indicate that no significant loss of esters or acids occurred

Table 1 TYPICAL RETENTION TIMES* (MIN) FOR ANALYSES OF ACIDS AND ESTERS

Materials	Acids	Esters	
		With acids	Reduced flow
Palmitic	19 7	3 2	11 8
Stearic	32 6	5 5	19 6
Oleic	38 8	6 5	23 2
Linoleic	49 3	8 3	29 3
Linolenic	65 5	11 0	38 6

* Adjusted (from first solvent disturbance)

Table 2 PERCENTAGE OF COMPONENTS, AND IODINE VALUES (I V), AS INDICATED BY AREA RESPONSE FOR DIFFERENT ANALYSES OF ACIDS AND ESTERS

Materials	Acids		Esters	
Palmitic	8 7	7 4	8 7	8 6
Stearic	2 9	3 3	2 9	2 7
Oleic	17 5	17 4	16 3	17 7
Linoleic	16 8	16 5	15 7	16 0
Linolenic	54 8	55 4	56 4	55 1
I V (calc)	196	197	188	186
I V (Wijs)	199		188	

Theoretical plate numbers[8] as determined for linolenic acid and ester peaks in the mixed analysis were identical (about 2,400) within experimental error The other ester peaks were too narrow to permit accurate measurement The comparable ester peak at reduced flow gave a lesser value (about 900) and palmitic acid and ester peaks under the two different operating conditions gave proportionate values of approximately 2,000 and 700

Table 3 SEPARATIONS OBTAINED IN VARIOUS ANALYSES OF ACIDS AND ESTERS

Materials	Acids			Esters			
				With acids		Reduced flow	
Stearic/palmitic	1 65	1 66	1 64	1 71	1 70	1 70	1 70
Oleic/stearic	1 20	1 19	1 18	1 18	1 17	1 18	1 18
Linoleic/oleic (6/9)*	1 28	1 28	1 28	1 27	1 27	1 27	1 27
Linolenic/linoleic (3/6)*	1 32	1 34	1 32	1 33	1 33	1 32	1 32
Linolenic/oleic (3/9)*	1 68	1 70	1 69	1 69	1 68	1 67	1 67

* See ref 11

In examining the separations between various acids (Table 3), the slightly lower stearic/palmitic separation with the acids in contrast to the esters parallels the different vapour pressure ratios obtained for these materials at lower temperature[9] If the oleic/stearic separation is slightly less with the esters this may be due to lengthening of the chain, since the saturated and mono-unsaturated ester log plot lines[10] converge for this polyester in this temperature range as fatty acid chain length increases The systematic type II separation factors for the unsaturated acids and esters are, as predicted[11], virtually identical in all cases

R G ACKMAN
R D BURGHER
J C SIPOS

Technological Research Laboratory,
Fisheries Research Board of Canada,
Halifax, Nova Scotia

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Effect of Ultrasonic Radiation on the Crystallization of Polyethylene from Dilute Solution

THERE is much interest at present in the morphology of polymer single crystals grown from dilute solution, and, using polyethylene, Bassett and Keller[1] have recently attempted to systematize the relationship between crystal habit and preparative method In some of their experiments involving quenching, crystals of an unfamiliar type were sometimes observed. These took the form of necklaces very similar to those prepared by van der Heijde[2] in his examination of the crystallization of polyoxymethylene from dilute solution

In our experiments, 'Rigidex 50' polyethylene with $\overline{M}_w$ of $1\ 12 \times 10^6$ and a branching frequency of less than 1 per 1,000 was crystallized by pipetting 1 ml of a 0 5 per cent wt. solution in boiling *p*-xylene into a suitable volume of *p*-xylene thermostated at the appropriate temperature in an ultrasonic field Figs 1 and 2 show the general appearance under the electron microscope of the crystals from the resulting suspension The necklaces consist of roughly hexagonal platelets strung along a central filament In none of the preparations was the normal lozenge-shaped crystals totally absent, but they could have been formed as the suspensions cooled to room temperature in the absence of ultrasonic radiation The characteristic necklaces were produced over a range of

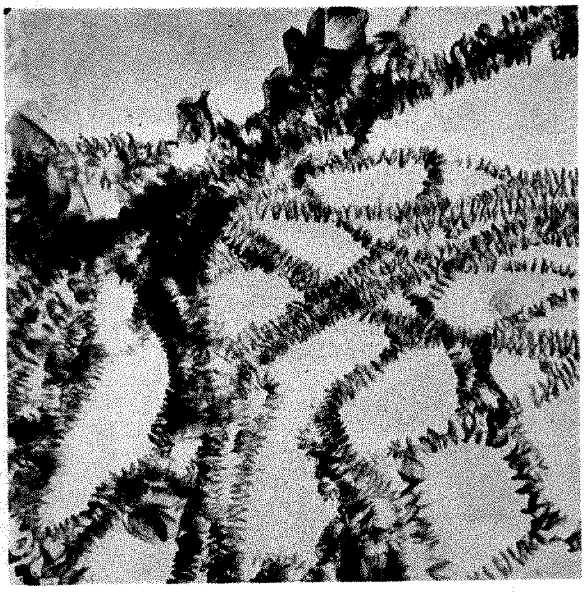

Fig. 1. Polyethylene. Gold–palladium shadowed at 30° C. (× 4,000)

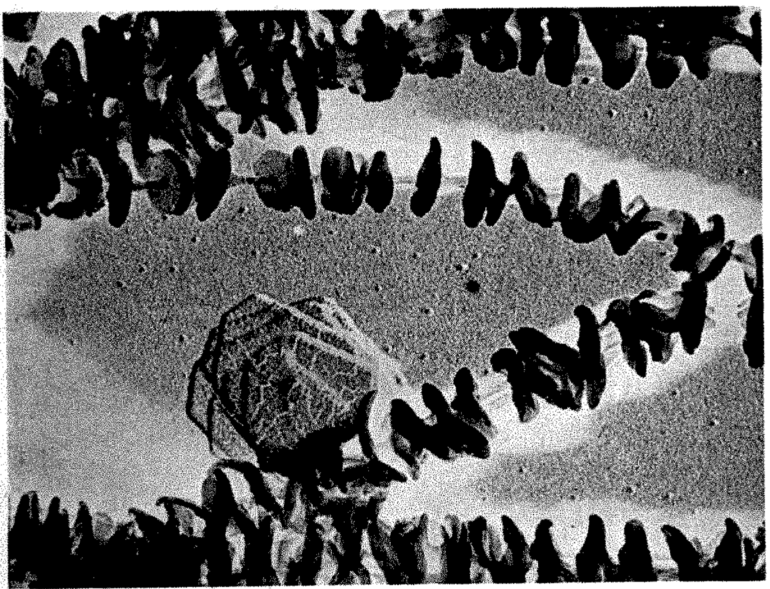

Fig. 2. Polyethylene. Gold–palladium shadowed at 30° C. (× 24,000)

conditions: (1) 0·05–0·1 per cent wt. solution, (2) 85–190 kc/s, (3) 82°–88° C, (4) 0·1–0·4 mW/cm^2. The experiments showed that 0·1 mW/cm^2 is the minimum power sufficient to produce the new crystals, but the other conditions do not necessarily represent limits. Niegisch[3] has produced similar forms at 75° C and 20 kc/s from dilute solutions of 'Marlex 50' polyethylene. The method of preparation we describe is evidently not unique, but polyethylene crystals of the new morphology are certainly typical of our technique which favours their production. Using a sample of Du Pont 'experimental polyoxymethylene' under similar conditions we were not able to produce necklaces though other anomalous crystals were detected.

At this stage it is not possible to say whether the effect of ultrasonic radiation on the morphology of polymer crystals is due primarily to a modification of the nucleation process or of the crystal growth stage, but the answer to this question is clearly relevant to the formulation of theories of crystallization mechanisms. We are at present using ultrasonic techniques as a means of investigating the crystallization process, and the observations reported here form part of a more general investigation.

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D. A. Blackadder
H. M. Schleinitz

Department of Chemical Engineering,
University of Cambridge.

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BIOPHYSICS

Molecular Lesions produced in Ribonuclease by Gamma-rays

Crystalline enzymes are very sensitive to inactivation by ionizing radiations. In general, one ionization within an enzyme molecule can result in the loss of catalytic activity[1-3]. Although these primary ionizations occur randomly within the molecule there is evidence from electron spin resonance investigations[4] that certain groups are preferentially damaged. There is, however, a scarcity of knowledge about the nature of the molecular lesions produced by ionizing radiation in biological macromolecules, and the forms of molecular damage responsible for the observed loss in enzyme activity are unknown.

Experiments are reported here which suggest that the direct action of ionizing radiation produces structural changes such as the breakage of certain disulphide bonds, and molecular conformity changes so that 'latent' tyrosyl groups become easily ionizable.

We have examined the radiosensitivity of ribonuclease (RNase) and the results have been reported elsewhere[3]. RNase is particularly well suited for studies on molecular damage, since a great deal is known about its structure. The amino-acid sequence of RNase has been determined[5], and it is known to possess four disulphide bridges[6]. The disulphide bridges are important in determining the conformation of the molecule, and their breakage results in loss of enzyme activity[7]. It appears, however, that not all the disulphide bridges are functionally important[8].

There is evidence that disulphide bridges may be broken in RNase[9] and in insulin[10] by ionizing radiation. We have assayed for the appearance of sulphydryl groups in samples of dry, purified, crystalline ribonuclease, irradiated in sealed tubes to a dose of 30 Mrads. This dose reduces the activity in air to 25 per cent.

The sulphydryl groups were titrated by the method of Boyer[11] using *para*hydroxymercuribenzoate (*p*OHMB) (Sigma Chemical Co., formerly catalogued as *para*chloromercuribenzoate (*p*CMB)). Increasing concentrations of ribonuclease were added to a constant concentration of *p*OHMB at *p*H 6·80 and the optical density at 255 mμ read at time zero. The optical density of irradiated RNase controls was also read at this time. The reaction vessels were closed and left for 12 h, at which time they were shaken and the optical density read. The results of an assay for RNase irradiated with 30 Mrads in air are shown in Fig. 1. The optical densities of the irradiated RNase were subtracted from those of the mixture to give a curve which represents the ultra-violet absorption due to the mercaptide formed from the combination of the *p*OHMB with sulphydryl groups. The equivalence point or 'knee' of the titration curve represents the point at which all the *p*OHMB is utilized. From this one can

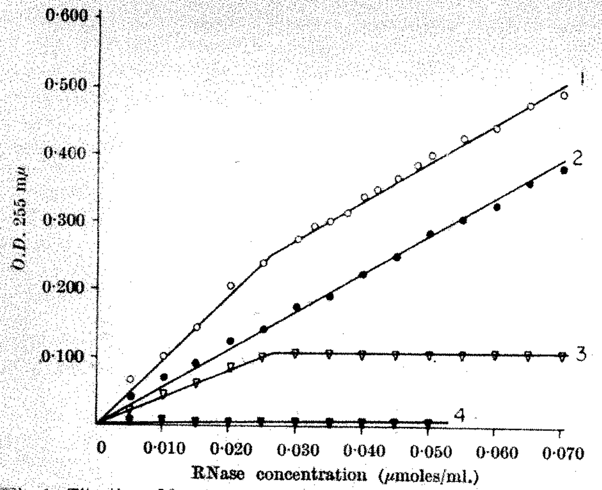

Fig. 1. Titration of free SH groups in RNase after a dose of 30 Megarads in air. The optical density (*O.D.*) is measured against that of a *p*OHMB blank. The knee of curve 3 represents equimolar concentrations of free SH groups and *p*OHMB

1, *p*OHMB (0·020 μmole/ml.) and irradiated RNase; 2, irradiated RNase; 3, 1 minus 2; 4, as in 3 for native RNase.

calculate the average number of radiation-produced, free SH groups per RNase molecule, and also the *G* value or yield of SH groups per 100 eV of γ-radiation absorbed. These are shown in Table 1 together with the yields for loss in enzyme activity under the same conditions. No free SH groups were detected in the native RNase. The appearance of SH groups following irradiation in air was also assayed at *p*H 4·5, and a slightly lower *G* value of 0·72 was obtained.

Table 1. Radiochemical Yields in RNase

Alteration observed	Units	Gaseous conditions during irradiation† O_2	N_2	NO
RNase molecules inactivated	/100 eV	3·00	1·53	0·93
Free SH* groups	/molecule	0·75	0·58	0·51
Disulphide bridges broken	/100 eV	0·95	0·67	0·63
Tyrosine groups unmasked	/100 eV	3·00	—	—

* Dose, 30 Megarads.
† Initial rate of breakage.

An experiment has been performed which shows that the total number of disulphide groups which are available for assay after chemical reduction remains unchanged following the irradiation. This suggests that the sulphur–sulphur bonds of the cystine groups (disulphide bridges) are broken by irradiation of RNase in the dry state, and it seems unlikely that C—S bonds of either cystine or methionine groups are broken.

The average titre of SH groups produced increased with dose, and appeared to approach an average of two per molecule. This is compatible with the assumption that only one disulphide bond per average molecule may be broken by the radiation.

RNase has six tyrosyl (phenolic) groups, three of which ionize easily with a *pK* of 10 and three of which are hidden within the molecule and are not readily ionized[12]. These ionized tyrosyl groups absorb strongly in the region of 295 mμ, and one can titrate them spectrophotometrically. Changes in the titration curve of these tyrosyl groups should indicate structural changes within the molecule.

Ionizing radiation caused a hyperchromic effect in the ultra-violet absorption curve of ribonuclease. Elevation of the hydrogen ion concentration also produced a hyperchromic effect (Fig. 2). The hyperchromic effect due to *p*H elevation was more marked in the region 290 mμ for irradiated ribonuclease than for the native enzyme, which suggested that tyrosyl group ionization was being changed. Spectrophotometric titrations at 295 mμ were carried out for native and irradiated ribonuclease after the method of Tanford[12]. The results are shown in Fig. 3. The increase in the extinction coefficient of native ribonuclease takes place in two equal steps, and Tanford interprets this as representing the ionization of three exposed tyrosyl groups followed, at higher *p*H, by the ionization of three tyrosyl groups which are hidden within the molecule. The ionization curve of irradiated ribonuclease is different, in that the first step is higher. This indicates that some latent tyrosyl groups have been unmasked so that they ionize at lower hydrogen ion concentrations. There is a striking similarity between the titration curve for irradiated RNase and that reported for RNase[13] in which hydrogen bonds were broken by guanidine hydrochloride.

At present, it is not possible to make a direct correlation between disulphide bond breakage and loss in enzyme activity although disulphide bonds might be important to enzyme activity[7]. The yield for sulphydryl group appearance is generally considerably lower than that for loss in enzymatic activity, so disulphide breakage may at the most be responsible for a fraction of the total

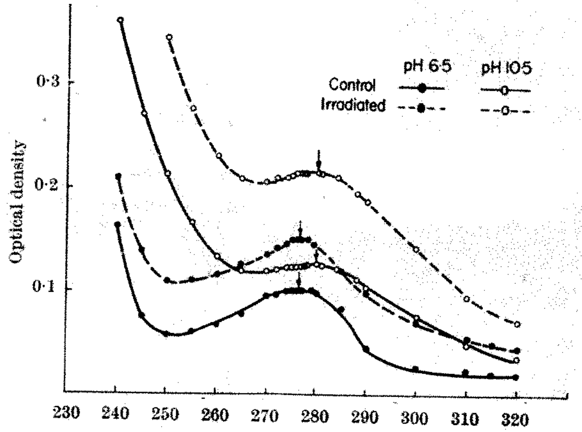

Fig. 2. Ultra-violet absorption spectra of irradiated and native RNase. Note the hyperchromic effect in the irradiated samples (dashed lines) after a dose of 30 Megarads in air, and the enhanced effect at higher *p*H. Enzyme concentration, 10^{-5} M

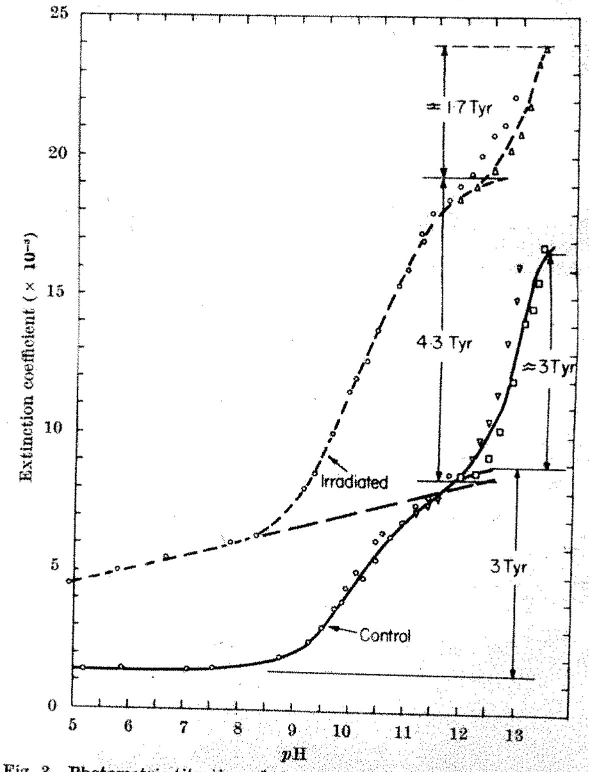

Fig. 3. Photometric titration of phenol (tyrosine) groups in native and irradiated RNase. After 30 Megarads, the number of unmasked tyrosine groups have increased from 3 to 4·3

Groups in RNase 295 mμ; absorbed dose, 30 Mrads damage. The possible exception to this is for irradiation in nitric oxide where both yields are of similar magnitude

It is possible that the appearance of the unmasked tyrosyl groups accompanies the breakage disulphide bridges, that is, the three tyrosyl groups are unmasked as a result of the breakage of a single disulphide bridge Tanford has suggested that the tyrosyl groups themselves might be involved in hydrogen bonding and that such bonds could be important in maintaining the tertiary structure of the molecule

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J F Williams
J W Hunt

Department of Medical Biophysics,
University of Toronto, and
Ontario Cancer Institute

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An Alpha-globulin Allotype in the Mouse (MuB1)

Polymorphism, detected by antibody from individuals of the same species (allotypy), has so far been observed only in the γ- (ref 1) and β-globulins (ref 2) In man, a class of α-globulin *Gc*1 and *Gc*2 is subject to genetic variations and has been revealed by antibodies of heterologous origin[3] Polymorphism of other α-globulins, the haptoglobins in several mammalian[1] species and recently the trypsin inhibitors in man[4,5] have been detected by other means So far, the polymorphism of serum proteins has been most thoroughly examined in man and rabbit In the mouse the genetic variation of the transferrins[6] and of an allelic pair of γ-globulins (*MuA*1 and *MuA*2) (refs 7–10) have been examined We wish to report here a new allotypic specificity among the α-globulins of the mouse

Antibody was induced in *DBA/2J* and *A/HeJ* mice immunized with the serum of *DBA/1J* and *C57L/J* mice, respectively Mice were given subcutaneously, or into the foot pad, six injections of serum incorporated in complete Freund's adjuvant On the fifth day after the last injection, the animals were bled from the tail, and the sera from the two groups of animals (*DBA/2J* and *A/HeJ*) were pooled separately Both pools of sera reacted in an identical manner, giving one zone of precipitation in double diffusion with the serum of *BALB/cJ*, *CBA/J*, *C57BL/10J*, *C57Br/cdJ*, *C58/J*, *C57L/J*, *129/J*, *SJL/J*, *C3H/HeJ* and *DBA/1J* mice The specificity was absent in mice of the *A/HeJ*, *A/J*, *AKR/J*, *RF/J*, *SWR/J* and *DBA/2J* strains A number of hybrid strains were also tested *AKR/J* × *DBA/2J*, *C57BL/6J* × *A/J*, *C57BL/6J* × *DBA/2J*, *BALB/cJ* × *A/J*, *C57L/J* × *A/HeJ*, *C3H/HeJ* × *DBA/2J*, *C3H/HeN* × *C57BL/6J*, and only the hybrid *AKR/J* × *DBA/2J* was found to lack the allotypic specificity under investigation In short, all hybrids having in their ancestry one or two of the strains containing the allotypic specificity also possessed the allotypic specificity in their serum

In all cases, 3 or more males and 3 or more females were tested for the presence of the allotypic specificity and in all cases the distribution of the allotypic specificity in males and females was identical

In addition to inbred mice examined, forty 'Swiss' mice, from the Connaught Laboratories and from our own colony, were found to possess the allotypic specificity under investigation Five serum samples of woodland deer mouse (*Peromyscus maniculatus gracilis*) were also examined and found to give a reaction of partial identity with the allotypic specificity of strains such as *DBA/1J* These samples did not produce any detectable reaction with a previously described *MuA*2 antiserum.

The antibody from *DBA/2J* and *A/HeJ* mice was shown by immunoelectrophoresis to react with an α-globulin, the mobility of the α-globulin was located by comparison with that of some well-defined human proteins (Fig 1) Since it has been previously proposed to call the γ-globulin allotypes *MuA*1, *MuA*2, MuA_n[8,9], we now propose to call the α-globulin family of allotypic specificities *MuB* and the specificity here described *MuB*1

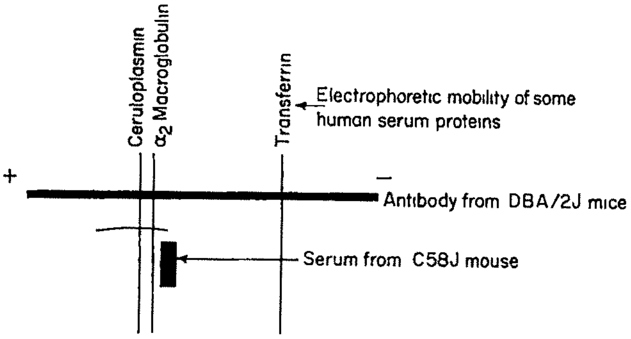
Ceruloplasmin
α2 Macroglobulin
Transferrin
Electrophoretic mobility of some human serum proteins
+
–
Antibody from DBA/2J mice
Serum from C58J mouse

Fig 1 Electrophoretic mobility of the α-globulin *MuB*1 in relation to human ceruloplasmin, transferrin and α-macroglobulin

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B Cinader
S Dubiski

Subdivision of Immunochemistry,
Division of Biological Research,
Ontario Cancer Institute, and
Departments of Medical Biophysics and
Pathological Chemistry,
University of Toronto

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BIOCHEMISTRY

X-Irradiation of Deoxyribonucleic Acid

One of the biochemical effects of X-irradiation is the inhibition of synthesis of DNA Although Okada and Hempelmann[1] showed that, after ionizing radiation, there is an inhibition of the activities of enzymes essential for DNA synthesis, the experiments of Bollum *et al*[2] indicate that regenerating rat liver can still synthesize thymidylic kinase and DNA polymerase in tissues where DNA synthesis has been suppressed by X-irradiation Walwick and Main[3] reported that the enzymes catalysing synthesis of DNA are unaffected by X-radiation at dose-levels (100–5 000 rads) ordinarily encountered in radiation biology. Stacey[4] found that the ability of X-irradiated DNA to 'prime' the action of polymerase is reduced, but only by relatively high doses

This communication describes another feature of the effect of X-irradiation of DNA, namely, a loss of ability of Ehrlich ascites carcinoma cells to take up irradiated DNA and an increased susceptibility of DNA to DNase after X-irradiation

DNA labelled with carbon-14 used in these experiments was prepared as follows Ehrlich ascites carcinoma cells from 8 mice, 5 days after inoculation, were collected by centrifugation and washed with saline free from erythrocytes, yielding about 7 ml of packed cells The ascites cells were incubated in 120 ml of isotonic Krebs–Ringer medium containing 2 4 m moles of *tris* buffer, *p*H 7 4, and 8 μc of thymidine-2-^{14}C (66 4 μg) for 3 h at 37° shaken aerobically in a Research Specialties Co water bath shaker After incubation, the ascites cells were collected by centrifugation and washed with saline DNA of the ascites cells was isolated by the method of Marmur[5] up to RNase treatment and then by the method of Kay, Simmons and Dounce[6] onwards from step 8 About 3 mg of DNA were obtained from 1 ml of packed ascites cells, yielding 26,000–30,000 c p m /mg DNA Heated DNA samples were prepared by keeping a solution of ^{14}C-DNA (0 3 mg per ml 0 01 M sodium chloride) at 100° for 10 min and then cooling it in an ice bath. X-irradiated DNA samples were prepared by irradiating (500 rads for 5 min) a solution of ^{14}C-DNA (0 3 mg per ml 0 01 M sodium chloride) in a Petri dish 1 ml (0 3 mg) of X-irradiated ^{14}C-DNA was used for each experiment

For the examination of cellular uptake of ^{14}C-DNA, 0 1 ml of Ehrlich ascites cells was incubated in 3 0 ml of isotonic Krebs–Ringer medium containing 60 μmoles of phosphate buffer, *p*H 7 4, and 0 3 mg of ^{14}C-DNA in an Erlenmeyer flask shaken aerobically in a water bath shaker for 60 min at 37° After incubation the contents of the flask were centrifuged, washed twice with 10 ml each of saline and once with 95 per cent ethanol, ethanol/ether (3 1) and then ether The residues were then suspended in a small amount of chloroform/ether (4 1), plated on aluminium planchets, and their radioactivities were counted The control counts of radioactivity, in which ^{14}C-DNA and ascites cells in the same medium were mixed in ice and washed immediately as already described, were subtracted from the counts found in experiments in which the incubation was carried out for 60 min at 37°

Table 1 CELLULAR UPTAKE OF ^{14}C-DNA BY EHRLICH ASCITES CARCINOMA CELLS

^{14}C-DNA preparation	Uptake (counts/2 min)
Native DNA	290
Heated DNA	139
X-irradiated DNA	0
Native DNA + DNase	0

As shown in Table 1 when ^{14}C-DNA is X-irradiated with 500 rads for 5 min no uptake is observed When heated DNA is used instead of native DNA the uptake is reduced The uptake is also reduced after treatment of DNA with pancreatic DNase (8 μg/ml) However, when DNA is radiated in the presence of 1 mM cysteine, the activity taken up by ascites cells is almost undiminished In the present experiments the localization of ^{14}C-DNA in the ascites cells was not studied This is now under investigation

The increased susceptibility of DNA to DNase activity after X-irradiation, or heating, was shown in the following manner 0 3 mg of ^{14}C-DNA was incubated in 2 0 ml of medium containing 80 μmoles of *tris* buffer, *p*H 7 4, 10 μmoles of magnesium chloride and 0 5 ml of 1 5 ascites homogenate in water for 60 min at 37°. After incubation, 1 ml of 30 per cent trichloroacetic acid (TCA) was added and the contents were centrifuged The clear supernatant was extracted three times with 6 ml each of ether to remove TCA Aliquots of the aqueous phase were plated and their radioactivities counted As shown in Table 2, DNase in ascites cells hydrolyses heated and X-irradiated DNA faster than native DNA The results indicate that not only is there a loss of ability of Ehrlich ascites carcinoma cells to take up irradiated DNA but also that a change occurs in DNA after X-irradiation whereby its susceptibility to DNase activity is increased

Table 2. DNASE ACTIVITY OF ASCITES CELLS WITH DIFFERENT SAMPLES OF DNA

^{14}C-DNA used as substrate	DNase activity total counts in TCA-soluble fraction
Native DNA	1,572
Heated DNA	3,060
X-irradiated DNA	2,310

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SHAN-CHING SUNG
J H QUASTEL

McGill-Montreal General Hospital Research Institute,
3619 University Street,
Montreal

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Participation of Purine and Pyrimidine Nucleotide Sequences in the Molecule of Calf Thymus Deoxyribonucleic Acid

A METHOD was recently developed in this laboratory by which it was possible to degrade DNA chemically to sequences of purine nucleotides[1,2] During this procedure the DNA is first freed of pyrimidines by heating with anhydrous hydrazine and then degraded in alkali, the fragments formed are separated by chromatography on DEAE cellulose in a lithium chloride gradient Accurate quantitative analysis revealed that most purine nucleotides occur in calf-thymus DNA in sequences of two or more purine nucleotides[3] The results make it possible to calculate the frequency of occurrence of the individual purine nucleotide sequences in the DNA molecule (Table 1)

The frequency of occurrence of the individual sequences corresponds to the probability of occurrence of analogous sequences in a model system arranged as follows A certain number (n) of nucleotides of the same type (marked a) is arranged in all possible ways so that the number of nucleotides in every arrangement would be equal to n and so that no arrangement would be repeated, the sequence of the individual units being of no concern Thus, for example, for $n = 5$ a total of 7 arrangements are possible, namely, $aaaaa$, $aaaa+a$, $aaa+a+a$, $aaa+aa$,

Table 1 DISTRIBUTION OF PURINE AND PYRIMIDINE* NUCLEOTIDES IN CALF-THYMUS DNA

Sequence	Purine nucleotide clusters: % of total purine nucleotides in this type of sequence	Purine nucleotide clusters: Frequency of occurrence of the cluster (%)	Pyrimidine nucleotide clusters: % of total pyrimidine nucleotides in this type of sequence	Pyrimidine nucleotide clusters: Frequency of occurrence of the cluster (%)
$p\,N\,p$	17 3	44 0	17 9	44 1
$p\,(N\,p)_2$	15 8	20 0	17 6	21 6
$p\,(N\,p)_3$	15 4	12 9	15 5	12 7
$p\,(N\,p)_4$	12 8	8 1	13 6	8 3
$p\,(N\,p)_5$	10 9	5 6	9 9	4 9
Longer sequences†	27 8	9 4	25 5	8 4

* The values referring to the pyrimidine nucleotide clusters are calculated from data of Spencer and Chargaff (ref 4)

† The average chain length of this fraction corresponds to 7 6 nucleotides

Table 2 DISTRIBUTION OF NUCLEOTIDES IN THE MODEL SYSTEM FOR DIFFERENT MEANINGS OF n

Sequence	% of total nucleotides in this type of sequence $n=12$	$n=13$	$n=14$	Frequency of occurrence % of this type of sequence $n=12$	$n=13$	$n=14$
a	21 1	20 6	19 7	49 0	48 9	47 7
aa	18 0	17 0	16 9	20 7	20 1	20 6
aaa	14 6	14 4	13 8	11 2	11 3	11 2
aaaa	12 1	11 6	11 6	7 0	6 8	7 1
aaaaa	9 2	9 6	9 3	4 3	4 5	4 5
Longer sequences	25 0	26 7	28 6	7 8	8 3	9 0

$aa+aa+a$, $aa+a+a+a$, $a+a+a+a+a$ (since the sequence of units makes no difference the following arrangements are taken as equal $aaa+a+a$, $a+aaa+a$, $a+a+aaa$) The whole set is composed of 20 sequences of five types, the sequence *aaaaa* occurring once, *aaaa* also once, *aaa* twice, *aa* four times and *a* twelve times. The probability of occurrence of the individual sequences is determined by the ratio of participation of the sequence type investigated to the total number of all sequences of the set investigated, for example, the probability of occurrence of sequence *aa* is 4 20 = 0 20, that is, 20 per cent The probability of occurrence of the individual sequences depends considerably on the number of nucleotides (n) which are taken into consideration Thus for $n=2$ the probability of occurrence of sequence *aa* is 33 3 per cent, while for $n=20$ only 19 8 per cent, etc

The frequency of occurrence of the individual sequences of purine nucleotides from calf-thymus DNA corresponds to the probability of occurrence of analogous sequences of a model system derived in the foregoing way for $n=13$ to $n=14$ (Table 2)

The same law holds for the distribution of pyrimidine nucleotide sequences in the molecule of this DNA if the data published by Spencer and Chargaff[4] on the participation of nucleotides in the individual sequences of pyrimidine nucleotides are expressed with respect to the frequency of occurrence of the given sequences quite analogous results are obtained (Table 1) Even here the frequency of occurrence of the individual sequences corresponds to the probability of occurrence of these sequences in the foregoing model system for $n = 12$ to $n = 13$

This regularity of occurrence frequency of the individual sequences makes it possible to assume that the longest monothematic sequences of purine as well as pyrimidine nucleotides occurring in calf-thymus DNA are formed by 13 nucleotides According to the laws of probability, a molecule containing a single purine 13-nucleotide must also contain another 555 sequences similarly as the corresponding model system for $n=13$ If now the purine nucleotides in all these 556 sequences are added the total number of nucleotides is 1,313 The numbers of pyrimidine sequences and nucleotides are the same, the molecule of calf-thymus DNA contains thus (for $n=13$) a total of 2,626 nucleotides in the polynucleotide chain which corresponds to a molecular weight of $8\ 1 \times 10^5$ The intact molecule of calf-thymus DNA formed by two basic polynucleotide chains arranged in the double spiral should thus have a molecular weight in the vicinity of $1\ 6 \times 10^6$ The number n is thus a function of the molecular weight of the fundamental polynucleotide chain

The equal participation of the individual purine and pyrimidine sequences thus confirms the correctness of the Watson–Crick model of the double spiral of DNA (ref 5) against a sequence of purine nucleotides there must always be an analogous one of pyrimidine nucleotides

VLASTIMIL HABERMANN

Institute of Medical Chemistry,
Medical Faculty, Charles University,
Plzeň,
Czechoslovakia.

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Effect of Injection on Nicotinamide Adenine Dinucleotide-levels

DURING an investigation of the possible *in vivo* effect of redox dyes on the distribution of oxidized and reduced nicotinamide adenine dinucleotides (NAD+ and NADH), it was noted that the ratios and/or levels of these co-enzymes can be altered by injection of distilled water alone

To standardize experimental conditions, only male rats, 11–12 weeks old and bearing 6-day Walker tumour implants, were used, and injection and killing (by cervical dislocation) of all the animals were carried out at the same time of day. The rats were divided into 3 groups Group I, 12 rats which had not received an injection , Group II, 5 rats injected intraperitoneally with 1 ml distilled water per 100 g body-weight , Group III, 5 rats injected intraperitoneally with 1 ml per 100 g body-weight of an aqueous solution of leuco base of indophenol blue (1 25 mg/ml).

Groups II and III were killed 0 5 h after injection.

The liver, kidney, spleen, tumour and left hind muscle were removed as quickly as possible, cut into small pieces, and frozen in liquid nitrogen The samples were immediately weighed (without being allowed to thaw), and were then homogenized and analysed according to the method of Caiger *et al* [1]

The results are presented graphically in Figs 1 and 2 Marked differences in the levels of nicotinamide adenine dinucleotides were observed only in the spleen, kidney and liver of Groups II and III as compared with Group I Two striking effects are apparent First, that under the conditions specified the leuco base of indophenol blue does not influence absolute or relative concentrations of NAD+ and NADH, since water gave virtually identical results. Second, the NADH concentration is distinctly less in both water and dye injected animals, resulting in an almost two-fold increase in the NAD+/NADH ratio Since no

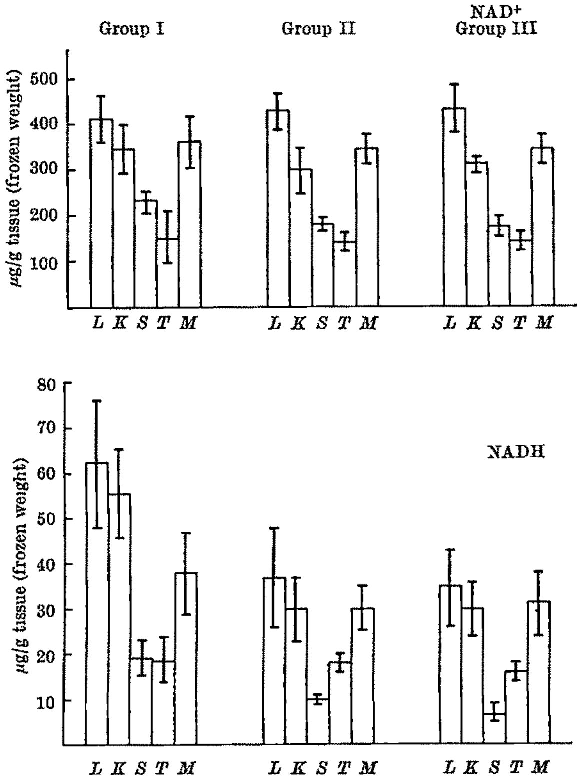

Fig 1 Distribution of NAD+ and NADH in the various tissues of the three groups of rats The standard deviations are indicated Group I, no injection , Group II, water injection, Group III, dye injection Tissues examined were liver (*L*), kidney (*K*), spleen (*S*), tumour (*T*), muscle (*M*)

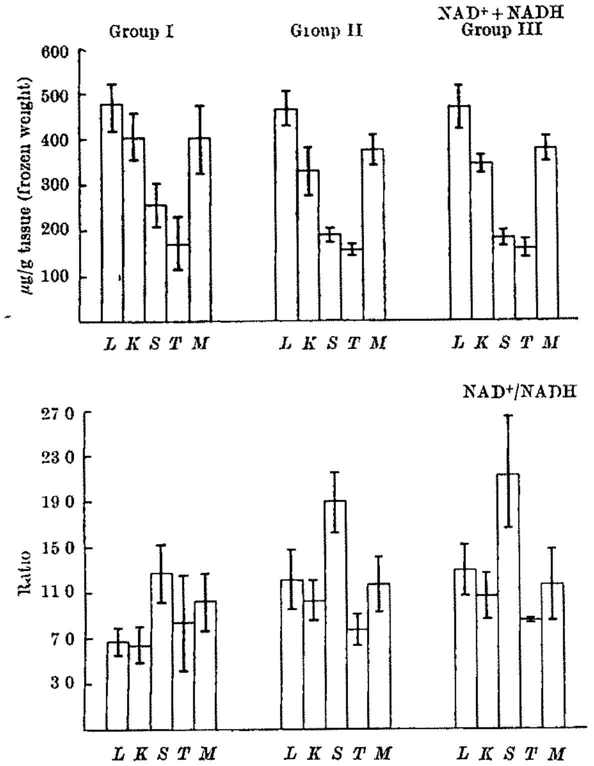

Fig. 2. Distribution of total coenzyme and NAD+/NADH ratio in the various tissues of the three groups of rats. The standard deviations are indicated. For details see Fig. 1

rise in the NAD+ level was observed (perhaps even a slight loss is indicated), it seems probable that a destruction or non-formation of reduced co-enzyme has occurred.

These findings suggest that injection *per se* can have a marked effect on the ratio and levels of nicotinamide adenine dinucleotides. Other workers have reported on the effect of stress on biological components, for example, non-protein sulphydryl groups[2,3] and blood sugar[4,5]. This investigation also confirms reports by other workers that NAD+ levels are low in tumours[6-8].

The question arises whether this stress affects NAD+ and NADH similarly in both normal and tumour-bearing animals. Extensive investigation on this problem is required. If qualitatively different reactions are obtained between normal and tumour-bearing animals this might yield further insight into the process of malignancy.

This research was carried out as a preliminary investigation to a co-operative study of the problem of biological and biochemical characterization of human cancers. We thank Miss P. Briggs for her assistance and Dr. D. A. Gilbert for his advice.

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R. YASIN
F. BERGEL

Chester Beatty Research Institute,
Institute of Cancer Research: Royal Cancer Hospital,
London, S.W.3

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Acid Mucopolysaccharide Content of Dermal Connective Tissue of Normal and Hypophysectomized Rats

PREVIOUS investigations conducted in our laboratory have shown a parallel relationship between the *in vitro* oxidative-reductive depolymerization of hyaluronic acid and the *in vivo* 'permeability' changes of dermal connective tissue[1-5]. These results suggest that hyaluronic acid may play a part in the permeability of dermal connective tissue. Our investigations concerning substances which act as activators and others which act as inhibitors of connective tissue 'permeability' also have shown that a different effect is obtained using hypophysectomized and adrenalectomized rats as compared with normal rats[6]. This result led us to investigate the content of mucopolysaccharides in the dermal connective tissue of normal and hypophysectomized rats in order to establish whether the amounts of these substances are affected by the pituitary function.

Experiments were performed on rats 3–5 months old, *CFE* strain of both sexes. The hypophysectomized animals were purchased from N. and J. Supply, Inc., New City, Rockland County, New York. The animals were fed 'Wayne Lab Blox' (mice and rats) diet[7]. The animals were hypophysectomized about 2 weeks prior to use. One group of female rats was used 2 months after being hypophysectomized in order to establish whether the time after hypophysectomy would affect the mucopolysaccharide content. These animals maintained a stationary weight throughout this period.

Both control and hypophysectomized rats were killed by bleeding them from the aorta. The skin of the dorsal and lumbo-dorsal region was shaved and taken for analysis. It was ground into powder, defatted in a Soxhlet extractor using acetone and dried at 80° C in a drying oven. The mucopolysaccharides were extracted and isolated following a slight modification of the technique described by Schiller *et al.*[8]. The mucopolysaccharides were finally precipitated with cetylpyridinium chloride and the precipitate was washed with 0.03 M sodium chloride. From the precipitate, hyaluronic acid, chondroitin sulphate and heparin were extracted successively with 0.4, 1.2 and 2.1 M sodium chloride. The mucopolysaccharide content of different fractions was determined by the carbazole method of Dische[9] which is based on the uronic acid content. Glucurone was used as standard. The results are shown in Tables 1 and 2. The values are expressed as μmg of glucurone equivalent per gram of dry skin, they do not correspond, therefore, to actual but relative values of mucopolysaccharide content. The results obtained on normal animals (Table 1) are closely similar to those reported by Schiller and Dorfman[10] for normal rats of the same age. Moreover, the present results show no difference in the concentration of mucopolysaccharides in male and female rats.

In comparing the results obtained with normal and hypophysectomized animals (Tables 1 and 2), no difference is apparent between the various fractions and total mucopolysaccharide content for both groups.

Schiller and Dorfman[11] reported data on the turnover of hyaluronic acid and chondroitin sulphate in normal and hypophysectomized rats. This was done by the administration of both acetate-1-^{14}C and $Na_2{}^{35}SO_4$. The authors found the half-life times of 2.9 and 4.8 days, respectively, for hyaluronic acid and chondroitin sulphate in normal rats and 5.1 and 11.7 days, respectively, for hyaluronic acid and chondroitin sulphate in hypophysectomized rats.

Schiller also reported in another study[12] that hypophysectomy resulted in a progressive increase of hyaluronic acid, while the concentration of chondroitin sulphate steadily decreased.

Our results do not confirm this last finding. The discrepancy can be, however, due to the difference in the age of animals used in both experiments. Schiller used 4-week-old rats, whereas the animals in our experiments

Table 1. CONCENTRATION OF MUCOPOLYSACCHARIDES IN THE SKIN OF NORMAL ADULT RATS

Animal (Exp. No. and sex)	Total conc. of mucopolysaccharides (μmg uronic acid/g dry skin)	Hyaluronic acid	Chondroitin sulphate	Heparin
		% of total uronic acid		
1 ♂	790	—	—	—
2 ♂	960	75	10	15
3 ♂	1,140	—	—	—
4 ♀	1,080	57	19	24
5 ♀	1,090	65	17	18
6 ♀	1,010	65	22	13
7 ♀	1,100	—	14	—
8 ♀	1,030	51	15	34
9 ♀	930	59	30	11
10 ♀	—	68	16	16
Average	1,015 ± 108*	63 ± 8	18 ± 6	19 ± 8

* S.D.

Table 2. CONCENTRATION OF MUCOPOLYSACCHARIDES IN THE SKIN OF ADULT HYPOPHYSECTOMIZED RATS

Animal (Exp. No. and sex)	Total conc. of mucopolysaccharides (μmg uronic acid/g dry skin)	Hyaluronic acid	Chondroitin sulphate	Heparin
		% of total uronic acid		
11 ♂	900	69	18	13
12 ♂	750	68	17	15
13 ♂	1,000	55	20	25
14 ♂	920	59	20	21
15 ♂	1,010	57	23	20
16 ♀	—	42	35	23
17 ♀	930	63	26	11
18 ♀	800	52	36	12
19 ♀	1,060	74	12	14
20 ♀	1,060	56	33	11
21 ♀	870	55	34	11
Average	930 ± 95	59 ± 9	25 ± 8	16 ± 5
Values for pooled sample from 4 female rats, 2 months after hypophysectomy:	840	71	23	6

were about 3 to 5 months of age. Schiller and Dorfman[10] found the concentration of hyaluronic acid and chondroitin sulphate in the skin of 44-day-old rats to be much greater than that of mature rats.

The results of the present work suggest that the *in vivo* 'permeability' changes of dermal connective tissue which follow the ablation of the pituitary may not be a result of a change in the concentration of mucopolysaccharides, but could possibly result from a decrease in the degree of polymerization of the hyaluronic acid or other mucopolysaccharides as suggested earlier[6].

In conclusion, the present findings show that hypophysectomy does not affect the mucopolysaccharide content of skin, at least not in adult rats. The concentrations of the different mucopolysaccharides are not affected even 2 months after the hypophysectomy.

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GO MATSUMURA
JOHN FABIANEK*
ANTHONY HERP
WARD PIGMAN

Department of Biochemistry,
New York Medical College,
New York 29,
New York.

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* Research Fellow of The John Polachek Foundation.

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Effect of Propanol–Water Mixtures on the Reactivity of Wool Cystine

THIS communication shows that the concept of hydrophobic bonds in interactions of soluble proteins[1] can also be applied to water-insoluble fibrous proteins. It has been observed[2,3] that lanthionine formation in wool at 65° C with sodium hydrogen carbonate or borate is much faster in mixed solvents than in aqueous solutions. This effect was not explained by the authors. It was also found by Atkinson, Filson and Speakman[4,5] that it is more difficult to extend wool fibres in water alone than in saturated aqueous butanol. Now there is evidence that addition of organic solvents will tend to overcome the resistance of the hydrophobic interior of proteins to penetration by the aqueous solvent and thus may increase the reactivity of protein groups located in hydrophobic regions.

New experiments (Fig. 1) on the relationship of propanol concentration to the wool cystine degradation by 0·1 normal sodium carbonate at 45° C gave a maximum cystine reactivity in 60 per cent (v/v) aqueous propanol. In Fig. 1 the results given in ref. 3 for the same solvent are included to show the close similarity of the solvent effect.

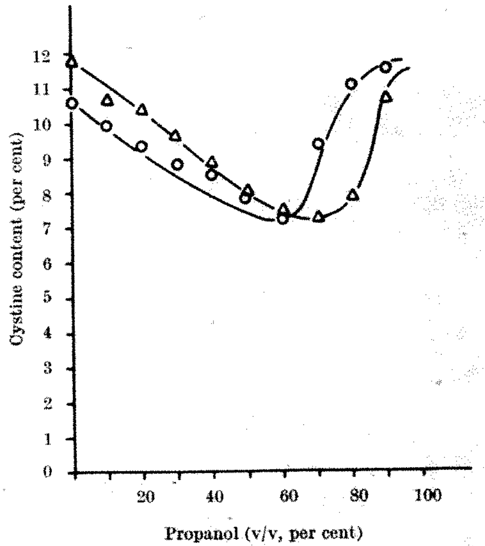

Fig. 1. Change in the cystine content in merino wool in 0·1 normal sodium carbonate: 45° C, 2 h (○) and in 0·5 molar borate solution: 65° C, 4 h (△) as a function of the concentration of propanol

The results suggest that the increased reactivity of wool cystine bonds in 10–80 per cent (v/v) aqueous propanol is best described as the consequence of a non-specific interaction of the solvent with the wool protein, resulting in the weakening of the hydrophobic interactions and the consequent exposure of wool cystine groups to the action of alkali.

Note added in proof. J. A. MacLaren (*Austral. J. Chem.*, **15**, 824; 1962) also regards wool as a protein linked by hydrophobic as well as hydrophilic interactions and found the extent of reduction of wool by thiols in aqueous alcohols increased.

H. ZAHN
T. GERTHSEN

Deutsches Wollforschungsinstitut an der
Technischen Hochschule,
Aachen.

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PHYSIOLOGY

Effects of Calcium Ions on Prolonged Action Potentials and Hyperpolarizing Responses

The membrane of excitable tissue is capable of maintaining two different states, namely, either the resting state or the acting state. These two states are defined in terms of the membrane permeability to sodium and potassium ions; P_{Na}/P_K is small in the resting state whereas it is large in the acting state. Our recent experimental results suggest that the membrane potential may be dependent on the state of the membrane calcium which is probably located in a layer of lipoproteins[1]. In other words, the resting state of the membrane will be the condition in which calcium ions are associated with the membrane, and the acting state, the condition in which these ions are dissociated from the membrane. This concept is supported by the recent findings that P_{Na}/P_K is markedly increased when the membrane calcium is removed[2]. It is expected that the dissociation of the membrane calcium will be impeded when the concentration of calcium in the external solution is high, and accelerated when it is low or nullified. The membrane, therefore, will tend to stay in the resting state or acting state depending on the external concentration of calcium. Indeed, the excitable membrane is depolarized and often initiates action potentials spontaneously when the concentration of calcium in the external solution is reduced. Furthermore, it has recently been found that the action potential of frog's pinal ganglion cells is markedly prolonged in Ringer's solution containing no calcium ions[3].

It is known that the action potential of various excitable tissues is prolonged under certain experimental conditions, and also that the depolarized membrane produces hyperpolarizing responses when anodal currents are applied. It is considered that these potentials are produced by the transition of the state of the membrane between two physicochemically stable states[4]. If the two stable states of the membrane actually correspond to the resting and acting states, it will be expected that the increase in the external concentration of calcium will prevent the prolongation of the action potential or prolong the hyperpolarizing response of the depolarized membrane.

Toad's (*Bufo vulgaris formosus*) spinal ganglion with the dorsal root and sciatic nerve attached was used. The changes in the membrane potentials were recorded continuously with an intracellular microelectrode during which time the external perfusing solution was changed from Ringer's solution to the control solution. The time needed for changing the perfusion solution was one-tenth of a second. At the end of each experiment, the solution was changed back to Ringer's solution to ascertain reversibility. The technique of using a single intracellular microelectrode for both recording and stimulating was essentially similar to that described elsewhere[5].

It is known that the action potential of ganglion cells is prolonged when tetraethyl ammonium (TEA) is partially or totally substituted for sodium in the external solution[5]. The superimposed responses in record *A* (Fig. 1) were taken approximately 3 min after the external solution was changed from Ringer's to the control solution (TEA-chloride 87 mM, potassium chloride 2·0 mM, calcium chloride 1·8 mM, potassium bicarbonate 0·5 mM and sucrose 55 mM). Immediately after the responses were recorded, the control solution was changed to a calcium-rich TEA solution (TEA-chloride 87 mM, potassium chloride 2·0 mM, potassium bicarbonate 0·5 mM and calcium chloride 20 mM), whereupon the prolonged potential was rapidly shortened. As seen in record *B*, 5 responses were superimposed at intervals of 2 sec.

As Tasaki[6] has demonstrated, barium ions markedly prolong the action potentials of cells. Record *C* shows such a potential of a cell during perfusion with barium solution (barium chloride 58 mM, potassium chloride 2·0 mM, calcium chloride 1·8 mM, potassium bicarbonate 0·5 mM and sucrose 55 mM). The response of the same cell shortened as soon as the barium solution was changed to a calcium-rich barium solution (barium chloride 58 mM, potassium chloride 2·0 mM, potassium bicarbonate 0·5 mM and calcium chloride 20 mM) as shown in record *D* in which 6 responses were superimposed every 2 seconds.

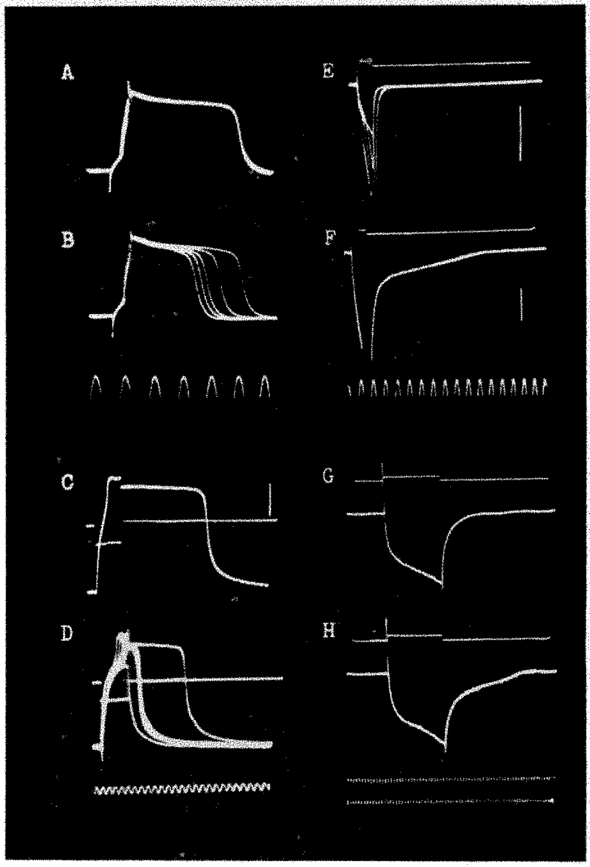

Fig. 1. Time marks: 60 cycles for all records except record *H* (1,000 cycles). Voltage calibration: the vertical bar in record *E* represents 50 mV calibration which is applicable to all records. Current calibration: the vertical bar in record *C* represents a calibration of 4×10^{-9} amp for records *C* and *D*, and that in record *F* represents a calibration of 5×10^{-8} amp for records *E*, *F*, *G* and *H*

The depolarized cell membrane in potassium-rich Ringer's solution is capable of producing a hyperpolarizing response during application of anodal currents[3]. The hyperpolarizing responses in a sodium-deficient potassium-rich solution, as a rule, abruptly returned to the initial depolarized potential level after the break of the anodal currents (duration: 20 msec), but in some cases showed a small plateau (step). Anelectrotonic potentials, induced by applying anodal currents ranging from 3×10^{-9} to 1×10^{-8} amp, of a cell perfused for 5 min with a sodium-deficient potassium-rich solution (potassium chloride 40 mM, sodium chloride 48·5 mM, calcium chloride 1·8 mM, sodium bicarbonate 0·5 mM and sucrose 55 mM) are shown in record *E*. In this case no plateau (step) formation could be observed following the break of the applied currents. However, when a calcium-rich potassium-rich solution (potassium chloride 40 mM, sodium chloride 48·5 mM, calcium chloride 20 mM and sodium bicarbonate 0·5 mM) was substituted for the sodium-deficient potassium-rich solution, a plateau soon appeared lasting for about 150 msec after the break of the applied currents (record *F*).

A similar effect of calcium ions could be observed when chlorine ions were completely replaced with glutamate ions (records *G* and *H*). In this experiment, the preparation was first perfused with a potassium-rich chlorine-free solution (sodium glutamate 58 5 mM, potassium glutamate 30 mM, sucrose 55 mM, calcium chloride 1 8 mM and sodium bicarbonate 0 5 mM) for about 5 min and then with a calcium-rich solution (sodium glutamate 58 5 mM, potassium glutamate 30 mM, calcium glutamate 20 mM and sodium bicarbonate 0 5 mM). The electrotonic potentials taken immediately before and approximately 30 sec after in the calcium-rich solution are shown in records *G* and *H*, respectively

These experimental results support the concept that the two stable states are the states in which the membrane calcium is either associated with or dissociated from the membrane[7] Presumably, the prolonged action potential is produced when the state of dissociation of the membrane calcium is sustained, and the hyperpolarizing response is produced when the association of membrane calcium takes place

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K Koketsu
S Nishi
H Soeda

Department of Physiology,
Kurume University School of Medicine, Kurume, Japan,
and
Research Laboratories, Department of Psychiatry,
University of Illinois College of Medicine,
Chicago

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Site of Action of Aldosterone on the Bladder of the Toad

The active transport of sodium by the urinary bladder of the toad, *Bufo marinus*, is increased when the animal[1] or the membrane itself[2–4] is treated with aldosterone Antidiuretic hormone also increases active sodium transport by the isolated toad bladder[5], this hormonal stimulation has been ascribed to a facilitated passive movement of sodium from the incubation solution on the mucosal side into the epithelial cells of the membrane[6], as a result, there is an increase of that fraction of tissue sodium concerned with the transfer of this ion across the preparation Hence the acceleration of the active extrusion of sodium, at the serosal border of the epithelial cells, can be taken as a mere consequence of an increased concentration of substrate—namely sodium—made available to the 'pump

An experimental approach among those devised by Frazier, Dempsey and Leaf[6] for the elucidation of the action of antidiuretic hormone on active sodium transport by the isolated toad bladder was adopted in order to obtain information concerning the site of action of aldosterone

Two subcutaneous injections of 50 μg *dl*-aldosterone-21-acetate, dissolved in 0 05 ml ethanol diluted to 0 25 ml with frog Ringer solution, were administered to toads, *Bufo marinus*, 16 and 4 h before death After pithing, both bladder halves of each toad were dissected and mounted between conical 'Lucite' chambers (inner diam 20 mm) according to Ussing and Zerahn[7] In a first series of experiments, incubation was carried out in frog Ringer fluid in which sodium was replaced by choline After 15 min part of this sodium-free solution on the mucosal side was removed and frog Ringer fluid containing tracer amounts of sodium-22 was introduced on that side The membranes were maintained short-circuited for 90 min, measured samples of incubation fluid on either side of the preparation were collected immediately before removal of the tissue which was blotted, care being taken not to contaminate one surface with the solution on the other side Bladder tissue was weighed in tared tubes before and after drying overnight at 95° C Radioactivity in the tissue and the incubation fluid was determined, and the amount of sodium-22 per unit tissue water was expressed as a function of the concentration of this radioisotope on the mucosal side The final concentration of sodium in the solution on that side was measured by flame photometry The pertinent results are given in Table 1, each value is the average of 4 determinations Mean short-circuit current and tissue labelling with sodium-22 were increased when the toads had been treated with aldosterone, mostly at the higher concentration of sodium used

Table 1 Influence of Aldosterone on Active Sodium Transport by the Isolated Toad Bladder and on Tissue Labelling with Sodium-22

	Membranes from untreated toads		Membranes from toads treated with aldosterone	
(1) Serosal surface exposed to choline–Ringer fluid				
Concentration of sodium on mucosal side (mM /l)	15 1	40 8	14 5	41 5
Short-circuit current* (μamp)	6	5	18	28
Tissue labelling† (% ± *S E*)	18 72 ± 4 42	10 98 ± 2 22	22 84 ± 3 82	18 46 ± 2 94
(2) Serosal surface exposed to sodium–Ringer fluid				
Concentration of sodium on mucosal side (mM /l)	10 0	40 0	10 0	40 0
Short-circuit current* (μamp)	33	47	91	106
Tissue labelling† (% ± *S E*)	7 57 ± 0 25	6 00 ± 0 53	20 00 ± 4 94	21 88 ± 6 49

* Average value of the last 30-min periods of incubation
† Concentration of sodium-22 in tissue water relative to that on the mucosal side

In view of the modesty of the short-circuit current—a quantitative expression of net sodium transport, even under such experimental conditions[6]—a similar number of investigations was performed on membranes the serosal surface of which was exposed this time to the usual frog Ringer solution, containing sodium, furthermore, incubation was interrupted after 45 min, which period of time is still amply sufficient for equilibrium to be reached between the 'active sodium transport pool' of the preparation and the solution on the mucosal side[6,8] In these circumstances, the behaviour of the untreated membranes was more satisfactory, the marked increase of sodium transport as well as that of bladder tissue labelling as a result of aldosterone treatment are thereby rendered more meaningful (Table 1)

Although quite different in their characteristics, the effects of antidiuretic hormone and aldosterone on active sodium transport by the isolated toad bladder are additive[1,2] The influence of this hormonal combination was therefore examined with regard to the size of the tissue sodium pool concerned with the active transport of this ion Experiments were carried out on paired bladder halves, one of which incubated as just described while the matched membrane was exposed simultaneously to 125 mU/ml vasopressin ('Pitressin', Parke, Davis and Co) It appears from Table 2 that antidiuretic hormone regularly increased active sodium transport and tissue water labelling with sodium-22, as has been reported by Frazier *et al* [6], this occurred even when the membranes were under the stimulating influence of aldosterone

These results thus are compatible with the interpretation that aldosterone stimulates the active transport of sodium by the epithelial cells of the toad bladder at least in part because this hormone exerts on the mucosal border of these cells an influence such that the amount of sodium delivered to the 'pump' is increased Indeed, the amount of sodium drawn into the tissue from the mucosal side was found to be increased at the time of an accelerated

Table 2 INFLUENCE OF ALDOSTERONE AND VASOPRESSIN ON ACTIVE SODIUM TRANSPORT BY THE TOAD BLADDER AND ON TISSUE LABELLING WITH SODIUM-22

Untreated animals				Animals treated with aldosterone			
Control	membrane	Paired membrane exposed to vasopressin		Control	membrane	Paired membrane exposed to vasopressin	
μamp*	labelling†	μamp*	labelling†	μamp*	labelling†	μamp*	labelling†
55	8 3%	136	9 9%	80	10 4%	145	15 3%
25	4 8%	63	8 0%	83	13 2%	136	14 3%
95	12 2%	215	25 8%	80	14 2%	121	15 1%
				144	13 1%	224	18 1%

* Mean short-circuit current during the last 30-min period

† Concentration of sodium-22 in tissue water relative to that on the mucosal side of the membrane The total concentration of sodium on that side was 25 mM /l for this series of incubations

rate of transfer of sodium across the epithelial cell layer In this respect, the influence of aldosterone resembles that of antidiuretic hormone, and both hormones actually combine their effects on this presumably passive albeit carrier-dependent[8] transfer of sodium across the cell mucosal border How aldosterone facilitates this transfer and whether it acts in addition on the 'pump' directly are still unanswered questions

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JEAN CRABBÉ

Laboratory of Experimental Surgery,
Hôpital St Pierre,
Louvain,
Belgium

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Mesial Surface of the Brain and Genital Function

WORK on the mesial surface of the cortex has produced conflicting observations on the part played by the cingulate gyrus in behaviour Smith[1,2], Ward[3] and Glees *et al* [4] reported that cingulectomy produces profound changes in the social and emotional behaviour of monkeys and similar observations have been made by Kennard[5] on the cat In contrast, Pribram and Fulton[6] and Mirsky *et al* [7] have observed little or no permanent effect following bilateral ablation in the monkey and this conclusion has been supported by investigations on the cat[8]

Our observations have been restricted to sexual behaviour Adult rhesus monkeys (*Macaca mulatta*), two males and two females, were used in the investigation and were observed over a period of eighteen months They were caged individually and their sexual activity was assessed by caging the mating pairs together for half an hour each day for thirty consecutive days In the pre-operative control phase copulation occurred daily for the period of the assessment in each pair and was unaffected by the female cycle

Cingulectomy (areas 23 and 24) was performed through a fronto-parietal craniotomy The dura was reflected toward the midline and the gyrus was approached by retraction at the sensory cortex The ablations extended to the sensory cortex of the mesial surface, particularly in the second animal

In the first male the operation was carried out in two stages No impairment of locomotion was produced by the right-sided ablation but an inability to grip with the right hind-limb followed the left-sided craniotomy The sexual activity of the animal was assessed from the third post-operative day of each operation Copulation occurred daily for a period of thirty days in each case, although the animal had some difficulty in maintaining the position due to the impairment of grip Bilateral carotid angiography was normal The female gave birth to a normal healthy monkey twenty-nine weeks after the first stage and eighteen weeks after the second stage Copulation continued to within twelve weeks of the delivery

In the second male the ablation was carried out in a single stage, a total paraplegia was produced The female was caged regularly with the male from the third post-operative day During the stage of total paraplegia erection was not impaired, but as the animal was unable to adopt the copulatory position ejaculation did not occur With some recovery in the paralysis, daily copulation occurred and was maintained for a period of thirty days even though a considerable motor deficit was present

These investigations suggest that the cingulate gyrus is not essential for sexual performance in the monkey and would support the observation of Pribram *et al* [6,7] that the gyrus is not intimately concerned in emotional behaviour The paralyses of the hind limbs, produced by the lesions extending to the somatic area 1, were accompanied by normal sexual activity This observation would be in accord with the conclusions of MacLean and his associates[9–11] from work on the squirrel monkey (*Saimiri sciureus*), that the somatic and autonomic aspects of genital function may be integrated by subcortical systems.

ANTHONY N NICHOLSON*
ERIC A TURNER

Department of Neurosurgery,
University of Birmingham

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* Present address Institute of Aviation Medicine, Royal Air Force, Farnborough, Hants

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Uptake of Tritiated Aldosterone by Rat Tissues

As Tait *et al* [1] have pointed out, the half-life of aldosterone in plasma does not reflect metabolism alone The calculated 'volumes of distribution' for the body compartments exceed that of total body water, suggesting that aldosterone is selectively taken up by some region of the body[2] The work recorded here shows that injected tritiated aldosterone is unevenly distributed in the various tissues, that the rate of its disappearance from the tissues varies, and that in the liver the radioactivity is found predominantly in the supernatant fraction

Male Holtzman rats, 275–300 g, maintained on a synthetic diet with a constant daily intake of sodium and potassium were used throughout Four microcuries of chromatographically pure 1,2-^{3}H-*d*-aldosterone (100 mc / mg) in 2 ml. of 5 per cent glucose were injected into the tail vein Groups of rats, consisting of four animals, were killed by decapitation at intervals after injection of 5, 15, 30, 60 and 90 min A sample of blood was collected The tissues (heart, lung, muscle, spleen, intestine, aorta, brain, adipose tissue, kidney and liver) were immediately removed, dropped into ice-cold 0 25 M sucrose and allowed to chill. They were then blotted, weighed, finely minced, and homogenized in 2 5 ml of 0 25 M sucrose/g tissue Blood was hæmolysed by allowing it to stand with ten times its volume of cold de-ionized water A portion of each homogenate was extracted with 7 5 volumes of dichloromethane The various extracts were analysed for aldosterone by a slight modification, as described by Ayers *et al* [2], of the double isotope derivative assay

The total dichloromethane-soluble radioactivity of each tissue was determined by extracting 3 ml of the homogenate with 7 5 volumes of dichloromethane, drying the extract with a stream of air, transferring the material to counting vials, adding 5 ml phosphor, and counting in a liquid scintillation spectrometer Suppression of counts by the chromogens in the tissues was corrected by adding a known amount of tritiated toluene to each sample, recounting, and determining recovery When a known amount of tritiated aldosterone was added to unlabelled homogenates and analysed by the foregoing procedure, 98 per cent of the added radioactivity was recovered

To determine the intracellular distribution of injected aldosterone in the liver, 20 μc of tritiated aldosterone in 2 ml of 5 per cent sucrose were injected into the tail veins of rats fasted overnight Fifteen min after injection, the rats were killed by decapitation The livers were perfused *in situ* with cold 0 9 per cent saline, immediately removed, and the subcellular components isolated by ultracentrifugation in a Spinco model *L* preparative ultracentrifuge according to the method recommended by Hogeboom[3] Nuclei were isolated by the method of Chauveau[4]

The histological distribution of radioactivity was shown in these rats by autoradiography Sections of brain, heart, aorta, duodenum, spleen, liver, kidney, skeletal muscle and skin were quick frozen using acetone plus dry ice With a cryostat at –20° C, sections 8μ thick were cut, mounted on slides, and dried The stripping film technique of Fitzgerald *et al* [5], using Kodak '*AR* 10 film', was followed to film the slides The slides were then sealed in dark boxes containing calcium chloride and kept at 5° for 21 days The film was developed in *D*19 developer (Eastman Kodak) for 7 min at 18° and fixed in acid fixer After washing in running tap water for 10 min, sections were stained with Harris hæmatoxylin Only areas showing a grain density much above background were considered indicative of the presence of radioactive material

The average results on the disappearance of dichloromethane soluble radioactivity from rat tissues are shown in Table 1 Five minutes after injection of tritiated aldosterone, radioactivity was more concentrated in the kidneys, heart and lungs than in blood Fifteen min after injection, all tissues investigated with the exception of brain and adipose tissue had a higher concentration of radioactivity than blood

Tritiated aldosterone plus its dichloromethane-soluble metabolites disappeared logarithmically with time for 60 min from blood, kidney, heart and muscle After an initial uptake period of 15 min, they also disappeared logarithmically from spleen, lung, aorta and brain The uptake of radioactivity by the adipose tissue was slower than by the other tissues The maximum concentration in this tissue was 30 min after injection The level of radioactivity in the small intestine, although as high initially as most of the other tissues, did not decrease over the first 60 min

The distribution and disappearance of tritiated aldosterone were determined in blood, kidney, heart, lung, duodenum, brain and liver They were shown to be closely related to the distribution and disappearance of dichloromethane extractable radioactivity in all tissues investigated other than the liver In other words, most of the lipid-soluble radioactivity was present as tritiated aldosterone rather than its metabolites

By preparative ultracentrifugation of liver homogenates obtained from four rats 15 min after injection of 20 μc tritiated aldosterone, 70 per cent of the dichloromethane-soluble radioactivity present was shown to be in the supernatant fraction The nuclei, mitochondria, microsomes and cellular debris each contained approximately 7 per cent of the radioactivity in the total homogenate

Table 1 DICHLOROMETHANE EXTRACTABLE RADIOACTIVITY IN RAT TISSUES AFTER INJECTION OF TRITIATED ALDOSTERONE*

	Min after injection				
	5	15	30	60	90
Blood	1,017	845	381	324	164
Kidney	3,232	2,609	1,848	788	809
Heart	1,490	1,248	802	427	217
Muscle	1,027	913	650	524	237
Spleen	1,007	1,053	677	341	172
Aorta	874	960	512	356	184
Lung	1,127	1,349	747	384	335
Liver	954	1,476	1,321	537	513
Duodenum	1,028	966	1,003	1,076	803
Brain	272	397	299	179	161
Fat	160	228	446	102	372

* Results are expressed as the mean level of radioactivity in tissues from four animals in c p m /g tissue

Preliminary investigations of the histological distribution of radioactivity in the tissues using autoradiography indicate that aldosterone is localized in the tubular epithelial cells of the kidney, along the muscle fibres both in skeletal and cardiac muscle, in the alveolar walls of the lungs, and in the parenchymal cells of the liver

Although the role of aldosterone in the renal re-absorption of sodium has been examined most extensively, other investigations indicate that aldosterone is also involved in sodium and perhaps potassium transport in extrarenal tissues It lowers the sodium/potassium ratio in the saliva, sweat, and the secretions of the gastrointestinal tract of adrenalectomized animals[6]. Renkema *et al* [7] demonstrated that the intestine of cows is involved in the excretion and retention of ingested sodium Moll and Koczorek[8] found that administration of aldosterone resulted in an increased absorption of sodium by the duodenum This effect was inhibited by aldosterone antagonists These observations suggest that circulating aldosterone influences the transmucosal transport of sodium in the intestine Perhaps the maintenance of a high level of injected aldosterone by both the duodenum and the kidney 90 min after injection as compared with the other tissues is a reflexion of its role in sodium transport in these tissues

The slow rate of concentration or binding of aldosterone by fat as compared with the other tissues is interesting. Parra and Reddy[9] suggested that the mechanism by which fat accumulates cortisol is a solubility phenomenon rather than the essentially physical phenomenon of protein binding Our results also suggest that the mechanism by which fat accumulates aldosterone is different from that occurring in other tissues

The appearance of tritiated aldosterone in brain shows that the blood-brain barrier is, to some extent, permeable to aldosterone

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L L SULYA
CONNIE S MCCAA
VIRGINIA H READ
DONALD BOMER

Department of Biochemistry,
University of Mississippi School of Medicine,
Jackson 6, Mississippi

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PHARMACOLOGY

Vitamin B_6 as an Antidote against the Rodenticide 'Castrix' (2-Chloro-4-methyl-6-dimethylaminopyrimidin)

THE 'Castrix' molecule (2-chloro-4-methyl-6-dimethylaminopyrimidin)[1,2] resembles the vitamin B_6-antagonist toxopyrimidin. In rodents poisoned with this rodenticide the symptoms are an initial depression followed in 40–60 min by a 'search and flight reaction', spasticity, and tonic-clonic seizures, often with tongue-bite. Sound and touch aggravate the severity of the seizures, which may end with death from respiratory failure, dependent on dosage. Survival for more than 5 h usually results in total recovery within 24 h. As these symptoms are almost identical with those seen after administration of certain carbonyl trapping agents and vitamin B_6-antagonists[3-6] we tried vitamin B_6 as an antidote.

A total of 540 mice were used for the experiments. In the strain of mice utilized the LD_{50} of 'Castrix' when administered subcutaneously was 1.3 mg/kg in males and 1.1 mg/kg in females. On oral administration the LD_{50} was 1.2 mg/kg in female mice.

In all experiments pyridoxin, pyridoxal or pyridoxal phosphate was administered subcutaneously in a dosage of 25 mg/kg, which dosage was chosen arbitrarily. Mice injected subcutaneously with twice the LD_{50} of 'Castrix' showed no symptoms when pyridoxin was injected simultaneously with the 'Castrix', while untreated controls showed almost 100 per cent mortality. In another experimental series 'Castrix' was injected intraperitoneally at a dosage of 2.5 mg/kg. The first muscular symptoms, spastic gait and raised tails, appeared in about 30 min, at which time half the animals were treated with pyridoxin. Among the treated animals no seizures were seen, and all symptoms of toxicity disappeared within 10 min. All mice in the control group died within 90 min. Pyridoxal and pyridoxal phosphate were found to have the same effect as pyridoxin, while thiamine, nicotinamide, riboflavin, glutamic acid, and pyruvic acid had no effect. The last two compounds were tested both separately and in combination.

As a further test of the antidotal effect, the LD_{50} was determined in 125 female mice pretreated with pyridoxal immediately before the intraperitoneal injection of 'Castrix'. In these circumstances the LD_{50} was found to be 137 mg/kg. At this high dosage no epileptiform seizures were seen. The only symptoms were somnolence, prostration, and a slight opisthotonus preceding death.

O. KARLOG
E. KNUDSEN

Department of Pharmacology and Toxicology,
Royal Veterinary and Agricultural College,
Copenhagen

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Amphotericin *B* in the Chemotherapy of Experimental *Schistosomiasis mansoni* in Swiss Mice

IN May 1963, Gordon, St. John and Olsen[1] reported the prolongation of life in Swiss mice experimentally infected with a Puerto Rican strain of *Schistosoma mansoni*, when treated for 5 days with 'Fungizone' (amphotericin *B* made soluble with deoxycholate, E. R. Squibb). In the foregoing experiment, treatment began at a level of 0.5 mg of 'Fungizone'/kg body-weight of mouse a day. The dosage was increased in two daily increments of 0.5 mg/kg to a final level of 1.5 mg/kg a day, which was maintained for three days. Treatment began twelve weeks after infection (approximately 6 weeks after the parasite had begun producing ova). By one week subsequent to the termination of treatment, 80 per cent of the untreated but only 27 per cent of the treated animals had died. A χ^2 test showed that the experimental results fit to a confidence limit of 98 per cent the hypothesis that treatment influenced survival.

The work described here was undertaken to demonstrate whether the observed prolongation of life was due to a reduction in the number of worm pairs per mouse (that is, a reduction of infection-level due to treatment with 'Fungizone').

The mice used in this experiment were strain *CD*–1 Swiss, obtained from Charles River Breeding Laboratories, Inc. The snails used to obtain cercariæ were laboratory-reared *Australorbis glabratus*, obtained from several sources. Both the snails and the parasites were of Puerto Rican origin.

Large numbers of snails were induced to shed cercariæ by placing them in artificial pond water, in a shallow 'Pyrex' baking pan, and exposing them to a fluorescent lamp at close range. By this method, a concentration of 100 cercariæ/ml. was obtained.

Mice were infected by intraperitoneal injection of 1 c.c. of the above-described cercariæ-water per mouse. Treatment with 'Fungizone' began 13 days after infection. Treated animals comprised four groups, receiving daily doses of 1.0, 1.5, 2.0, and 2.5 mg of 'Fungizone'/kg mouse for 40 days. A fifth group received no treatment of any kind subsequent to infection. Treatment in all groups began at the 0.5-mg/kg level and increased in daily increments of 0.5 mg/kg until the final levels were reached. For the first seven days, all treatment was intravenous, thereafter intraperitoneal. The animals were killed within five days after terminating the treatments. The individual infection-levels of all animals were determined by counting the number of viable worms per mouse. The worms in the mesenteric venules were counted by carefully searching these structures at a magnification of ten times with a dissecting microscope. Those which remained in the liver were counted by crushing this organ flat between two glass plates and carefully searching at a magnification of 10 times. No parasites were found associated with any other organ or system of the mice.

Statistical methods by which treatment groups were compared included a one-way analysis of variance for unequal sample sizes, and a calculation of the value of Student's *t* for each treatment group compared with the group that received no treatment.

Table 1 presents the treatment groups and the mean number of worms per group (that is, mean infection-level per group) with the standard deviation of each group mean.

Table 1

Group No.	Daily dosage 'Fungizone'	No. mice in group	Mean level infection	Standard deviation
A	none	11	32.7	16.9
B	1.0 mg/kg	12	30.8	20.2
C	1.5 mg/kg	19	18.9	11.4
D	2.0 mg/kg	9	18.1	9.7
E	2.5 mg/kg	10	14.6	6.4

An analysis of variance comparing the infection-levels for all groups is presented in Table 2.

Table 2

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance estimate)
Between samples	(5—1)=4	(*SSB*) 3,025.74	$MSB=\frac{SSB}{4}=756.435$
Error	(61—5)=56	(*SSE*) 10,305.67	$MSE=\frac{SSE}{56}=184.048$
Total	(61—1)=60	13,331.41	

$$F=\frac{MSB}{MSE}=\frac{756.435}{184.048}=4.01$$

With the numerator at 4 degrees of freedom and the denominator at 56 degrees of freedom, at the 99 per cent confidence-level, $F \simeq 3{\cdot}7$. Therefore, variance due to treatment is significantly greater than the individual variance within each group.

In order to demonstrate which groups are responsible for the observed group variance, values of Student's *t* comparing the mean infection-level of each group with that of the untreated group were computed and are presented in Table 3.

Table 3

Group No.	Student's *t*	Confidence limits (%)
A	—	—
B	0·24	(10–20)
C	2·68	(98–99)
D	2·29	(95–98)
E	3·18	(99·0–99·9)

From the foregoing analysis of variance and computation of Student's *t* the following inferences can be drawn: (1) treatment with 'Fungizone' effectively lowers the level of infection of treated animals below the infection level of untreated animals; (2) 1·5–2·5 mg/kg administered in a daily series of 40 injections represents an effective range of therapy; (3) no significant effect was observed using 1·0 mg/kg a day for 40 days.

The reduction of the mean number of worms per mouse (mean infection-level) in mice treated with 1·5–2·5 mg/kg 'Fungizone' a day undoubtedly explains the earlier observations by Gordon, St. John and Olsen that treatment with 'Fungizone' significantly prolongs the life of infected animals.

The two types of observations suggest to us that amphotericin *B* is an effective agent in the chemotherapy of experimental *Schistosomiasis mansoni* in the Swiss mouse.

Since the agent has been pharmacologically characterized in man in the treatment of disseminated mycoses, we suggest its use in clinical trials to determine its effectiveness in the treatment of human schistosomiasis.

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Benjamin L. Gordon
Philip A. St. John

Department of Biology,
Brandeis University,
Waltham, Massachusetts.

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Action of Puffer Fish Poison

Puffer fish (Tetraodontoidea) is an important cause of food poisoning in China and Japan[1,2]. Murtha *et al.*[2] claimed that the poison acts centrally and peripherally to produce respiratory and neuromuscular paralyses. They offered as proof of its central action the more rapid onset of respiratory depression when the toxic extract was injected into the carotid artery than when it was injected intravenously. This is disputable because the carotid artery normally supplies blood to the cerebrum while the brain stem is supplied by the basilar artery[3]. Secondly, the peripheral neuromuscular paralysis as described by Murtha *et al.*[2] appears to be a late effect because the transmission in the phrenic nerve diaphragm preparation in their experiment was not blocked until 20–30 min after artificial respiration was resorted to for the respiratory arrest.

In the present investigation the crude extract of liver and gonads of puffer fish, prepared as described by Hashimoto and Migita[4], was separated chromatographically into three fractions. The fraction with the highest R_F was found to increase the contractility of isolated rabbit gut preparation, and the fraction with the lowest R_F inhibited this action. The middle fraction with R_F about 0·26 was proved to be the toxic fraction when injected into animals. In mice, the intraperitoneal *MLD* of the toxic fraction was $3{\cdot}08 \pm 0{\cdot}06$ μg/100 g body-weight, as computed from the probit-log dose relationship.

A progressive arterial hypotension is a constant feature in rats given the toxic fraction irrespective of the dosage. This was also found in dogs and cats after administration of extract of puffer fish[2]. In the rat administration of a small sub-lethal dose produced hypotension only, without respiratory or other apparent changes; therefore, it seems that this is an independent action. Preliminary investigation showed that cardiac output as measured with a cardiometer was unaffected, which suggests that hypotension is attributable to direct action of the puffer fish toxin on peripheral blood vessels. Further lowering of diastolic pressure when the toxic fraction was given after injection of a ganglion blocking agent (tetraethylammonium bromide) supports the foregoing hypothesis. The administration of antihistamine (diphenhydromine) before injection of a sub-lethal dose of toxic fraction prevented the fall in blood pressure, therefore, the hypotension may probably be due to the histaminic action of the puffer fish toxin. The hypotension is unlikely to be due to autonomic effect because the pressure response to carotid occlusion or adrenaline was not affected by administration of the toxic fraction, and atropine did not influence the hypotensive effect. Infusion of noradrenaline following injection of toxic fraction maintained the arterial blood pressure at normal level for about 1 h.

Large sub-lethal or lethal dose of toxic fraction caused respiratory depression in addition to hypotension. When the lethal dose was large, the respiration stopped when the fall in blood pressure was still slight. The respiratory arrest occurred more rapidly when the toxic fraction was injected into the subclavian artery (retrogradely via the carotid artery) than when the same dose was injected into the carotid artery or a vein. When Evans blue was injected into the subclavian artery of a rat the dye stained the brain stem only, while injection of the dye into the carotid artery stained the cerebrum only. Therefore, the rat brain stem was supplied, as in other animals[3], with blood from the basilar artery. Thus, the cause of death following a lethal dose of the toxic fraction appears to be respiratory arrest from central action of the toxin on the brain stem respiratory centres. The view is supported by the finding that intracisternal injection of the toxic fraction, in a dose which was non-lethal when given intraperitoneally, caused immediate cessation of respiration.

K. M. Li

Department of Physiology,
University of Hong Kong.

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HÆMATOLOGY

A Hæmagglutination Phenomenon between Tanned Erythrocytes and Sera of Guinea Pigs

In the course of experiments designed to examine the effect of serum on the streptolysin-*S* susceptibility of tanned erythrocytes[1], guinea pig erythrocytes treated with tannic acid in the cold were suspended in 1 : 100 dilution of fresh serum from tuberculous guinea pig. A heavy hæmagglutination appeared a few minutes after mixing. No such heavy agglutination was observed either with

Table 1. TITRATIONS OF SERA FROM TUBERCULOUS AND NORMAL GUINEA PIGS FOR THEIR TANNED ERYTHROCYTE-AGGLUTINATING CAPACITY

Serum No.		Dilution of serum									
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024
Tuberculous	1	·	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ + +	+ +	±	—
	2	±	+ + +	+ + +	±	±	+	+ +	±	—	—
	3	—	—	—	—	—	+ +	+ +	+	—	—
	4	—	—	—	—	—	+	+ +	—	—	—
	5	—	—	—	—	—	+ + +	+ +	±	—	—
	6	—	—	—	—	—	+ +	+ + +	+ +	±	—
	7	—	—	—	—	±	+ + + +	+ + + +	+ + +	±	—
	8	—	—	—	—	—	—	±	±	—	—
	9	—	—	—	—	+	+ + +	+ +	±	—	—
	10	—	—	—	—	+ + +	+ + +	+ + +	±	—	—
Normal	1	—	—	—	—	—	+	+	—	—	—
	2	—	—	—	—	—	±	—	—	—	—
	3	—	—	—	—	—	+ +	±	—	—	—
	4	—	—	—	—	—	±	—	—	—	—
	5	—	—	—	—	—	—	—	—	—	—
	6	—	—	—	—	+	+ + +	—	—	—	—
	7	—	—	—	—	—	±	—	—	—	—
	8	—	—	—	—	—	—	—	—	—	—
	9	—	—	—	—	—	+	±	—	—	—
	10	—	—	—	—	—	—	—	—	—	—

The figures are the final readings taken 25 min after mixing the cells and the serum. The hæmagglutination was graded by noting the gross amounts of settled cells on the bottom of the tube ; —, negative ; ±, clumpings with little or no settlings ; + + + +, almost complete settlings ; +, + + and + + +, intermediate settlings.

heat-inactivated tuberculous serum or with fresh serum of normal animals. These observations led us to further investigations on this curious hæmagglutination phenomenon, provisionally designated as 'tanned erythrocyte (TE-)agglutination' to differentiate it from the Boyden's antigen–antibody hæmagglutination[2].

Erythrocytes from normal healthy guinea pigs, weighing 400–700 g, were used. Blood was collected by heart puncture in 1·2 vol. of modified Alsever's solution and centrifuged. The packed red cells were washed 4 times in phosphate-buffered saline (*p*H 7·2) consisting of 10 vols. of 0·85 per cent saline and 1 vol. of 0·1 M phosphate buffer. The washed cells were finally suspended in buffered saline to the original volume. The preparation of tannic acid-treated erythrocytes was carried out in a cold room (2° ± 2° C). Two ml. of the washed erythrocyte suspension were added to 40 ml. of 1 : 10,000 tannic acid (J.P.) diluted in buffered saline. The mixture was left for 30 min in the cold, then centrifuged, the cells washed 3 times in buffered saline, and finally re-suspended to the original volume. Guinea pig sera were recovered after clotting blood samples at room temperature. The tuberculous sera used were obtained from guinea pigs infected, at least one month beforehand, with 0·2–0·5 mg (semi-moist weight) of a human tubercle bacillus *H37Rv* grown on Sauton's medium. Dilution of serum was made in buffered saline. For agglutination test centrifuge tubes (12 mm × 100 mm) were used. To the tube containing 4 ml. of a dilution of test serum was added 0·2 ml. of tanned erythrocyte suspension. The tube, after agitation, was placed at room temperature, and the readings made at 5-min intervals.

As shown in Fig. 1, agglutination of tanned erythrocytes by tuberculous serum occurred, within 5–15 min after mixing the cells and the serum, as coarse clumpings which soon aggregated into gross masses and then settled down on the bottom of the tube. The reaction reached its end-point within 30 min. As shown in Table 1, the titrations of sera from tuberculous and normal guinea pigs for their TE-agglutinating capacity revealed that there were considerable differences between the individual serum specimens in the appearance of agglutination in relation to serum dilutions. Tuberculous serum No. 1 gave a titre of 1 : 512, and the occurrence of the agglutination increased with increase of the serum concentrations. Tuberculous serum No. 2 gave more or less definite agglutination in all dilutions ranging from 1 : 2 to 1 : 256, the grades of which were, however, quite irregular with respect to the serum concentrations.

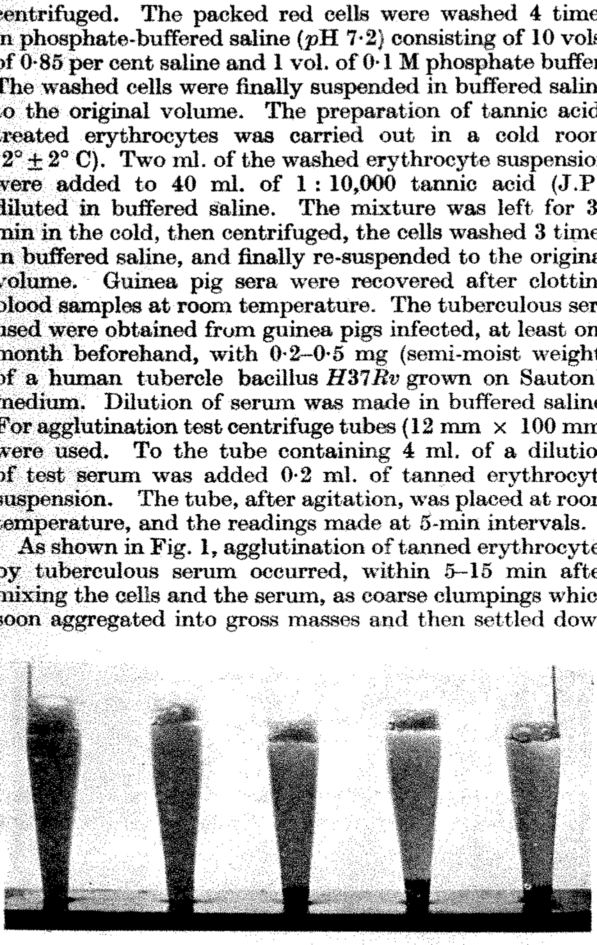

Fig. 1. Time-course of tanned erythrocyte agglutination by tuberculous guinea pig serum (1 : 100). Left to right : after 5, 10, 15, 20 and 25 min

Other sera showed no agglutination up to dilution of 1 : 16 or 1 : 32 (inhibiting prozone), and only when further diluted beyond this point they caused varying degrees of agglutination. It is worthy of note that the positive sera of the last group showed the maximum agglutination at dilutions of 1 : 64 to 1 : 128. It is interesting to note that the capacity of the most normal sera to produce the hæmagglutination was much weaker, as compared with that of the tuberculous sera. Further experiments carried out with tuberculous sera revealed that the TE-agglutination was dependent on a number of variables : when the tanned red cells of guinea pig were replaced with the cells of sheep, goat, ox, rabbit, rat, mouse or chicken, no hæmagglutination occurred, while those of horse, pig, man or cat were more or less sensitive to the agglutination. Tanning of red cells should be carefully carried out in the cold (0°–5° C), and no consistent results were obtained with the erythrocytes tanned at higher temperatures. The TE-agglutinating activity of the serum was completely annulled by heating it at 56° C for 30 min, and the lost activity of the heated serum was not restored by the addition of fresh serum of normal animals. The activity was non-dialysable and not affected by pretreatment with weak ammonia or zymosan.

These results suggest that complement[3,4] is not involved here. Electrophoresis investigations indicated that the TE-agglutinating activity of the guinea pig serum is present in γ-globulin fraction. In addition, it was found that the 'inhibiting prozone' demonstrated in the titration tests could be easily removed by a single, careful pretreatment of the serum with tanned erythrocytes, resulting in a marked enhancement or an unmasking of the serum capacity to produce the TE-agglutination. From all these results it was supposed that there are at least two factors in guinea pig serum, one of which is capable of agglutinating tanned erythrocytes, that is, agglutinator, the other being capable of inhibiting the agglutination, that is, inhibitor, and we are at present of the opinion that a strongly positive TE-agglutination test, as was observed on the tuberculous guinea pigs in this investigation, may be an indication of a disturbed state of serum in its 'agglutinator-inhibitor' relation.

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RYO ITO
MARIKO AKIYAMA

Department of Pharmacology,
Research Institute of Tuberculosis,
Kanazawa University,
Japan.

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HISTOLOGY

A Unique Pattern of Localization within the Cerebellum

IT has been generally accepted that the microscopic structures of the individual layers of the cerebellum are invariant in all animals. For example, Kappers *et al.*[1] state that "different cytoarchitectonic regions, such as are demonstrable in the various areas of the cerebral cortex, are not present in the cortical portion of the cerebellum". This absence of localization of histological structure has been a hindrance to research in view of the known localization of function in different regions of the cerebellum.

During the course of certain experiments in this laboratory it was noted that there was a microscopically observable difference in the 'staining' intensity in the histochemical test for the enzyme 5′-nucleotidase between the anterior and posterior portions of the cerebellum in mice. This difference was found in the molecular layer (Fig. 1). Microdensitometry indicated a 2-fold difference in activity between the two portions. The enzyme was demonstrated by a modification of Naidoo's[2] method, using 10μ thick cold-microtome sections. In coronal section, on the other hand, the enzyme was seen to be distributed in a series of bands which ran in an antero-posterior direction (Fig. 2). These bands were wider posteriorly than anteriorly, thus accounting for the antero-posterior differences originally noted (Fig. 1).

This unique pattern was observed in mice of all ages from 11 days to 13 weeks but not of 4 or 7 days of age. There were no sex differences and no such distributional pattern was seen with any of 16 other enzymes examined.

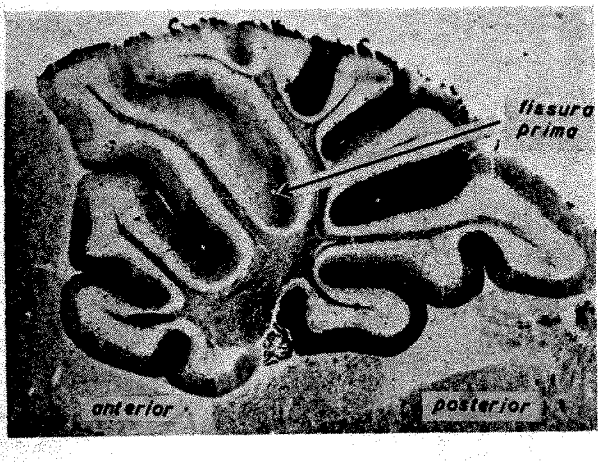

Fig. 1. Paramedian section of cerebellum of 8-week-old mouse showing 5-nucleotidase activity (no counter stain). (× *c.* 15)

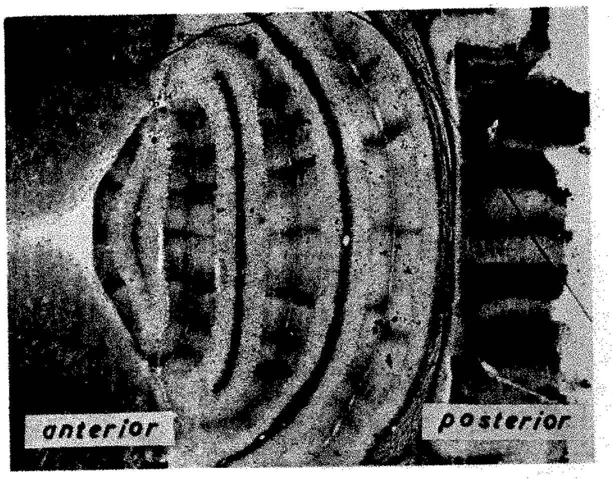

Fig. 2. Coronal section (slightly oblique) of cerebellum of 10-week-old mouse showing the banded distribution of the enzyme. (× *c.* 19)

The significance of this discovery is not immediately apparent. The transition between the two zones occurred at the apex of the folium immediately posterior to the fissura prima in all the mice examined. The increased enzyme activity of the zone is present right up to the posterior margin of this layer in the roof of the 4th ventricle. It therefore does not differentiate paleocortex from neocortex, and in consequence it must be supposed that it has little or no phylogenetic significance.

Ontogenetically it would suggest that the posterior wall of the fissura prima is to be included in the anatomically anterior portion of the cerebellum, and that any functional differences between anterior and posterior terminate not at the base of the fissura prima, as might be supposed, but caudal to it.

The significance of the antero-posterior banding is not known.

T. G. SCOTT

Department of Neuropathology,
National Hospital for Nervous Diseases,
Queen Square, W.C.1.

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Histochemical Reaction for Steroid-3β-ol-dehydrogenase in the Interrenal and the Corpuscles of Stannius of *Anguilla anguilla* and *Conger conger*

SINCE Giacomini's[1] demonstration that the interrenal tissue of the 'head-kidney' in teleosts is embryologically and anatomically independent of the corpuscles of Stannius, doubt has remained as to the participation of these corpuscles in the production of corticosteroids.

Phillips and Mulrow[2] and Nandi and Bern[3] have given direct evidence for the secretion of these hormones by the interrenal tissue. Ford[4] did not manage to identify corticosteroids in extracts of corpuscles of Stannius in the pacific salmon, *Oncorhynchus nerka*, just as Phillips and Mulrow[5] had shown that *in vitro* cultures of the corpuscles of Stannius from *Pseudopleuronectes americanus*, incubated in the presence of tritiated progesterone, lack the capacity to synthesize adrenocorticosteroids. The latter results show indirectly that the enzymes responsible for steroidogenesis are absent from the corpuscles of Stannius.

In order to obtain direct proof of the absence of steroidogenesis in this gland, we have carried out a histo-

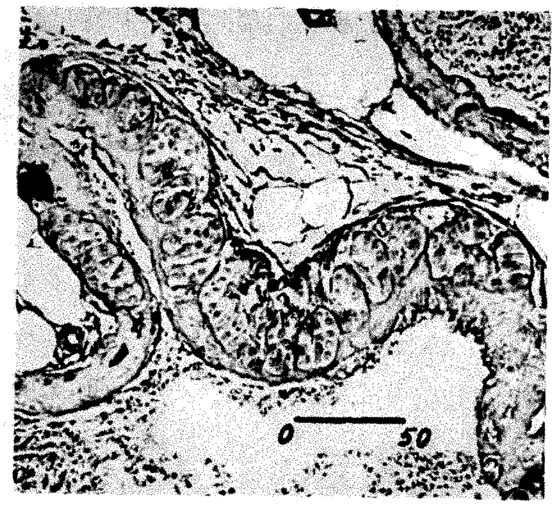

Fig. 1. Cross-section of the 'head-kidney' of *Anguilla anguilla*. The cords of interrenal cells are scattered in the wall of the blood vessels (hæmatoxylin and eosin)

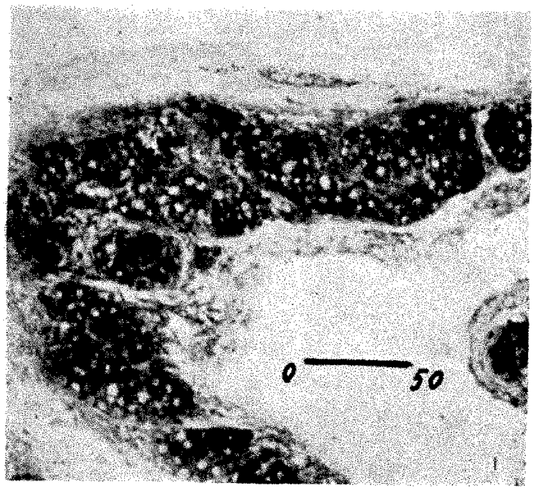

Fig. 2. Cross-section of the 'head-kidney' of *Anguilla anguilla*. Positive reaction for steroid-3β-ol-dehydrogenase in the interrenal cells: note the precipitation of granules of formazans

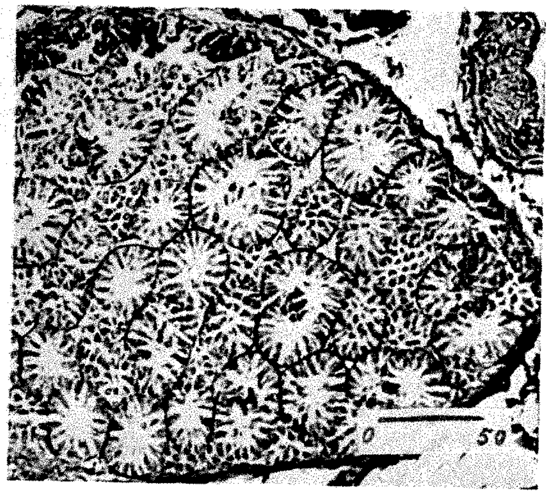

Fig. 3. Cross-section of the corpuscle of Stannius of *Anguilla anguilla* (hæmatoxylin and eosin)

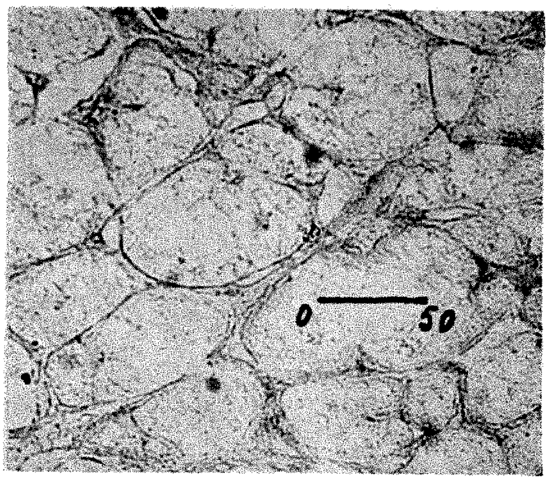

Fig. 4. Cross-section of the corpuscle of Stannius of *Anguilla anguilla*. Reaction for steroid-3β-ol-dehydrogenase: note the absence of the granular precipitation of formazans. Counterstaining with carmalum

chemical reaction for steroid-3β-ol-dehydrogenase, both on the interrenal tissue and on the corpuscles of Stannius from *Anguilla anguilla* and *Conger conger*, following the technique of Wattemberg[6], modified by Levy *et al.*[7,8]. This enzyme, which is responsible for the transformation of pregnenolone into progesterone, has been shown to be present only in the steroid-secreting glands of vertebrates[6-11].

The interrenal tissue, which is found along the blood vessels of 'head-kidney', consists of cords of cubical or cylindrical cells, arranged in the form of alveoli (Fig. 1). The corpuscles of Stannius, which are found on the ventral or dorsal surface of the kidney, are formed of alveoli of cylindrical cells, with very characteristic spaces in the middle (Fig. 3). The reaction for steroid-3β-ol-dehydrogenase was positive only for the interrenal cells (Fig. 2), but was constantly negative in the corpuscles of Stannius (Fig. 4).

The specificity in the location of this reaction has been further emphasized by the ubiquitous histochemical distribution of the lactic-dehydrogenase, which, like the other enzyme, is DPN-dependent.

Therefore it can be concluded that, while the function of a gland of steroid secretion is confirmed for the interrenal tissue, this function is definitely excluded for the corpuscles of Stannius in *Anguilla anguilla* and *Conger conger*.

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G. CHIEFFI
V. BOTTE

Department of Histology and Embryology,
University of Messina,
and
Zoological Station of Naples.

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RADIOBIOLOGY

Determination of Radiation-induced Mutation Rates of Recessive Lethal Alleles in *Saccharomyces*

COMPARING X-ray inactivation curves of haploid and diploid strains of *Saccharomyces*, Latarjet and Ephrussi[1] were the first to suggest that radiation inactivation of yeast cells may be caused by an induction of recessive and dominant lethal mutations. The first evidence of X-ray-induced recessive and dominant lethal mutations was obtained by Mortimer[2] by means of tetrad analysis and specific mating experiments respectively. Recently the number of loci which may be induced by ultra-violet or X-rays to mutate to corresponding recessive lethal alleles as well as the average mutation rate of these loci has repeatedly been estimated after taking into consideration inactivation by dominant lethal mutations and other lethal effects[3,4]. Although the mean mutation rates determined in different laboratories do show a good correspondence, they are much higher than ultra-violet or X-ray-induced mutation rates from auxotrophic to prototrophic conditions or vice versa[4,5]. This striking difference in mutation rates demands a new independent investigation of the frequencies of radiation-induced recessive lethal mutations. If the estimated high mean mutation rates of recessive lethal mutations do correspond with reality, an investigation of specific mutation rates of a number of individual loci should reveal certain loci exhibiting high mutation rates.

An attempt to determine radiation-induced mutation rates of specific genes to recessive lethal alleles has been undertaken by comparing the survival rates of irradiated diploid cells heterozygous for one recessive lethal allele ($S_{+/l}$) with those of diploid cells homozygous for the corresponding vital alleles ($S_{+/+}$). The mutation rate t_m of a specific vital allele to a recessive lethal condition can be calculated using a general equation for cell inactivation[6]:

$$S_{+/+} = (1 - D) \; . \prod_{i=1}^{m} (1 - t_i^2) \qquad (1)$$

The survival rate of diploid cells heterozygous for one recessive lethal allele is given by:

$$S_{+/l} = (1 - D) \; . \prod_{i=1}^{m-1} (1 - t_i^2)(1 - t_m) \qquad (2)$$

In these equations D stands for the rate of inactivation by effects other than recessive lethal mutations and m for the number of loci per chromosome set which can mutate to a recessive lethal condition. It follows:

$$t_m = \frac{S_{+/+}}{S_{+/l}} - 1. \qquad (3)$$

Saccharomyces strains heterozygous for one recessive lethal mutation (+/l) were isolated from the diploid strain 241 of our strain collection after irradiation with small doses (≈ 90 per cent survivors) of X-ray or ultra-violet. Strain 241 is carrying no recessive lethal allele and therefore is of the genotype +/+. An induced heterozygosis for one recessive lethal mutation is recognizable by a 2 : 2 (colony-forming ability : non-colony-forming ability on complete medium) segregation of ascospores in tetrad analysis. At least 10 tetrads of every isolated strain were analysed. Only those strains the ascospores of which exclusively exhibited a 2 : 2 segregation were selected as heterozygotes for further inactivation experiments.

The accurate reproducibility of experimental results necessary for the determination of relatively small differences in survival rates could only be achieved in inactivation experiments with X-rays. The cultures were prepared according to the method described by Wood[7] with minor modifications being applied. In Table 1 the survival frequencies after irradiation with 85 krads X-ray are given for 3 X-ray-induced +/l-strains and the +/+-parental strain 241. The maximum mutation rate obtained was 0·25. The most sensitive +/l-strain, 241*x*-2, was chosen for an investigation of the dose dependence of t_m. From the dose effect curves given in Fig. 1, t_m-values were calculated, which show a linear increase with dose up to 85 krads (Fig. 2).

Table 1. SURVIVAL FREQUENCIES AND CALCULATED MUTATION-RATES OF FOUR *Saccharomyces* STRAINS AFTER IRRADIATION WITH 85 KRADS X-RAYS

Strain	Genotype	Frequency of survivors (%)	No. of independent determinations	Mutation-rate t_m (+ → l)
241	+/+	21·3 ± 0·5	15	—
241*x*-2	+/l	17·1 ± 0·4	16	0·25
241*x*-10	+/l	18·6 ± 1·0	3	0·15
241*x*-17	+/l	20·7 ± 1·2	3	(0·03)

Besides the 3 X-ray-induced +/l-strains there were also 5 ultra-violet-induced +/l-strains isolated, the X-ray-sensitivity of which was determined. None of these strains proved to be more X-ray-sensitive than the parental +/+-strain. Two of the ultra-violet-induced +/l-strains were also checked for their ultra-violet-sensitivity and compared with the ultra-violet-sensitivity of the parental +/+-strain. Although the results obtained in different ultra-violet experiments could not be reproduced very accurately the +/l-strains generally did show an increased ultra-violet-sensitivity. t_m-values, tentatively calculated, were of the same range as those given in Table 1 for X-ray experiments.

Since it is of interest to know also whether homozygous +/+-strains with a significantly increased X-ray sensitivity do occur, survival rates after irradiation with 85 krads X-rays of several sub-clones isolated from strain 241 were determined. The results obtained are in good agreement with those of the parental strain 241 (Table 2).

As may be seen in Table 1 two out of three investigated +/l-strains did show a significantly increased X-ray sensitivity as compared with the values obtained for the parental +/+-strain. Mutation rates of 0·15 and 0·25 were calculated from these data. The occurrence of

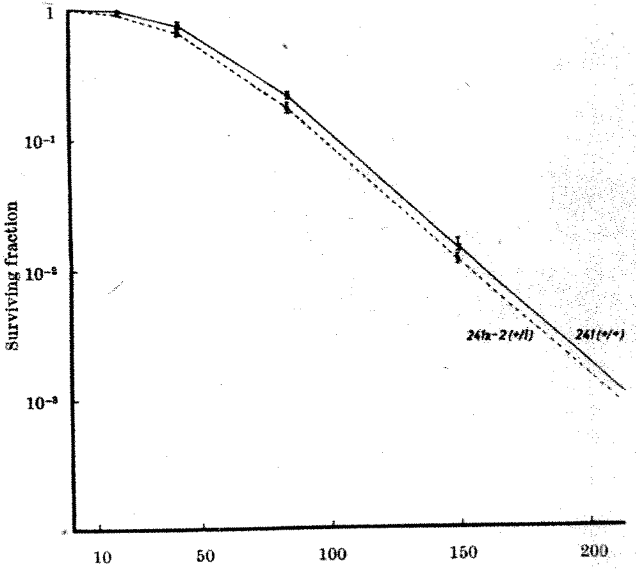

Fig. 1. X-ray inactivation curves for the diploid strain 241*x*-2 heterozygous for one recessive lethal allele (+/l) and for the parental strain 241 homozygous for the corresponding vital alleles (+/+). Vertical bars indicate the mean standard deviation. Irradiation source was a Machlett X-ray tube, operated at 50 kV, 9 m.amp, beryllium window without additional filter

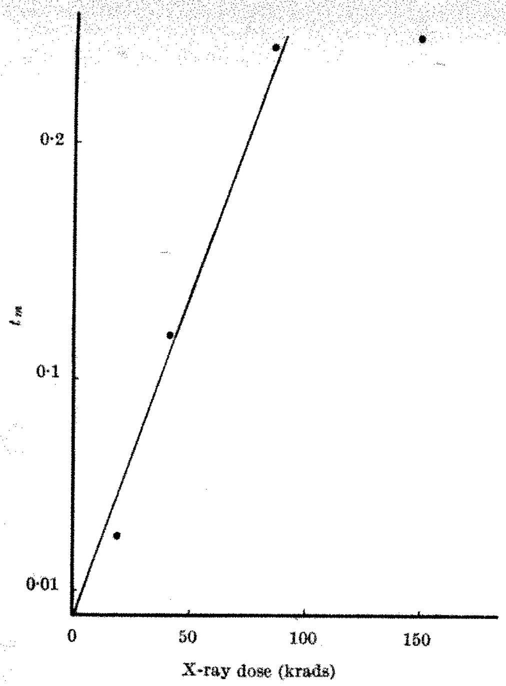

Fig. 2. X-ray dose dependence of t_m calculated from Fig. 1 with the help of equation (3)

extremely high mutation rates to recessive lethal alleles in *Saccharomyces* is thus confirmed. It must be taken into account, however, that the method used for obtaining heterozygous +/l-strains is highly selective for the isolation of mutants with high mutation rates. Therefore, one has to expect that besides the observed highly mutable groups of genes there are also others with definitely smaller mutation rates to recessive lethal conditions. Although radiation-induced recessive lethal mutations evidently do occur, definite evidence is still lacking that they play a major part in killing of haploid cells. About possible reasons for the observed high mutation rates only speculations are possible. They may be caused by radiation-induced inactivation of especially long critical chromosome regions.

Table 2. SURVIVAL FREQUENCIES OF SIX SUB-CLONES OF STRAIN 241 AFTER IRRADIATION WITH 85-KRADS X-RAYS

Sub-clone / Experiment	1	2	3	4	5	6
A	20·6	20·2	22·5	21·3	20·9	23·7
B	20·8	21·7	22·7	21·7	19·5	22·2
C	23·2	22·1	18·6	20·4	20·7	20·3
Mean	21·5	21·3	21·3	21·1	20·4	22·1
Total mean	21·3 ± 0·3					

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W. LASKOWSKI
K. HAEFNER

Institut für Biophysik,
Freie Universität Berlin,
Berlin-Dahlem, Germany.

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BIOLOGY

Resorption of Necrotic Lens Material by a Newly Formed Lens Capsule in Certain Types of Cataract

THE formation of a new lens capsule by regenerated epithelial cells under a superficial area of necrosis has been described by several authors. It may easily be observed by means of optical microscopy in some senile cataracts or in localized traumatic cataracts after periodic acid–Schiff staining.

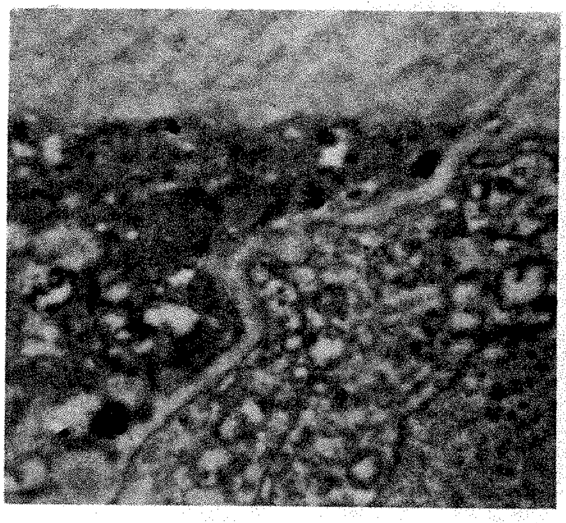

Fig. 1. Senile cataract. Under a normal capsule, a necrotic area (on left) is separated from the normal epithelium (on right) by a newly formed capsule which is in continuity with the normal capsule (right upper corner)

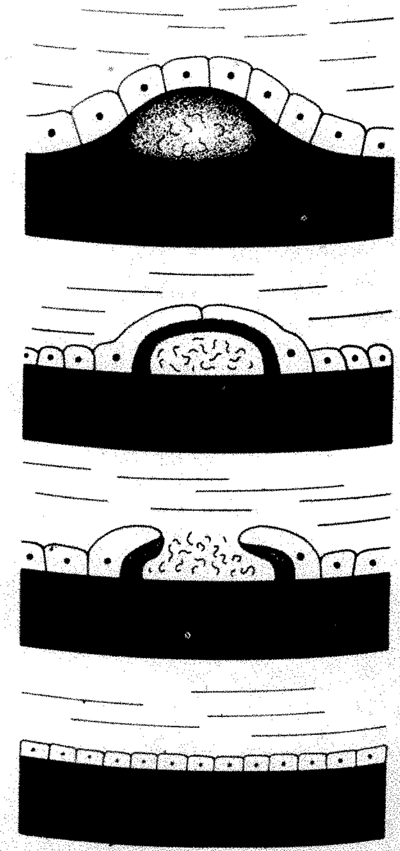

Fig. 2. Diagram showing the successive stages of the resorption of a small necrotic area inside the newly formed capsular material

Electron micrographs of senile cataracts[1] show the newly formed capsule between the normal epithelium and the necrotic area (Fig. 1). This newly formed capsule shows a definite tendency to grow more and more closely to the normal one, and finally to merge with it. Thus, the necrotic material is included in the capsular substance. The necrosis will disappear progressively. The end of this evolution is a localized area of a thickening of the capsule. The stages of this process are shown diagrammatically in Fig. 2.

We had the opportunity of observing the same features in experimental traumatic cataracts.

This seems to be the interesting point of these electron microscopic observations. It explains the well-known localized areas of the thickening of the capsule in cataracts, and the possible disappearance of some small superficial opacities of the lens.

A. Brini
A. Porte
M. E. Stoeckel

Clinique Ophtalmologique,
Hôpital Civil,
Strasbourg (Bas-Rhin),
France.

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Wounds and the Polarity of Implant-induced Accessory Urodele Limbs

Previous work[1-3] has established that xenoplastic implants made into the limbs of larval and adult urodeles would initiate the formation of accessory limb structures and, further[4], that the position of the superficial wound used for insertion of the implanted material seemed to have a very decided effect on the stimulus-response system. The implants would not take advantage of wounds made proximal to the implant so readily as they would of distal wounds. In larvæ, as well as adults, very few growths were produced from proximal wound areas. These few supernumerary limbs which did form in association with proximal wounds, however, showed interesting growth patterns which are better understood to-day than they were at the time of the previous writing.

The purpose of this communication is to supply newer information on the relationship between a superficial insertion wound and the formation and polarity of the resultant supernumerary limb and in particular to correlate these relationships with long-standing morphogenetic rules, Barfurth's rule[5] and Bateson's rule[5].

First, it should be noted that the wound relationships found previously apply if an implant of moderate size is placed in such a way as to be completely included beneath the skin cover without protruding through the open wound. If, however, larger quantities of implant material are used which are allowed to protrude through the open insertion wound, thereby delaying the wound healing phase, supernumerary limbs may be obtained in many instances regardless of the position of the wound. With this information, then, it became possible to study readily the polarity of implant-induced accessory limbs in association with wounds made in a variety of positions.

Thirty adult *Triturus viridescens* from Petersham, Massachusetts, were utilized as hosts, while the kidneys of a single adult male *Rana pipiens* from Oshkosh, Wisconsin, served as donor material. Six different experimental situations were established, using both forelimbs of each animal. These situations differed in the position of the insertion wounds, which were made proximal, distal, anterior or posterior to implants placed on the dorsal surface of the radio-ulnar portion of the forelimb; and proximal or distal to implants on the ventral surface of the limb. Our previous experience, as already noted, had shown that the position of the wound does play a hand in establishing the early growth pattern of the induced accessory limb.

Fig. 1 illustrates a limb which developed in association with a distal wound on the dorsal surface. (The figures are labelled with *D* and *P* for distal and proximal.) In this case, the axes appear to be identical with those of the 'mother' limb. Those accessory limbs which develop in association with wounds which are other than distal begin their development in the direction of the wound and later, as their development progresses, tend to adjust to the axes of the 'mother' limb. For example, accessory limbs formed on the dorsal surface in association with proximal wounds follow the pattern described in a previous paper[4]. They originally grow proximad, then straighten up, with a concavity usually on their proximal surface, and finally shift to a more distal direction, the concavity normally becoming filled in. Those which form with anterior or posterior wounds on the dorsal surface tend also to grow initially in the direction of the wound and then later to shift more distad. Fig. 2 shows a limb growing in association with an anterior wound. At the time this supernumerary was photographed it was changing toward a distal direction.

Only one accessory structure of multiple character was obtained from a ventral surface distal wound situation, and it was impossible to judge its axial relationships. Fig. 3 is strikingly illustrative of an accessory structure formed in association with a proximal wound on the ventral surface, which began its development in a proximal direction and subsequently shifted so that its entire distal portion became hooked around and grew almost directly distad.

Implant-induced accessory limbs, then, appear to follow Barfurth's rule in that the insertion wound supplies to the morphogenetic system what is in effect an oblique amputa-

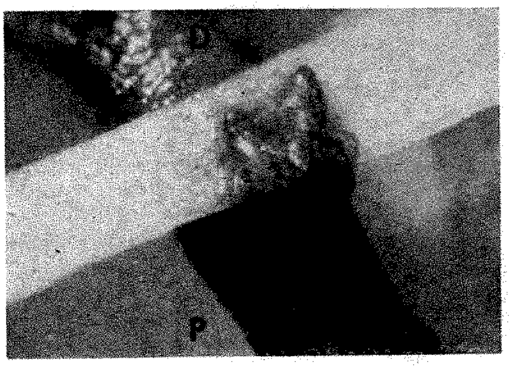

Fig. 1. Implant-induced accessory limb which formed in association with a distal insertion wound on the dorsal limb surface. Its axes correspond with those of the 'mother' limb. (× *c.* 40)

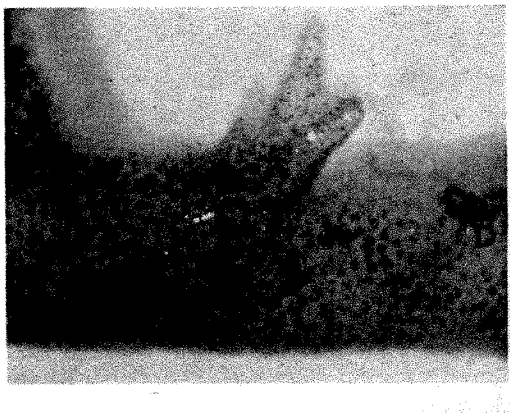

Fig. 2. Implant-induced accessory limb which formed in association with an anterior insertion wound. Its axis, although originally entirely anteriad, had shifted somewhat distad. (× *c.* 40)

tion surface and the original blastemal polarity is that of this oblique surface. Afterwards, as development progresses, the growing accessory limbs tend to adjust to the polarity of the 'mother' structure so that in their advanced stages they tend to deviate only slightly from it.

Multiple accessory limbs are of particular interest, since if the growth-centres are in close association, they tend to follow Bateson's rule and mirror-image. If their centres are so close as to form a common base external to the level of the limb surface, one obtains 'hydra-like' accessory structures where as many as 7 digits have been found arranged in a whorl around the apex of the stalk. Fig. 4 shows two such formations on the same host.

Other situations have been found in which the developmental centres fail to join external to the surface of the limb but established a common base internally with the digits arranged much like the spikes on a crown. One of these 'corona-like' arrangements is illustrated in Fig. 5. These digital arrangements have in both instances been interpreted by us as having resulted from mirror-image development of multiple morphogenetic systems which are so close as to be interacting. In any event, it is known that they result when secondary wounds are established near the insertion wounds through the activities of the implants. This type of secondary wound formation by implants has previously been reported[4]. The foregoing postulate that these formations are really multiples further obviates the necessity of supposing that a single blastema has been created capable of forming a greater number of digits than one would normally find on an amphibian forelimb. Such an occurrence would be contrary to previous experiences in the limb regeneration field. Histological examination of the bases does indeed show multiple skeletal elements.

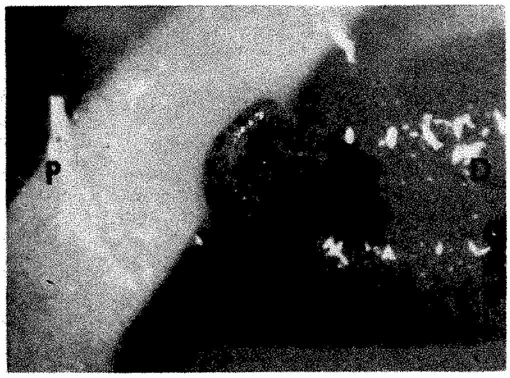

Fig. 3. Implant-induced accessory limb which formed in association with a proximal insertion wound on the ventral limb surface. Note that the entire distal end had hooked about and was heading toward the hand of the 'mother' limb. (× c. 40)

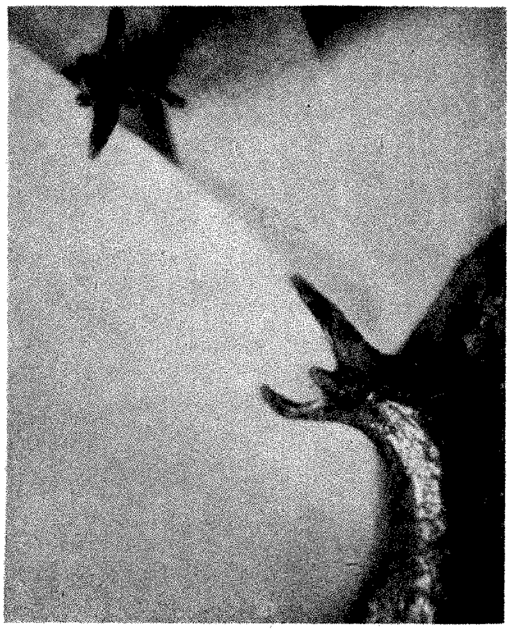

Fig. 4. Two 'hydra-like' accessory limb structures. These appear to arise from multiple-interacting morphogenetic systems which form a common stalk external to the limb surface. (× c. 40)

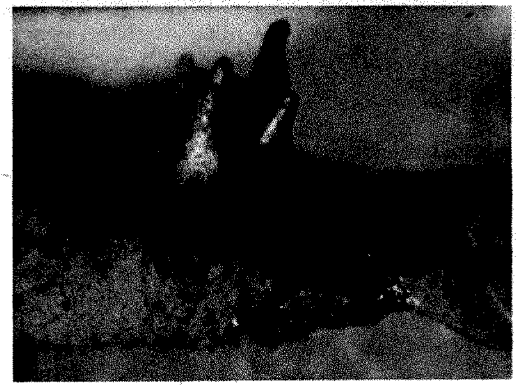

Fig. 5. An example of a 'corona-like' formation. These appear to arise from multiple-interacting morphogenetic systems which form a common base internal to the limb surface. (× c. 40)

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Laurens N. Ruben
Jean Stevens

Department of Biology,
Reed College,
Portland, Oregon.

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Hæmoglobin in Barnacles

HÆMOGLOBIN is known from many groups of Crustacea, excluding Malacostraca; but it has not previously been found in the non-parasitic Cirripedia[1]. It is, therefore, interesting to record the occurrence of hæmoglobin in *Balanus perforatus* Bruguière, a common intertidal barnacle of southern Britain, south-west Europe, the Mediterranean and north-west Africa.

When freshly collected specimens are dissected under a low-power binocular, certain of the muscles can be seen to be pale pink, though the majority are colourless. Examined with an eyepiece spectroscope, the darkest of these muscles produce faint absorption lines centred at 577 and 545 mμ, and reduction with sodium dithionite produces a single diffuse line centred at 555 mμ. These spectra are characteristic of hæmoglobin[2].

Treatment of fresh specimens with benzidine and hydrogen peroxide[3] gives a blue or brown stain in the pink muscles, the nervous system, the outer part of the gut wall and sometimes in the muscular walls of the vasa deferentia. There is no great difference between the staining of fresh and boiled specimens, indicating that tissue peroxidases are not responsible for the reaction in these organs[4]. After fixation in alcohol the distribution of the benzidine-positive muscles can be followed by dissection. They are most abundant in part of the prosoma around the adductor scutorum, but there are also some among the oblique muscles of the thorax and in the basal joints of the cirri. Of the three pairs of large muscles which depress the operculum, only the lateral depressors contain hæmoglobin, in a small number of flattened fibres on the inner side, which show up as pink when fresh. In some specimens two or three fibres of the adductor scutorum are also benzidine-positive.

From the distribution of 'pink' muscle outlined here, one may deduce that hæmoglobin is concentrated in muscles performing fairly continuous and rapid movements[5], especially in the region of the blood-pump[6]. The blood itself is very pale yellow or orange in colour, but has no perceptible absorption lines. However, it does give a slight positive reaction with both benzidine and *o*-tolidine[4], which, if not due to contamination, may indicate the presence of a low concentration of hæmoglobin.

No other species of barnacle examined has sufficient hæmoglobin to give a pink colour to the muscles; but *Elminius modestus* Darwin and *Balanus crenatus* Bruguière would appear to contain a small quantity, since some muscles of the prosoma give a positive benzidine reaction.

EVE C. SOUTHWARD

The Laboratory,
Citadel Hill,
Plymouth.

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Moulting Behaviour of *Birgus latro*

THE moulting behaviour of *Birgus latro*, the coconut or robber crab, has never been described even though this crab has been the subject of many observations. Weins[1] reviewed these observations most recently, and Harms[2] reviewed the older literature. Neither moulting nor the moulting behaviour is mentioned in these reports with the exception of a photograph of a recently moulted *B. latro* by Harms. Furthermore, in several years of observation of *B. latro* in the Marshall Islands, exuviæ or freshly moulted crabs were never found although scores of burrows have been examined, often with the aid of local residents. Small individuals with a cephalothorax length of less than about 30 mm are relatively rarely found even when special efforts are made. Prof. Donald C. Matthews of the University of Hawaii has had similar experiences (personal communication, December 1962).

On September 21, 1961, a living *Birgus*, with a cephalothorax-length of 20·8 mm, was obtained from Mr. Emos, magistrate of Rongelap Village, Rongelap Atoll, Marshall Islands. The crab was found in the early morning in an opened coconut in a grove where copra was being prepared on Rongelap Island. The specimen was carried in a plastic jar to the University of Washington, Seattle, Washington, and put in a terrarium on October 2, 1961, where the observations reported here were made.

The temperature in the terrarium was maintained at 26°–30° C and the relative humidity ranged from about 70 to 90 per cent. A red incandescent light bulb, burning continuously, served both to supply heat and permit observations at night. Both fresh water and sea water were available to the crab at all times. Coral sand, soil, and coconut husks from Rongelap were contained separately in the terrarium. Rye grass was grown in the soil. Food was always available and consisted variously of copra, fresh coconut, bread, apples, oranges, bananas, beef and fish meal. As in Nature, the crab was omnivorous but showed a clear preference for fish meal.

During the first month in the terrarium the crab remained hidden beneath the coconut husks in the daytime and was active at night, feeding, digging and usually visiting both fresh water and sea water. On the 32nd day it hid in the soil in a burrow ending in a spherical chamber with smoothly packed walls which it had made. The tunnel leading from the chamber to the surface was filled with loose soil. The closed entrance to the burrow was scarcely distinguishable from the soil around it. Larger individuals had been observed in burrows in the field but never with the entrance closed with soil.

During the second and third months the crab continued its nocturnal activity and remained hidden and covered in the soil during the day. Its abdomen appeared distended towards the end of this period and its weight had increased from 6·8 to 7·3 g. On the 86th night the crab did not leave the burrow and remained hidden without food or water for the ensuing 30 days.

In an attempt to observe the crab while in hiding, the soil was excavated to make an opening in one side of the crab's chamber and a glass window with an opaque shutter was put in place. This attempt was unsuccessful because the crab persisted in covering the glass surfaces with moist soil even when light was excluded. These attempts at observation were discontinued for fear of disturbing the 'normal' activity of the crab.

When the crab emerged from the burrow the length of the cephalothorax had increased from 20·8 to 22·7 mm. The crab continued to follow the pattern of alternating hidden and active periods (Fig. 1). During the active period it fed at night and hid during the day, sometimes in its burrow and sometimes under coconut husks. Whenever the burrow was occupied the entrance was found plugged with soil, which probably explains why small open burrows investigated in the field were always found empty.

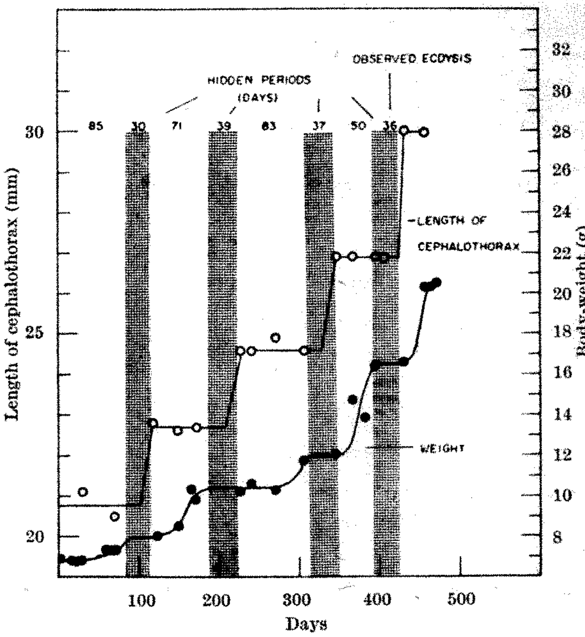

Fig. 1. Growth and moulting cycle of *Birgus latro* in a terrarium

A year after being brought to the Laboratory the crab had completed three hidden periods, and was transferred to a larger terrarium in which four clear celluloid trays containing either sand or soil were placed. The trays, which were about 20 cm × 25 cm × 10 cm deep, were suspended on stainless-steel racks so that they could be removed from the terrarium and observed from below and all sides. Within a few days the crab excavated a new chamber with one side a few millimetres from a lower corner of a tray containing soil. A flap was cut in the celluloid and folded back to afford a view into the chamber. A thin layer of soil adhered to the flap, which was pushed back in place and sealed with tape. Three weeks after the crab had entered its fourth hidden period, the flap was opened and the crab was observed daily.

Ecdysis occurred on the 28th day of hiding. The freshly moulted crab was pale blue, fading to near white in proximal areas. The following day the colour had become much darker, approaching the colour of the exuviæ, and a portion of the carapace of the exuviæ had been consumed. Day by day the exuviæ were consumed, the last remaining part being a claw. The night following the 36th day the crab emerged from hiding and at the time of writing has continued in an active period.

In 463 days this young *B. latro* moulted four times, increased in cephalothorax length from 20·8 to 30·0 mm, and in weight from 6·8 to 20·2 g (Fig. 1).

If the assumptions are made that this rate of growth remains constant and approximates that found in Nature, then specimens of *B. latro* with cephalothorax lengths in excess of 100 mm, which are common, must be about five years old.

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EDWARD E. HELD

Laboratory of Radiation Biology,
University of Washington,
Seattle, Washington.

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Identification of Species of *Scenedesmus*

AMONG the organisms frequently encountered in investigations of freshwater habitats are members of the genus *Scenedesmus*. Species are delimited on the basis of the size and shape of the cells, as well as position and number of spines, if present. Although Smith[1] in his monograph of the genus shows some variation in these characters, one can, with some degree of confidence, identify species quite easily.

However, when one reviews other literature concerning identification of species, especially the monograph of Chodat[2], as well as earlier supporting work by Chodat and others, one finds that these investigators had pointed out the extreme variability found in many *Scenedesmus* species. The bulk of this earlier work centred on the ability of these organisms, while in culture, to fragment into unicellular forms which resemble *Chlorella*, *Raphidium* (*Ankistrodesmus*), *Oocystis*, etc., or to form a *Dactylococcus*-like stage. Chodat emphasized that one must culture these organisms in order to determine correctly their taxonomic position. The formation of 100 per cent unicells by *S. dimorphus* in a two-membered culture with a soil bacterium[3], as well as the occurrence of a *Dactylococcus*-like stage when the soil alga was cultured in yeast extract[4], were reported.

Recently we encountered another extremely variable *Scenedesmus* species, *S. longus*, and have examined it in unialgal, bacteria-free culture. All observations discussed are with this axenic culture. When grown in liquid medium of inorganic composition under the usual laboratory conditions, the organism does not appear to be a *Scenedesmus*. Almost all the cells of the culture are unicells bearing three or four spines at each pole. When the organism is in this condition, it could be confused easily with *Chodatella subsalsa*. In fact, the unicell fits the description of the latter exactly.

By altering the conditions of growth, for example, culture on firm agar, or by use of a thermal shock, the percentage of cœnobes was found to increase markedly, almost to the exclusion of unicells. A three-day-old culture grown on 2·5 per cent inorganic agar at 22° C produced 98·6 per cent cœnobia. Only then could the organism be recognized as *Scenedesmus longus*. Although there was some variability in spine number and location, the majority of the cœnobes had the typical six spines.

The organism was also grown in soil extract[5]. This medium has been used in the past not only to supply nutrients absent in basal inorganic media, but also in an attempt to simulate natural conditions. Apparently some organisms exhibit the form seen in field collections only when grown in soil extract or in soil-water bottles[5]. When the organism described here was grown in liquid culture in soil extract, the majority of the forms were unicellular, with the cœnobe present only in the bottom of the culture. In addition, numerous unicells were suspended in the liquid. Cell size, shape and spine condition did not vary from that described here.

Further experimentation with *S. dimorphus* demonstrated that one can stimulate the production of unicells in a number of complex media. The role of an organic carbon source in reducing *S. acutus* to the unicellular condition was first reported almost 75 years ago[6].

Although these investigations have been conducted in the laboratory and one can now only speculate as to the extent of variability in Nature, we believe that pleomorphism could exist in Nature. Growth of *S. longus* in soil extract indicates that in a body of water the unicellular form might be abundant in the plankton, whereas the cœnobe could be found in bottom sampling. Reports by Prescott[7] of the occurrence of both *S. longus* and *C. subsalsa* in the lakes of Wisconsin and Michigan give some support to this argument.

With *S. dimorphus* it also appears that the introduction of organic waste in a pond or stream might change the morphology of the organism. If sufficient numbers of bacteria were present, cœnobe formation would be affected[3].

Apparently there are many species of *Scenedesmus* in which cœnobe formation is quite stable. The fact that Smith made no mention of pleomorphism in pure culture studies connected with his monograph attests to this[1].

Even though we know little, if anything, of the extent to which pleomorphism in *Scenedesmus* exists in Nature, we re-emphasize the word of caution of Chodat concerning precise identification of species of *Scenedesmus*. For a better understanding of variability in this genus field identification of unicellular and cœnobial stages from the same site, followed by pure culture studies, appear essential.

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FRANCIS R. TRAINOR
RICHARD L. HILTON

Department of Botany,
University of Connecticut,
Storrs.

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A Fungistatic Action of Coumarin

VAN SUMERE *et al.* showed that coumarin affects the germination of wheat stem rust uredospores[1], and Bellis showed[2] that *Penicillium jenseni* and *P. nigricans* are able to grow on a modified Czapek's nutrient medium containing coumarin as the only one carbon source. So far as I am aware, no other experiments have been done to examine the ability of coumarin to regulate the growth of moulds*, in spite of the fact that some fungi normally produce coumarin precursors and derivatives[3], and the level of coumarin-like substances increases in the fungus-attacked cell[4].

Cultures of *Aspergillus niger E*–32, *Penicillium glaucum* and *Rhizopus nigricans* were obtained by dropping 0·1 ml. of spore suspension into 100-ml. Erlenmeyer flasks containing 25 ml. of nutrient media with coumarin in proper concentration. After a suitable period of time of incubation at 26° C mycelia were taken out of flasks, dried for 12 h at 105° C and weighed. Two different nutrient media were used: (*a*) yeast water supplemented with 4 per cent of glucose[5]; (*b*) a synthetic medium consisting of glucose, 45·0 g; $NaNO_3$, 4·5 g; KH_2PO_4, 3·0 g; KCl, 0·75 g; $MgSO_4.7H_2O$, 0·75 g; $FeSO_4$, 0·02 g; distilled water up to 1 l. (ref. 6). The results were analysed statistically for significance at $P = 0·05$.

The visual examination revealed that coumarin in higher concentrations (100–1,000 p.p.m.) inhibits or markedly retards the germination of spores (lengthening of lag-phase). The detail picture of effects depends on the kind of mould and nutrient medium used. The most sensitive are spores of *A. niger*; these did not germinate in yeast water containing 1,000 p.p.m. of coumarin, and under the influence of 100 p.p.m. they germinated 20–30 h later than did the controls. In a synthetic medium, however, coumarin in concentrations of 1–10 p.p.m. exerts an insignificant stimulatory effect on germination of *A. niger*.

Spores of *P. glaucum* are much more resistant. Coumarin at a concentration of 100 p.p.m. only slightly retards their germination, and spores in flasks containing 1,000 p.p.m. of coumarin germinate 20–30 h later than control ones.

Germination of *R. nigricans* spores was considerably stimulated by 5–10 p.p.m. of coumarin dissolved in yeast water; an insignificant retardation occurred only at concentrations 500–1,000 p.p.m.

It follows from the curves in Figs. 1 and 3 that coumarin exerts only an inhibitory effect on dry matter accumulation of *A. niger* and *P. glaucum* mycelia cultivated on yeast water; the former mould is much more sensitive than the latter one. The analysis of results reveals that 50 p.p.m. appears to be a threshold concentration of coumarin for normal growth of *A. niger*, and 100 p.p.m. for *P. glaucum*. Below this quantity the growth is slightly affected; to the contrary the more concentrated solutions of coumarin are markedly inhibitory. This inhibition is transient, however, and after a suitable period of time moulds seem to develop normally, though slowly.

According to the retardation of growth the sporulation is also affected by coumarin, particularly in the case of *A. niger*.

Different results are observed on a synthetic nutrient medium (Figs. 2 and 3). In this case the fungi are much more sensitive to coumarin, as a rule. Also the picture is much more complicated owing to appearance of stimulation of growth of mycelia cultured on relatively diluted solutions of coumarin. When the threshold concentration is exceeded, the inhibition of growth is expressed much more sharply than on the yeast water.

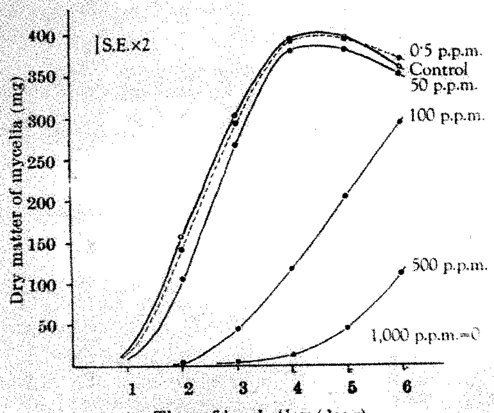

Fig. 1. Effect of coumarin on the growth of *A. niger* cultured on the yeast water containing 4 per cent of glucose

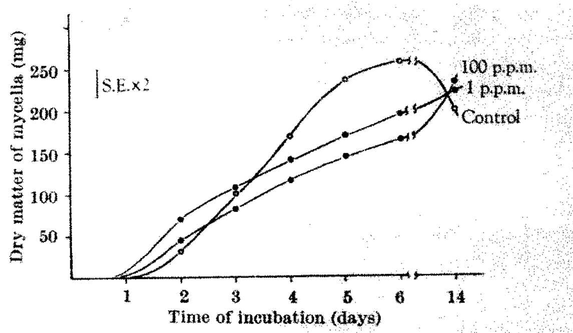

Fig. 2. Growth of *A. niger* on the synthetic nutrient medium supplemented with coumarin

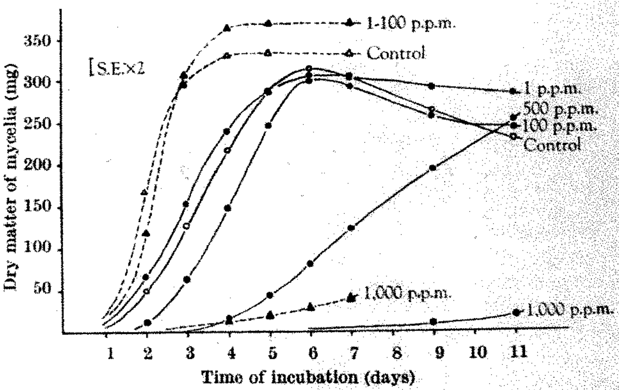

Fig. 3. Effect of coumarin on the growth of *P. glaucum* cultured on the synthetic nutrient medium and on yeast water with glucose. – – –, Yeast water + glucose; ——, mineral medium + glucose

It is striking that coumarin markedly stimulates the sporulation of *P. glaucum* grown on a synthetic medium. In series subjected to 1–10 p.p.m., spores were formed on the second day of cultivation and were green on the subsequent day. In the controls the spores appeared on the third day, but they were not green even on the fifth day of incubation. The case of *A. niger* was the same; on the third day of incubation the quantity of spores in sample 100 p.p.m. was nearly equal to control—in 100 p.p.m. they were black; in the control grey.

The most interesting results have been obtained with *R. nigricans*. This substance in concentrations 5–10 p.p.m. markedly stimulates the development of mycelia (Fig. 4). In affected series the mature spores were formed between 36–48 h, while in the control their appearance was noted in the fourth day of experiment.

Coumarin is metabolized by moulds. It was revealed by paper chromatography of ether and chloroform extracts of post-incubation media that during cultivation the spot of coumarin partially disappears, and new unidentified fluorescent spots occur. The velocity of synthesis of brown pigments, secreted to the medium by *A. niger*, is also retarded by coumarin. Thus it would seem that the process of autotoxication by products of mycelium metabolism is retarded by coumarin present in a nutrient medium in small quantities[7].

On the basis of these results it may be stated that: (*a*) coumarin is a fungistatic agent but not a fungitoxic one; (*b*) a physiological adaptation to this compound occurs. The nature of this adaptation still remains obscure because nothing is known about the primary mode of coumarin action[8]. Bearing in mind that moulds are much more sensitive to coumarin when they are cultured on the simple synthetic nutrient medium than on the yeast water, it can be assumed that this substance blocks the synthesis of an unknown metabolite (or metabolites) of fundamental importance to later stages of spore germination and

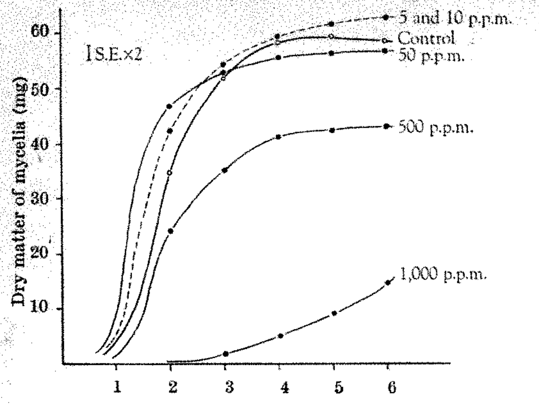

Fig. 4. Effect of coumarin on the growth of *R. nigricans* cultivated on the yeast water containing 4 per cent of glucose

initial growth of mycelia. This unknown metabolite is necessary either for nucleic acids and/or protein turnover, or for the utilization and storage of energy delivered in the breakdown of carbohydrates.

Coumarin does not directly affect the nucleic acid turnover in lettuce[9] and protein synthesis in sunflower[10]; however, it influences the spectrum of free amino-acids present in a cell, and some nucleic acid precursors partially reverse the coumarin-induced inhibition of growth of maize mesocotyle[10]. In connexion with the fact that coumarin affects the respiration[11] and phosphorus turnover in plants[12], these favour the supposition that coumarin controls the pathway of metabolism involved in the utilization of energy.

The stimulation of germination and growth caused by coumarin present in a medium in low concentrations may be elucidated on the basis of supposition that the affected cells react by the mechanism of super-compensation.

* *Addendum.* The Editor has directed my attention to a paper[13] describing the effects of some growth-regulators on the development of *Sclerotium rolfsii* (Sacc.), that is, the imperfect form of a basidiomycete attacking apple-trees. Coumarin in concentrations of 50–100 p.p.m. only slightly affects the growth-rate of mycelia (however, there is no growth at 500 p.p.m.); but it markedly inhibits the germination of sclerotia and reduces the number of progeny sclerotia per culture. Such sclerotia are darker in colour and larger than the normal ones; they germinate faster when planted on media without coumarin, but they are as sensitive to coumarin as control sclerotia are.

I thank Dr. Waclawa Maciejewska-Potapczyk for advice.

J. S. KNYPL

Department of Plant Physiology,
University of Lodz,
Poland.

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Non-climatic Factors affecting the Shape of Apple Fruits

SOME factors affecting fruit shape of apples are known. Partially seeded fruits are one-sided, the less fleshy side being adjacent to seedless carpels. Horticulturists have long observed that climate may also affect shape. Fruits grown in hot climates, especially where nights are warm, are flatter than those from cooler climates. In 1914, Shaw[1] stated: "There is a pretty constant fluctuation between the form of the apple and the temperature for a period following bloom; the cooler this period the more elongated the apple". In an effort to delimit this period, he concluded that the period from the sixth to the sixteenth days following full bloom fitted the observed fluctuations in form more closely than any other period. A recent study by Westwood[2], however, showed that very young fruits were distinctly elongated but became more flattened as they grew, the ultimate shape being determined about 100 days after full bloom. Thus, any effect of climate on shape might possibly extend well beyond a brief period after bloom.

Obviously, factors other than these affect shape because fruits from the same tree often differ strikingly. The investigation reported here was initiated to determine non-climatic factors which affect the shape of apples.

Test trees of Golden Delicious and three genetic strains of Delicious (Regular, Starking, Starkrimson) apples were grown under uniform cultural conditions at the Lewis-Brown Research Farm at Corvallis. All trees were 6–15 years of age. A minimum of pruning was done. The orchard received regular sprinkler irrigation during the season. The strains of Red Delicious were grown on several East Malling clonal rootstocks and on seedlings. A fruit-thinning test was carried out on matched leaders of 15-year-old Golden Delicious trees on East Malling IV rootstock. Heavy thinning was performed at full bloom on half the leaders. Only one flower in each eighth cluster was left, of which half were centre blooms and half were side blooms. Control leaders were not thinned. All trees in the thinning test had a uniformly heavy bloom (90 flower clusters per 100 growing points). For comparison, fruits from separate trees with light bloom (5 flower clusters per 100 growing points) were used.

Length/transverse diameter (*L*/*D*) ratio was used as an index of final fruit shape. The *L*/*D* ratio did not indicate subtle differences in shape but was adequate to distinguish between flat and elongated fruits.

Statistical differences (*L.S.D.* values) were calculated from error mean squares in a standard analysis of variance[3].

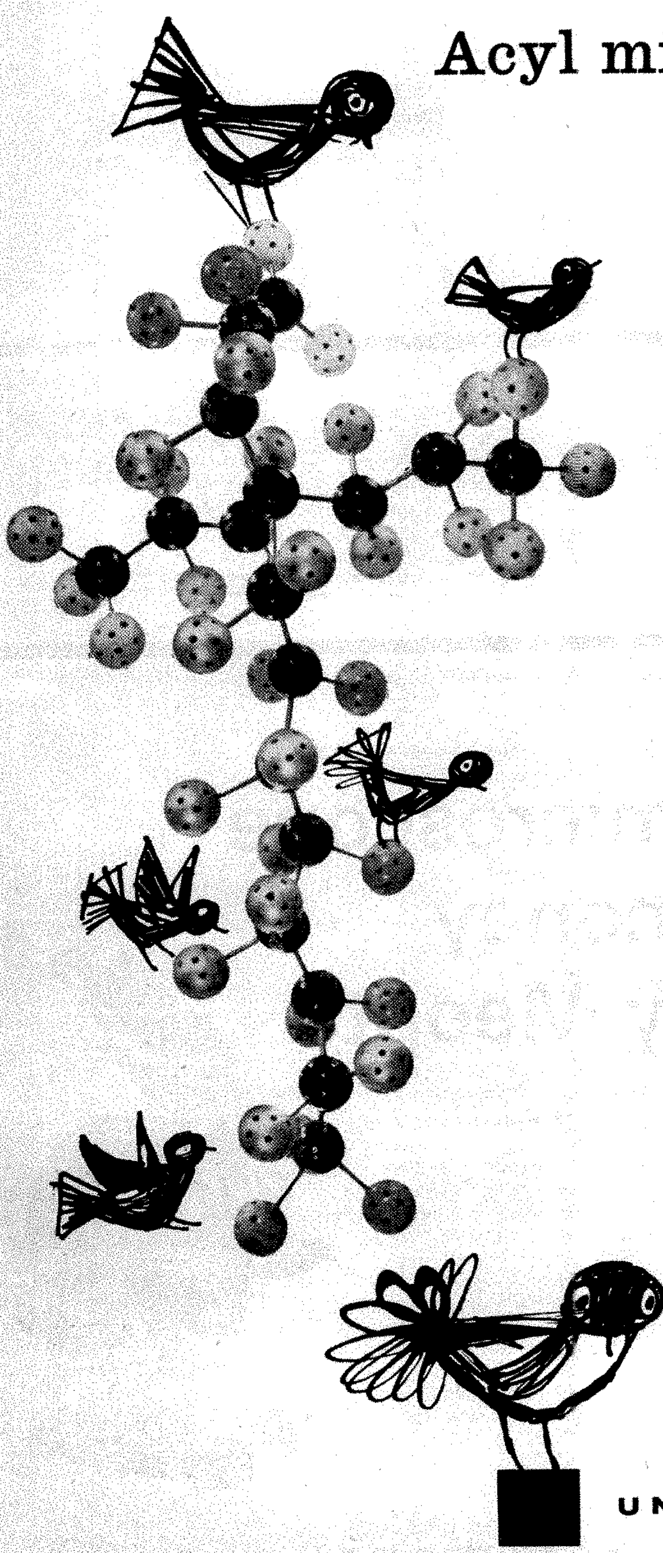
Rootstock type significantly influenced shoot growth and fruit shape of Red Delicious (Table 1). Fruits from trees with non-dwarfing rootstocks (seedling, XVI, I, II) were relatively more elongated than were those from dwarfing stocks (IV, VII, IX). Thus, relative fruit-length varied with rootstock in the same way as shoot growth. Shoot growth was shorter in every case on fruiting branches than on non-fruiting ones.

Table 1. EFFECT OF ROOTSTOCK ON FRUIT SHAPE AND SHOOT GROWTH OF RED DELICIOUS APPLE

Rootstock	Shoot growth Non-fruiting (cm)	Shoot growth Fruiting (cm)	Fruit* shape *L*/*D* ratio
EM XVI	46	39	0·967
Seedling	50	36	0·965
EM I	47	36	0·965
EM II	44	34	0·945
EM IV	31	15	0·938
EM VII	40	24	0·933
EM IX	24	9	0·920
L.S.D. 5% level			0·014

* 300 fruits measured from each rootstock.

Both crop density and cluster position affected the shape of Golden Delicious fruits (Table 2). Centre-bloom fruits were longer than side-bloom fruits, and light cropping (whether from heavy early thinning or from light bloom) resulted in longer fruits than did heavy cropping



Acyl migration

How welcome is the evocative word in the literature of science. Migration—geese in flight... swallows twittering on telephone wires... lemmings streaming blindly to sea. What of acyl migration? Alas, an element of bathos intrudes. At first we may see things in flight; but in truth, when we examine the concept, the vision changes to one of chimps swinging cheekily from branch to branch. Exactly so with partial glycerides: identifiably so with tri-glycerides, but the behaviour pattern is a little more complicated!

That acyl groups in glycerides can migrate remains a matter of surprise—even though rearrangement in fatty esters was first reported by Claisen so long ago as 1887, and in diglycerides by Fischer in 1920. Delayed interest in the subject has been due largely to the absence of good analytical methods, but the recent spate—differential thermal analysis, infra red spectrometry, partition chromatography, enzymic splitting, radio tracers—has opened the field, and today we are beginning to understand the mechanisms whereby an acyl group can swing between the 1- and 2- position in a partial glyceride, and interchange between triglycerides.

Work on acyl migration is of theoretical importance in the understanding and control of these mechanisms, and of practical importance both in the preparation of model substances for systematic study, and in the large scale treatment of oils and fats to modify their physical behaviour.

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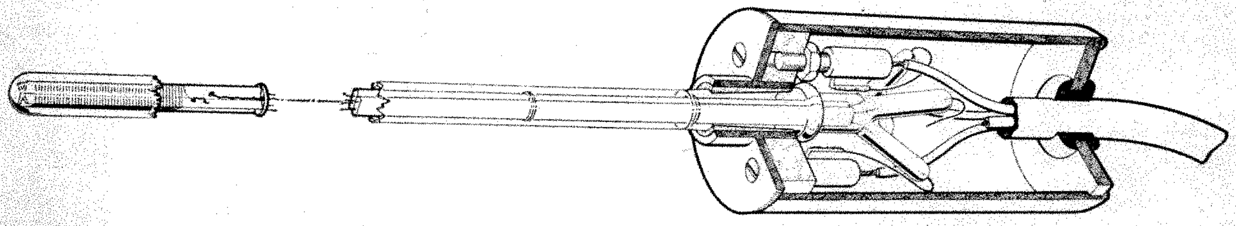

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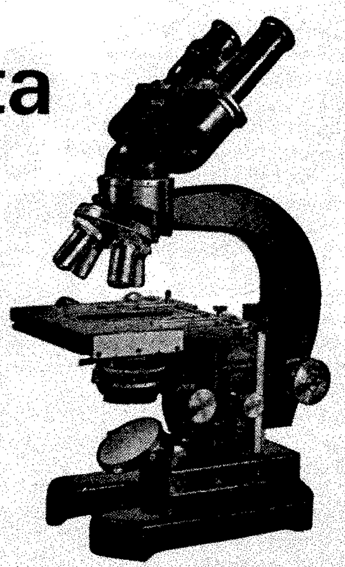

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Table 2. EFFECT OF FRUIT POSITION AND DEGREE OF THINNING ON THE SHAPE AND SIZE OF GOLDEN DELICIOUS FRUITS

Amount of thinning	Amount of bloom	Cluster position	No. of fruits	Fruit weight (g)	Fruit shape *L/D* ratio
Heavy (full bloom)	heavy	centre	80	236	0·987
	heavy	side	80	218	0·954
None	heavy	centre	80	174	0·960
	heavy	side	80	151	0·920
Light (late)	heavy	—	80	170	0·935
None	light	—	80	207	0·960
L.S.D. 5% level					0·013

leaders or trees. Fruit size *per se* did not correlate well with shape.

Centre-bloom fruits of Red and Golden Delicious were longer than side-bloom fruits both when grown on separate or on the same spur cluster (Table 3). Variability was less, and the difference was more striking when centre- and side-bloom fruits were paired on the same cluster. This would be expected because such factors as leaf/fruit ratio and spur vigour were identical for each pair.

Table 3. EFFECT OF FRUIT POSITION ON THE SHAPE OF APPLE FRUITS GROWN ON THE SAME OR SEPARATE CLUSTERS

Variety and fruit position	No. of fruits	Fruit shape *L/D* ratio	*L.S.D.* 5% level
Golden Delicious :			
Separate cluster—centre	350	0·942	0·017
Separate cluster—side	350	0·911	
Red Delicious :			
Separate cluster—centre	350	0·922	0·014
Separate cluster—side	350	0·897	
Same cluster*—centre	17	0·891	0·011
Same cluster —side	17	0·854	

* Each cluster contained one centre- and one side-blossom fruit.

Fruit shape of three genetic strains of Red Delicious was significantly different (Table 4). Common Red Delicious fruits were markedly flatter than Starkrimson or Starking fruits.

Table 4. FRUIT SHAPE AS RELATED TO THREE GENETIC STRAINS OF RED DELICIOUS APPLE GROWN ON NON-DWARFING ROOTSTOCKS*

Strain	No. of fruits	Fruit shape *L/D* ratio
Common Red Delicious	200	0·903
Starking Delicious	100	0·932
Starkrimson Delicious	200	0·980
L.S.D. 5% level		0·016

* Grown on seedling or *EM* XVI rootstocks.

The fleshy part of the apple, a pome fruit, is non-ovarian tissue, being considered either a proliferation of the stem[4], or a fusion of floral parts[5]. In any event, it is derived from vegetative tissue. The inflorescence is determinate with usually 5 or 6 flowers, one of which, the centre-bloom, terminates the axis. Thus the centre-bloom fruit has a position analogous to that of a primary shoot arising from a terminal bud ; a side-bloom fruit is analogous to a shoot arising from a lateral bud. The lengths of terminal and lateral shoots of Starking Delicious (not shown in tables) were found to be 65 cm and 46 cm, respectively. Visual inspection showed terminal shoots to be longer than lateral shoots in the other varieties also. The same length relationship holds for centre- and side-fruits (Tables 2 and 3). Non-cropping branches produce longer shoots than heavy-cropping ones irrespective of rootstock (Table 1). The trend also (Table 1) is for the fruits to become shorter as the shoots on fruiting branches become shorter.

The results presented show that differences in shoot length resulting from rootstocks, crop density, or shoot position vary in the same way as do comparable fruits from the same trees. This close parallel of fruit and shoot length suggests a close physiological relationship in the two organs which continues to persist even perhaps after an extensive evolutionary development of the fruit from vegetative tissue. Since cell elongation is mediated by auxin, we suggest that the factors which affect fruit shape do so by regulating the auxin available to them. The existence of local differences in auxin, which result in such phenomena as apical dominance, while generally accepted, are not well understood.

An apparent inconsistency exists with the mutant strain Starkrimson, which has longer fruits (Table 4) than the standard strains, but the shoots of which are noticeably shorter. This anomaly, however, appears to be a part of the built-in genetic difference between strains, because the relationship between fruit and shoot length (as related to rootstocks and position) held for Starkrimson as for other strains of Delicious. The supporting data for Starkrimson are not given, because they essentially duplicate the data of Tables 1 and 3.

It is evident that several factors besides climate can affect shape of the apple. These studies were done in a relatively cool temperate marine climate, and it is not known whether the same relationships would be found in a climate conducive to very flat-shaped fruit. The effect of climate on shape of fruit may be more important than the factors examined here, but our results suggest several ways one might alter shape of fruit within a given climate, if it were commercially advantageous to do so. In the past, buyers have favoured the long conical-shaped Red Delicious over the round or flat ones. Tests are being made to determine the effects of different climatic variables on shape of apple fruits.

M. N. WESTWOOD
L. T. BLANEY

Department of Horticulture,
Oregon State University, Corvallis.

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Starch-gel Electrophoresis of Proteins as an Aid in Identifying Fungi

MANY species of fungi are difficult to identify, particularly if they lack reproductive structures. Even if reproductive bodies are developed, the extent to which their morphological features, such as shape, size and colour, can be used as taxonomic criteria is often controversial. Species of *Pythium* are not easily characterized by conventional morphological criteria. This paper describes the application of starch gel electrophoresis of mycelial proteins to the identification of *Pythium* species.

Electrophoretic patterns of proteins in plasma and in eggs have been used as guides in the taxonomy of many animal species[1-3], and Chang, Srb and Steward[4] have recently shown marked differences between protein patterns obtained from different *Neurospora* species and smaller but significant differences between patterns obtained from strains of *N. crassa*.

In the work described here cultures of *Pythium* species were grown in liquid Czapek–Dox medium supplemented with 0·1 per cent yeast extract and incubated at 25° C on a rotary culture shaker. Mycelium was collected by filtration after 5, 8 and 10 days, washed with distilled water, frozen and broken in a Hughes press[5]. The suspensions of broken mycelium were mixed with one-tenth of their volume of buffer (0·76 M *tris*-citrate containing 5 mM ethylenediamine tetraacetic acid, *p*H 8·5) and centrifuged at 130,000*g* for 45 min. As little as 1 g of fresh mycelium could be used in 5 ml. centrifuge tubes (Spinco *SW* 39*L* rotor), but in this case paraffin oil was added to the centrifuge tubes to make up the required volume. The supernatant liquids and mycelia were stored at −15° C without deterioration. Samples (about 0·05 ml.) of supernatant were applied to pieces of Whatman 3*MM* paper (5 × 8 mm) and these were inserted in slots in 12 per cent starch gel in 0·076 M *tris*-citrate containing 0·5 mM EDTA, *p*H 8·5. Gels were 10 cm wide, 15 cm long and 0·5 cm thick and were used horizontally. Electrophoresis was carried out at about 5 V/cm and the gels were cooled by resting containers of crushed ice on them. Poulik's[6] discontinuous borate–citrate buffer system (0·3 M sodium borate, *p*H 8·5 in the electrode vessels) was used; resolution of components with this system is better than in continuous buffers. Protein components were detected by staining with amido-black 10*B* as described by Smithies[1].

Fig. 1 shows patterns obtained with six *Pythium* species. The patterns are easily distinguishable and have only one band in common. Samples of the same species isolated from different localities were either indistinguishable or showed only minor differences (Fig. 2). Samples of any one culture harvested at three different ages showed identical electrophoretic patterns of their proteins.

Similar experiments with *Pullaria pullulans*, *Fusarium oxysporum* var. *pisi* (races 1 and 2), bakers' yeast and other Ascomycetes showed that protein patterns of *Pythium* species differ as much from one another as they do from the protein patterns of these representatives of other fungal groups.

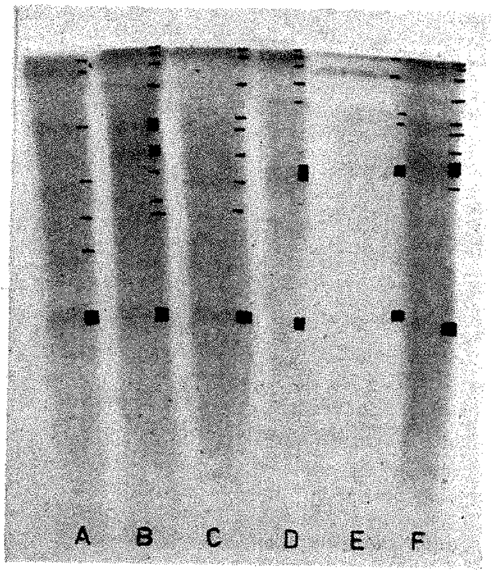

Fig. 1. Patterns obtained from mycelial extracts of six *Pythium* spp. (*A*) *P. echinulatum*; (*B*) *P. debaryanum*; (*C*) *P. irregulare*; (*D*) *P. ultimum*; (*E*) *P. dissimile* (Vaartaja sp. nov. ined.); (*F*) *P. violæ*

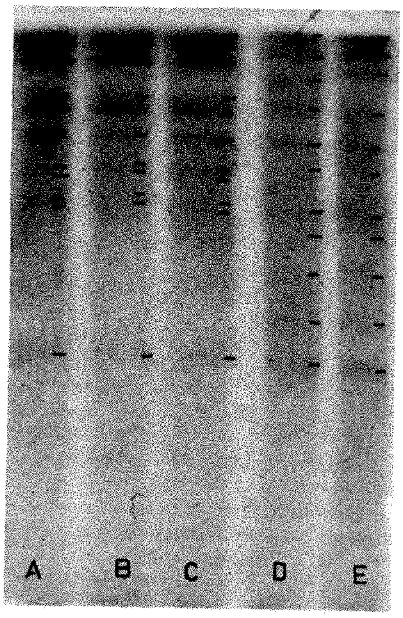

Fig. 2. Extracts from isolates of any one species collected at different localities gave similar patterns. Patterns (*A*)–(*C*) were obtained from different isolates of *Pythium intermedium* and (*D*)–(*E*) from different isolates of *P. myriotylum*

It seems likely that patterns of this sort will be useful aids in the delimitation of fungal species.

I thank Dr. M. R. Atkinson, of the Department of Agricultural Chemistry, for suggesting this approach to the problem and for advice on analytical methods, and Dr. L. O. Vaartaja for supplying cultures of *Pythium* species.

B. G. CLARE

Department of Plant Pathology,
Waite Agricultural Research Institute,
University of Adelaide.

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MICROBIOLOGY

Bacteriophages Specific for some *Mycobacteria*

MYCOBACTERIOPHAGES are generally known to lack specificity. However, due to chance isolation or to selection by enrichment with certain types of *Mycobacteria*, isolation of bacteriophages specific for *Mycobacterium phlei*, *M. pellegrini* and *M. tuberculosis* var. *hominis* and *bovis* was reported.

In the course of screening earth samples from Lausanne soil for the presence of mycobacteriophages (Hauduroy and Tanner, 1962), bacteriophages lysing mycobacteria were found in the majority of filtrates prepared according to the procedure described by Froman *et al.*[1]: In each case the earth samples were incubated at 37° C, enriched periodically by 13 rapid growing mycobacterial strains, centrifuged at 3,000 r.p.m. for 30 min and the supernatant filtered through a Chamberland *L*3 filter. The presence of bacteriophages lytic for mycobacteria was detected by the agar layer technique of Gratia as modified by Froman *et al.*[1]. 4 out of 16 bacteriophages thus obtained were of special interest:

(1) Filtrate No. 5 was found specific for *M. rubrum* (*F* 180, Froman, Los Angeles) from the beginning of its isolation. (2) Filtrate No. 7, initially active against *M. rubrum* (*F* 180), *M. smegmatis*, *A.T.C.C.* 607 (*F* 21, Froman, Los Angeles) and *M. fortuitum* (*F* 3816, Froman, Los Angeles), lost its capacity to lyse *M. smegmatis* (*F* 21) and *M. fortuitum* (*F* 3816) after 5 serial transfers on *M. rubrum* (*F* 180), and behaved afterwards as a specific bacteriophage for *M. rubrum* (*F* 180). (3) Filtrate No. 10 showed in the beginning a lytic activity for both the strains *M. smegmatis* (*F* 21) and *M. phlei* (*F* 89, Froman, Los Angeles). However, it lost its lytic activity for *M. smegmatis* (*F* 21) on 5 serial transfers on *M. phlei* (*F* 89) and *vice versa*: no lysis of *M. phlei* (*F* 89) was detectable after 5 serial passages on *M. smegmatis* (*F* 21).

Thus, in the case of filtrate No. 7, a specific phage against *M. rubrum* (*F* 180), and in the case of filtrate No. 10 two specific phages, one against *M. smegmatis*, the other against *M. phlei* (*F* 89), were obtained. It is suggested that in both cases this was due to 'adaptation' of a bacteriophage to specific host strains, although the probability of the coexistence of two distinct bacteriophages in filtrate No. 10 cannot as yet be excluded.

The 4 mycobacteriophages—differing with regard to the origin of their specificity—were tested against a variety of mycobacterial species. These included various strains of 'same' species, that is, strains received from different laboratories but having in common that they were designated as either *M. phlei* or *M. smegmatis*. As can be seen from Table 1, the bacteriophage active against *M. phlei* (*F* 89) lysed 5 out of 8 strains of *M. phlei*, that is, 4 strains in addition to the homologous host strain *F* 89. The

Table 1

Name of species	No. of strains	Lytic activity of Phage *phlei F* 89	Phage *smegmatis F* 21	Phage *rubrum F* 180 No. 5	No. 7
Mycobacterium phlei	8	5	0	0	0
M. rubrum	1	0	0	1	1
M. smegmatis A.T.C.C.607	3	0	1 (*F* 21)	0	0
M. smegmatis	12	0	0	0	0
Various rapid-growing mycobacteria	14	0	0	0	0

bacteriophage active against *M. smegmatis*, on the other hand, did not lyse but the homologous host strain F 21 did.

The confusion existing at present in the classification of mycobacteria : the arbitrary grouping of several strains, the frequent changes of species designation, the circulation of Collection strains with obscure past histories, makes it difficult to decide if the lysis of several mycobacterial strains by the same bacteriophage can be considered as a sign of their close relationship (sub-groups of same species), or only shows the common derivation (sub-cultures) of the strains tested, while those strains non-lysable by the specific bacteriophage bear the name of the species without any justification.

Nevertheless, this investigation shows the theoretical possibility of obtaining specific lytic activity against distinct groups of mycobacteria by serial passages from initially polyvalent filtrates.

I thank Prof. Paul Hauduroy for the initiation of this research project, for advice and for the facilities provided while I was guest in his Institute.

S. E. JUHASZ*

Institut de Bacteriologie et d'Hygiene,
Faculté de Médecine,
Université de Lausanne,
Lausanne, Switzerland.

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* Present address: Department of Bacteriology and Immunology, University of British Columbia, Vancouver 8, B.C., Canada.

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Water-soluble Products of Methyl Cholanthrene and Their Effect on Bacterial Growth

THE production of tumour cells in certain mammalian systems by carcinogenic agents stimulated considerable interest in testing the effects of these compounds on other kinds of cells. Micro-organisms, because of their apparently simpler structure and their adaptability to growth under controlled cultural conditions, have been repeatedly used in such assays. For different bacteria, yeasts, or moulds carcinogens have been reported : (*a*) to produce large and sometimes abnormal cells[1] ; (*b*) to inhibit early phases in the growth curve[2] ; (*c*) to increase the total number of cells in cultures[3] ; (*d*) to be involved in photodynamic lethal effects[4] ; (*e*) to induce mutations[5].

Results described in these assay tests are not always reproducible, and even now little can be said, other than to quote the literature concerning definite effects of carcinogens on bacterial and fungus cells. The method of administering the carcinogen (usually methyl cholanthrene, benzpyrene, or 1,2,5,6-dibenzanthracene) may be one main cause of the variability or indefinite results obtained. These compounds are not soluble in water and the usual method of their application has been either : (*a*) to grind the crystals in water, making a finely divided suspension which is incorporated into a culture medium (agar or broth) ; (*b*) to filter similar suspensions after considerable periods of time, resulting in so-called 'saturated solutions' which are then used as the basis for an agar or a broth ; (*c*) to add the carcinogen to the medium in solution in some organic solvent (as acetone or alcohol), later evaporating the solvent by heating or during the sterilization process. This communication describes a method to circumvent this difficult problem by selecting an assay organism (*Bacterium aliphaticum*) which can utilize hydrocarbons such as pentane, heptane, or other paraffins as the sole carbon source[6]. Since the carcinogens are themselves hydrocarbons exposure can be achieved with the carcinogen in solution in the bacterium's normal food. There is greater assurance in this case that the carcinogen will come in contact with the micro-organism; further, metabolic and biologic observations are quite easily made.

Cultures were prepared in test-tube-like cuvettes preselected for uniformity for use in fluorimetric analysis. After filtration through No. 1 Whatman filter paper 10 ml. Tausz's solution (MgNH$_4$PO$_4$, 1·0 g ; KH$_2$PO$_4$, 0·8 g ; CaSO$_4$, 0·1 g ; FeCl$_3$, trace ; KI, trace ; distilled water, 1,000 ml.) was placed in each tube and sterilized for 20 min at 15 lb. pressure. Inoculations (2 drops per tube) were made with sterile pipettes from cultures previously grown one week in Tausz's solution with paraffin oil as the sole carbon source. Sterile paraffin oil (2 ml.) was then added, forming an oil layer over the basal medium ; this layer contained the desired amounts of methyl cholanthrene in carcinogen-treated cases. Cultures were maintained at 28° ± 2° C in incubators or in a diffusely lighted room.

Dilution plate analyses using nutrient agar were made to assay the number of organisms present at various periods of time (up to 10 years) in cultures of the kind described. Methyl cholanthrene, in all concentrations, placed in the oil layer (62·5–500 μg/ml.) adversely affected both the population-size and long-term viability of the cultures. After 2 years the population-sizes of the methyl cholanthrene-treated cultures were usually less than half those of the controls. With only an occasional exception, all carcinogen-treated cultures examined between 2 and 10 years of age showed smaller populations than controls.

The smaller population size indicated toxicity leading to death of cells in tubes containing carcinogen, a suggestion further supported by the data which show expiration of the cultures from time to time (Table 1). The data are based on 300 methyl cholanthrene-treated cultures (50 replicates each of six concentrations of methyl cholanthrene 62·5–500 μg/ml. added in the paraffin oil) and 300 cultures without carcinogen as controls. After 4 years, cultures containing carcinogen were 47 per cent expired; there were no losses among controls. After 7 and 10 years respectively, cultures containing carcinogen were 70 and 100 per cent expired compared with 7 and 15 per cent among controls. Further, cultures containing the heaviest concentrations of carcinogen (250–500 μg/ml.) began to die out first. With the exception of complete kill obtained at a concentration of 250 μg/ml. after 7 years, the per cent of kill at any given age was roughly proportional to the concentration of added methyl cholanthrene.

Table 1. EFFECT OF METHYL CHOLANTHRENE ON VIABILITY OF *Bacterium aliphaticum*

Supplement to medium	No. of cultures	No. of cultures surviving after : 2 yr.	4 yr.	7 yr.	10 yr.
Methyl cholanthrene	300	290	140	90	0
None (controls)	300	300	300	280	255

Under certain conditions fluorescence measurements can be used to follow changes in the concentration of methyl cholanthrene and structurally related compounds. The water layer of the cultures established was not fluorescent at zero time. Surprisingly, fluorescence developed in this layer, increasing rapidly and linearly for the first ten weeks, and also increasing thereafter, but at a lesser rate. The fluorescence was similar to that of methyl cholanthrene and absent in controls without carcinogen. This suggested that water-soluble products of methyl cholanthrene were formed within a relatively short period of time. An appreciable concentration of these products develops spontaneously in diffuse light, and more rapidly under ultra-violet than with visible light. However, cultures containing *B. aliphaticum* develop much greater fluorescence at any age than appropriate spontaneous controls (no organism). Fluorescence measurements on the water layer before and after the cells were removed by centrifugation indicated that most of the fluorescence originated in the culture fluid; the contribution of the cells to the total was relatively small.

The fluorescent material in the water layer was chromatographed on paper with chloroform–water mixtures, yielding a definite spot moving slightly behind the front. The substance is not methyl cholanthrene, as this compound remains in the original spot and does not advance in the solvents used. The material is noticeably fluorescent on the paper. Its chemical nature has not been further established. However, both the water layer itself and the chromatographically isolated fluorescent spots proved weakly toxic to *Bacillus subtilis* and *Staphylococcus aureus*. Small inhibition zones (disappearing after 48 h) developed when 1 ml. of the water layer from methyl cholanthrene-treated cultures was used in the cup in place of penicillin following standard penicillin cup assay procedures[7]. Similar weak inhibition zones were noted when fluorescent filter paper disks from the chromatograms were placed on seeded nutrient agar plates.

It seems logical to associate this weak toxicity with the small populations and long-term loss of viability of the cultures containing carcinogen. Further, these results suggest that water-soluble oxidation products may be directly involved in this toxic effect. The existence of these substances also raises the question as to what extent such effects as death, cell enlargement, mutagenicity, etc., reported by other workers[1-5] and usually attributed to methyl cholanthrene (or other carcinogens) in the existing literature, might be due to reaction products of the carcinogen rather than to the compound itself.

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G. T. JOHNSON

Department of Botany and Bacteriology,
University of Arkansas,
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Evaluation of Colorimetric Methods in the Estimation of Sialic Acid in Bacteria

NUMEROUS reports have appeared claiming the presence of the sialic acids in various Gram-negative bacteria[1-9]. In several communications[7-9] the detection and estimation of these acids were made solely by the colorimetric procedure of Warren[10]. In others, a modified direct Ehrlich procedure was used[1-5].

The estimation of sialic acid by Warren's procedure involves several steps. These include hydrolysis to release the sialic acid, periodate oxidation of the acid to β-formyl pyruvate, reduction of excess periodate with sodium arsenite, addition of thiobarbituric acid followed by extraction of the chromogen with an absorption maximum of 549 mμ into *cyclo*hexanone. In addition to the sialic acids, 2-keto-3-deoxyoctonate is present in certain bacteria[11] and yields β-formyl pyruvate on periodate oxidation. Thus, as no method is available to determine the origin of β-formyl pyruvate, a positive result by the Warren assay may not necessarily indicate that sialic acid was present. The Warren method is further complicated by the existence of 2-deoxyribose and the 3,6-dideoxyaldohexoses in bacteria. On periodate oxidation these substances yield malonaldehyde which forms a chromogen with thiobarbituric acid with an absorption maximum at 532 mμ and considerable absorption at 549 mμ. This chromogen may be removed by extraction with isoamyl alcohol[10] prior to the *cyclo*hexanone extraction. An alternate procedure has been devised[10] to correct for absorbance due to the malonaldehyde-thiobarbituric acid chromogen in mixtures of the two chromogens.

In contrast, the estimation of sialic acid by the modified direct Ehrlich method is simple. It involves heating the cells in a solution containing mineral acid, aluminium sulphate and dimethylaminobenzaldehyde. The pyrrole derivative formed from degradation of sialic acid is immediately trapped by the dimethylaminobenzaldehyde and gives a chromogen with an absorption maximum at 530 mμ. In view of the popular usage of the thiobarbituric acid procedure for detection and estimation of the sialic acids, it was of interest to compare the results obtained by using the direct Ehrlich and Warren procedures on Gram-negative bacteria grown under similar conditions.

All enteric bacteria were grown in 1 l. of sterile nutrient broth containing 30 ml. of 50 per cent dextrose and 7 ml. of the filtered, sterile and concentrated dialysate obtained from 5 g of Difco yeast extract. Each culture, seeded with 0·2 ml. of a 3 ml. slope wash of an overnight culture, was grown for 6 h at 37° C with æration. *Rhodopseudomonas palustris* was grown at 25° C for 48 h without æration and under illumination by a 200-W tungsten lamp placed 6 in. from the flask. The bacteria were killed with chloroform and collected by centrifugation. The cells, after washing and recentrifugation, were suspended in water and lyophilized. A recovery of 70–400 mg of material was obtained. The *Veillonella* cells were kindly given by Dr. S. Mergenhagen of the National Institutes of Health, Bethesda, Maryland.

Analyses for sialic acid were performed on 30–100 mg of dried cells by a modified direct Ehrlich procedure[5] and on the clarified supernatant of cell hydrolysates by Warren's method[10]. An examination was first undertaken to determine the conditions of hydrolysis for maximum release of sialic acid. The results, obtained by using *Salmonella ngozi*, are given in Fig. 1. It can be seen that maximum amounts of sialic acid are released by heating in 0·1 or 0·5 N sulphuric acid at 100° C for 60 min and in 1·0 N acid for 20 min. Hydrolysates were, therefore, routinely prepared by heating 10 mg of dried cells for 20 min at

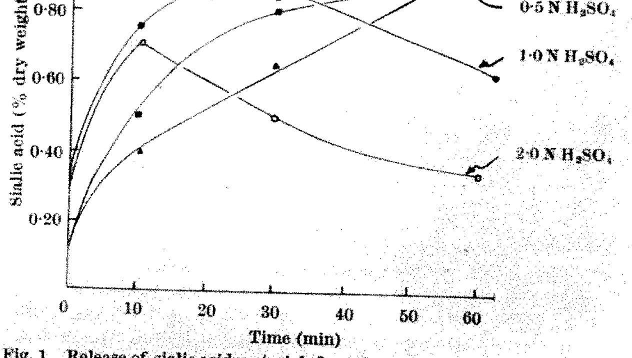

Fig. 1. Release of sialic acid materials from *Salmonella ngozi* in sulphuric acid at 100° C

100° C in 1 ml. of 1·0 N acid followed by centrifugation. The percentage of sialic acid was calculated using reference curves in which ovine sialic acid (*N*-acetylneuraminic acid) served as standard.

The per cent sialic acid content of the bacteria analysed is recorded in Table 1. The determinations made by the thiobarbituric acid method are recorded as uncorrected and corrected values obtained after extraction of the malonaldehyde-thiobarbituric acid chromogen with isoamyl alcohol. It is to be noted that for all the bacteria which give a positive Ehrlich test, the assay for sialic acid determined by this procedure is greater than that obtained by the thiobarbituric acid assay. As the latter method measures free and terminally bound sialic acid, it would appear that some of the acid is either destroyed during hydrolysis or remains attached to the cell debris. Although temperature, time or normality of the acid were varied in the preparation of the hydrolysates, the values for sialic acid obtained by the thiobarbituric acid procedure when using isoamyl alcohol extraction never equalled the higher values found by the direct Ehrlich method. A similar result was obtained when absorption measurements were made at various wave-lengths on the mixture of chromogens and calculations made for sialic acid by the alternate procedure of Warren[10].

Table 1. SIALIC ACID CONTENT OF VARIOUS BACTERIA

Organism	Serotype	Sialic acid per cent dry weight		
		Direct Ehrlich	Warren	
			Uncor.	Cor.†
S. kisingen	48 : a–1, 5, 7	0·97	0·81	0·49
S. dahlem	48 : k–e, n, z_{15}	0·60	0·65	0·34
S. djakarta	48 : z_4, z_{24}	0·39	0·45	0·29
S. sakaraha	48 : k–z_{39}	0·93	0·82	0·34
S. ngozi	48 : z_{10}–1, 5	1·11	0·85	0·53
C. freundii	5 : 30	1·02	0·65	0·35
Arizona	5 : 1, 2, 5, 6	0·63	0·64	0·35
Arizona	5 : 1, 7, 8	0·64	0·69	0·34
Arizona	5 : 13, 14	0·56	0·63	0·51
Arizona	5 : 26–31	0·64	0·96	0·40
Arizona	5 : 24–28	0·69	0·81	0·49
Arizona	5 : 17, 20	1·04	0·94	0·53
Arizona	29 : 23–31	1·25	0·64	0·34
Arizona	29 : 32–31	1·25	0·64	0·32
Arizona	29 : 33–31	0·60	0·41	0·19
Arizona	29(5) : 33–21	0·66	0·46	0·29
S. marcescens	N.D.*	0·00	0·30	0·23
P. vulgaris	N.D.	0·00	0·18	0·14
R. palustris	N.D.	0·00	0·43	0·17
A. aerogenes	N.D.	0·00	0·34	0·23
Veillonella sp.	N.D.	0·00	0·37	0·22
E. coli	0111 : $K58(B4)$: HNM	0·00	0·44	0·12
E. coli	06 : $K53(L)$: HNM	0·00	0·20	0·11
E. coli	N.D.	0·00	0·22	0·16

* N.D. Not determined.
† After extraction with isoamyl alcohol.

Several bacteria listed do not appear to contain sialic acid as determined by the Ehrlich assay, but have an 'apparent' content of sialic acid[8,9] when analysed by Warren's method. Paper partition chromatographic analyses of hydrolysates of these bacteria failed to show sialic acid, whereas in hydrolysates of strains which give positive Ehrlich and Warren assays, such as *Arizona*, *Salmonella* and *Citrobacter freundii*, the acid is readily detectable. Moreover, the presence of ovine sialic acid was definitely established in the *Citrobacter freundii* strain by isolation[12,13]. Thus, it is strongly indicated that sialic acid is present in bacteria which give a positive Ehrlich test but not in those which only give a positive Warren test. The chemical nature of the substances in bacteria which give a positive Warren and negative Ehrlich assay is unknown. However, in the case of *E. coli* 0111 : $K58(B4)$: HNM, which contains colitose (3,6-dideoxy-L-galactose) and 2-keto-3-deoxyoctonate, the positive result obtained in the thiobarbituric acid assay may be due to the latter substance.

It is concluded that Warren's method for estimation of the sialic acids in micro-organisms gives lower values than are obtained by the direct Ehrlich procedure. Although the thiobarbituric acid assay is some 80-fold more sensitive than the modified direct Ehrlich, it is also less specific. The results clearly reveal that sialic acid is present in *Arizona* 029, *Arizona* 05 and *Salmonella* 048 serotypes. Moreover, it is of interest to mention that *Salmonella* 048, *Arizona* 029 and *Citrobacter freundii* 05 are serologically related to *Arizona* 05 (refs. 5 and 14). As sialic acid is absent in other *Salmonella* O serotypes and has not been detected in other *Arizona* O serotypes, it is suggested that the chemical basis for the serological relationships among these strains involves sialic acid.

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GUY T. BARRY
JAMES D. HAMM
MEL G. GRAHAM

University of Tennessee Memorial Research Center,
Knoxville, Tennessee.

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CYTOLOGY

In vivo Incorporation of Tritiated Thymidine in Acute Leukæmia Chromosomes

THE DNA metabolism of normal human blood cells has recently been investigated at chromosome-level by means of an autoradiographic technique and 2–4 day *in vitro* culture of peripheral leucocytes[1–3]. The application of this type of investigation to the study of human leukæmia cells was prompted by recent reports of abnormal chromosome number and/or shape in these cells[4–6].

Three points must be borne in mind in undertaking such an investigation. First, account must be taken not only of circulating blood cells but also of bone marrow elements. Secondly, long-term cultures should be avoided in view of the discrepancies in the chromosome complement recently observed between *in vitro* data and direct *in vivo* observation (false-negative results)[7]. Thirdly, additional information could be expected by improving the resolution and efficiency of the method.

With this in mind, the *in vivo* incorporation of tritiated thymidine into the chromosomes of a patient with acute leukæmia was investigated. The patient was a male with untreated acute myeloblastic leukæmia : 76 per cent of the bone marrow cells and 91 per cent of the circulating leucocytes were myeloblasts and paramyeloblasts ; the peripheral white cell count was 72,000/mm³. With regard to the chromosome complement, 60 per cent of the cells had 46 chromosomes (modal number), the remainder 45, 47, 48 and 49 chromosomes. No chromosome marker was found ; the extra chromosomes belonged, in most cases, to groups 13–15 and 16–18 ; a few could be included in group 19–20. Ten mc. of tritiated thymidine (s.a. 1·4 c./m.mole) was injected intravenously. It is known that a single administration of thymidine *in vivo* serves the purpose of pulse-labelling[8]. The autoradiographic and chromosome technique used in the investigation recorded here has been reported in detail elsewhere[9]. The cells to be examined were obtained by sternal puncture performed 105 min after the administration of thymidine and 60 min after *in vivo* colchicine blocking[10,11].

50·6 per cent of the mitotic figures (out of a total count of 420 mitoses) showed evidence of labelling: owing to the experimental conditions, tagged mitotic figures could only be found in the cells which had incorporated the precursor in the final stages of the synthetic period, whereas the unlabelled figures correspond to cells which were past the *S* stage at the time of labelling.

For this investigation we selected a total of 30 labelled mitotic figures with a clearly outlined morphological pattern and with a total grain count ranging from 80 to 350 (Fig. 1). The chromosome number was 46 in 18 mitoses and more than 46 in 12. Karyotypes were arranged according to the Denver classification: when the intensity of labelling precluded identification of the chromosomes, the nuclear emulsion on the slides was removed after photographing the mitotic figures and localizing and counting the grains[9].

The rate of precursor incorporation was established by counting the grains on the chromosomes and on their arbitrarily chosen segments: a 'specific incorporation rate' was calculated by relating the number of grains to the relative length of the chromosomes or chromosome segments, as recently proposed[3]. Owing to the small number of mitotic figures featured in this preliminary experiment, the incorporation rate was calculated for chromosome groups rather than pairs where the chromosomes of neighbouring pairs were interchangeable. The 'specific incorporation rate' was consequently calculated for the following pairs or groups of chromosomes: 1,2,3, 4,5, *X*–6–12, 13–15, 16,17,18, 19–20, 21–22, *Y*. When the individual extra-chromosomes could not be identified with sufficient accuracy, they were included in the pairs or groups to which they were morphologically related. Finally, the 'specific incorporation rate' was calculated for each of the segments of the various chromosome pairs or groups.

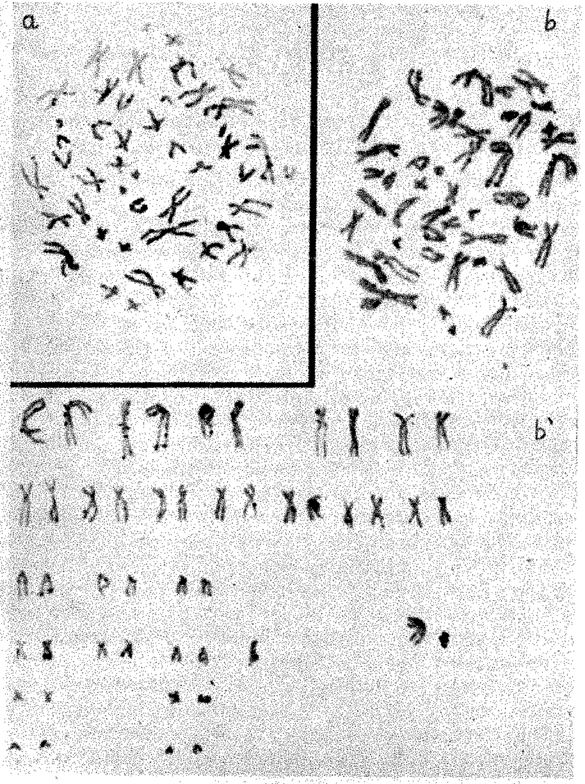

Fig. 1. *a*, labelled mitotic figure of bone-marrow cell with 46 chromosomes; *b*, labelled mitotic figure of bone-marrow cell with 47 chromosomes; *b*¹, karyotype of *b*

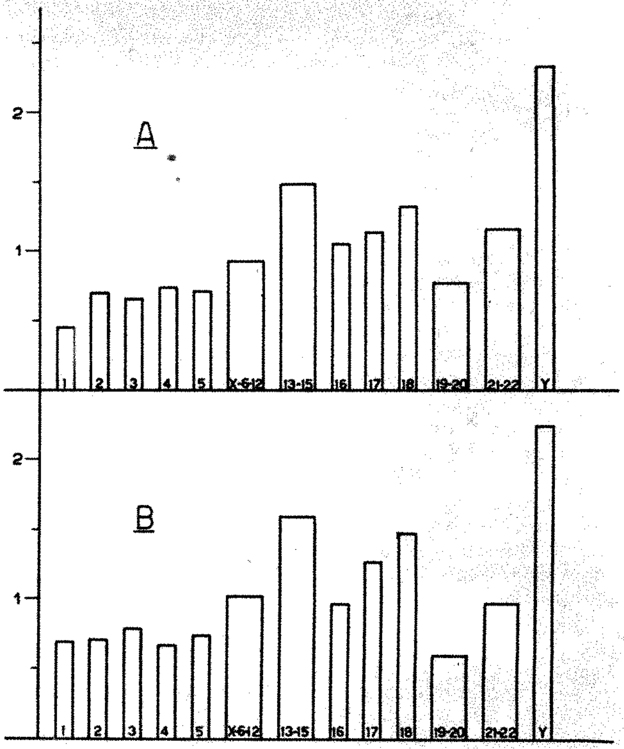

Fig. 2. 'Specific incorporation' (ordinate) of tritiated thymidine (pairs and groups of chromosomes)
A, euploid mitoses; *B*, aneuploid mitoses

Fig. 2 shows the mean values for each pair or group; the values of euploid and aneuploid mitoses are reported separately. The two types of mitoses (euploid and aneuploid) displayed similar behaviour, and significant differences could not be demonstrated between the corresponding pairs or groups of chromosomes. On the contrary, both in euploid and aneuploid mitoses a significant degree of variation was observed among the different pairs or groups of the same mitotic figures and considerable differences could also be demonstrated between the segments making up the various pairs or groups of chromosomes. All these differences in the 'specific incorporation rate' could be confirmed statistically by means of variance analysis. The highest incorporation rates were found in the chromosomes of group 13–15 and pairs 17 and 18, the lowest in the first pairs and in group 19–20. In the segments no constant pattern could be demonstrated: there was a higher incorporation rate in the segments close to the centromere in pairs 1 and 3, in the distal segments of the long arms of pairs 16 and 17, in the short arms of pair 2 and in the distal segments of the chromosomes of group 19–20. Both in euploid and aneuploid mitoses the incorporation rate in the chromosome *Y* was higher than in any of the autosomes.

A comparison was also made between the incorporation rate of the extrachromosomes and the corresponding autosomes, and between the incorporation rate in the pairs or groups in which extra chromosomes had been included and the corresponding pairs or groups with no extra-chromosomes. A higher incorporation rate was noted in the extra-chromosomes; however, this finding requires confirmation, and further investigations are under way on this point.

From the foregoing preliminary findings certain conclusions can be drawn. (1) Duplication of DNA follows not a random course but a particular time sequence not only in normal blood cells[3] but also in acute leukæmia cells. (2) This time sequence presents the same features in euploid cells as in aneuploid cells. (3) Incorporation of the DNA precursor is also achieved by the extra-chromosomes. (4) The synthetic phase of the *Y* chromosome in the present case of acute leukæmia differed from that of the small acrocentric chromosomes. (5) The ratio of labelled to non-labelled mitotic figures in the present experiment suggests the possibility that the post-synthetic period is shorter *in vivo* than *in vitro*. (6) The present investigation testifies that a more direct approach to the study of nucleic acid metabolism in leukæmic chromosomes is allowed by the *in vivo* injection of the labelled precursors.

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F. Gavosto
A. Pileri
L. Pegoraro
A. Momigliano

Medical Clinic,
Medical School,
Turin, Italy.

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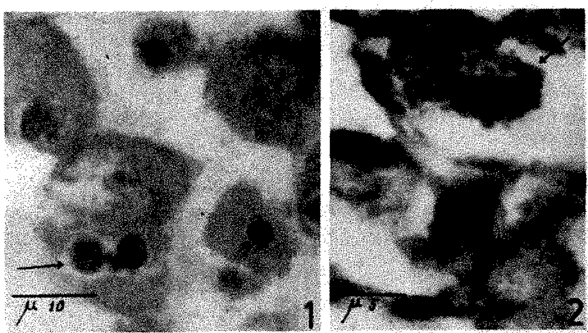
Aggregation of Protein Particles in Tumour Cells

In Walker tumour cells, during experiments carried out with deoxyribonuclease, the formation of particular globules, namely those rising from the aggregation of protein particles (ribosomes), were noted[1]. A similar aggregation occurs[2,3], normally in the oocytes of some animal species (*Patella coerulea, Aplysia depilans, Haliotis lamellosa, Paracentrotus lividus, Sphaerechinus granularis, Arbacia lixula*), which form yolk globules. Observations on the effect of deoxyribonuclease on supravital material confirmed the concept that in the tumour cell there are a large quantity of unorganized ribosomes ('scattered ergastoplasm'), which are not oriented to form identifiable products of synthesis.

Further investigations were undertaken not only on Walker tumour, but also on mammary carcinoma and uterine carcinoma of woman. As formerly pointed out[1], fragments of tumour were placed at room temperature (about 22° C) in an aqueous solution of deoxyribonuclease ('Crystalline' of Light and Co., 10 mg/ml.). Controls were carried out on fragments not submitted to any action, and on fragments kept in distilled water for the same time (minimum, 1 h; maximum, 5 h) at room temperature. The usual fixatives were then used, in particular Bouin's mixture. The results can be summarized as follows:

(1) In Walker tumour cells, rather large globules reduced in number (Fig. 1), and small and very numerous globules appeared in the cytoplasm (Fig. 2); also, in the nuclear area, artificial globules can be seen (Fig. 1).

(2) Artificial globules are also found in the mammary carcinoma (Fig. 4) and in the uterine carcinoma cells (Fig. 3), though in some cases they were rather small.



Figs. 1 and 2. Walker tumour treated with deoxyribonuclease; note in Fig. 1 large globules also in the nuclear area (arrow), and in Fig. 2 the very numerous small globules in the two cells (arrows)

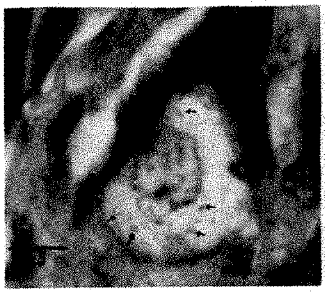


Fig. 3. Uterine carcinoma of a woman treated as in Fig. 1; note small globules (arrows) in the cytoplasm of the cell

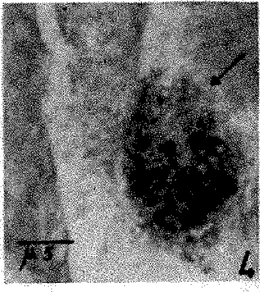


Fig. 4. Mammary carcinoma of a woman treated as in Fig. 1; note the high number of small globules (arrow) in the cell

(3) In the fragments of the three tumours it was observed that the formation of the globules occurs only in the cells of the outer layers of the sections of the preparations treated with enzyme. Very seldom, and especially in some Walker tumour cells of the control preparations kept in distilled water, the presence of globules was noted; this has never been found previously in tumours not submitted to enzyme action before fixation.

(4) In the three tumour cells the globules stain red with hæmatoxylin and eosin and with Mallory's method, yellow with Galgano's (first)[4] method, and blue with Dominici's method. In the same globules, a positive reaction for RNA and for polysaccharides (McManus) has been demonstrated. RNA has been demonstrated with the usual Unna–Pappenheim's method and control with ribonuclease according to Brachet; also with the fluorescence microscope. The observations made with this microscope on sections treated with acridine orange revealed an intense red coloration of the globules; but this colour disappears after the action of ribonuclease. The presence of RNA was established, too, by affinity of the globules for toluidine blue according to Dominici's method; this affinity was suppressed with ribonuclease.

The foregoing observations have confirmed the formation, by artificial means, of protein products, in the form of globules, in the cells of malignant tumours. Subsequent work on several forms of malignant tumours, and also of benign tumours, may render it possible to generalize the process, which could be considered as an artificially induced protein synthesis. In this connexion, it must be ascertained whether the process is to be regarded as a consequence of a change of state of the medium, or rather as a consequence of a modification (also of a chemical nature) of the ribosomes. Experiments must then be carried out in many directions in order to ascertain whether other substances, different from deoxyribonuclease, are capable of provoking an ordered aggregation of such particles.

The question involves, as a side issue, the nature of real alteration of tumour cells, and also the protein synthesis, as apparent from the action of ribonucleoproteins. At present we have no concrete results of the action and the destiny of ribosomes when the incorporation of amino-acids in them is activated by adenosine triphosphate[5,6]. If it were possible to demonstrate that in any event the formation of determined protein substances is realized by aggregation of more or less transformed ribosomes, a new direction would be offered for work on the synthesis of proteins.

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A. BOLOGNARI
M. P. ALBANESE
A. DONATO

Istituto di Zoologia e di Anatomia comparata,
Università di Messina.

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GENETICS

Pre-albumin Variations in Primates

THE pre-albumin of the monkey, *Macaca rhesus* (*Macaca mulatta*), has been examined electrophoretically by Blumberg and a possible polymorphism is described. Three types of pre-albumin were evident. In each instance the pre-albumin was presented as a single band.

In an investigation of various primate serum albumins, it was observed on starch-gel electrophoresis, using the lithium–borate discontinuous buffer system of Ashton, that the pre-albumins of higher primates could be distinguished from those of lower primates as shown in Fig. 1. The pre-albumins of man, gorilla, and chimpanzee were each composed of two bands. These three species could not be distinguished by their pre-albumin, as the two bands had a common electrophoretic mobility respectively among the three species. However, in three species of macaques, *irus*, *rhesus*, and *radiata*, pre-albumin variations were evident. *M. irus* and *M. radiata* each presented with a single electrophoretic band which is similar in mobility to the first pre-albumin of the higher primates. *M. rhesus* revealed two pre-albumin bands, neither of which corresponded to either of the two bands of the higher primates. The second band of this monkey may correspond to the single band of monkey type 3 of Blumberg.

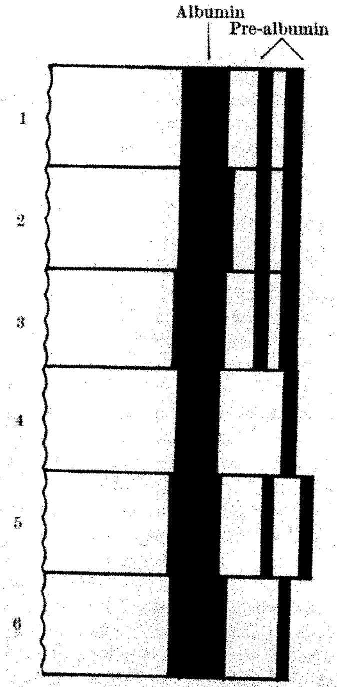

Fig. 1. Diagrammatic representation of the pre-albumins of the following primate serum: (1) human, (2) gorilla, (3) chimpanzee, (4) *Macaca irus*, (5) *Macaca rhesus*, (6) *Macaca radiata*

Thus in the pre-albumin region there appears to be a phylogenetic distinction between higher and lower primates as has been observed in the transferrins. The higher primates, though separated by greater species barriers, appear to have a common pre-albumin pattern, whereas the lower species, all of the same genus, have a polymorphic pre-albumin pattern.

All sera used in this work were supplied by Lars Beckman. I was a trainee of the U.S. National Institutes of Health, at the Institute for Medical Genetics, Uppsala, during the period of this investigation.

J. BRIAN MILLER

Institute for Medical Genetics,
Uppsala.

Frequencies of Teratologies among Homozygous Normal Mice compared with those Heterozygous for Anophthalmia

TRYPAN blue has long been known to possess teratogenic properties[1]. Several years ago Barber[2] reported being able to distinguish mouse fœtuses heterozygous for anophthalmia from those which were homozygous normals by the higher incidence among the former of eye defects in response to trypan blue administered to their dams as a teratogenic agent. All cases of defective eye development were unilateral anophthalmia; the expectation, in the absence of the teratogen, was complete bilateral normality. The anophthalmic mutant has been described in detail elsewhere[3,4].

The significance of Barber's findings prompted me to repeat and elaborate on her work. This communication confirms the observation that under appropriate conditions the anophthalmic mutant (*ey*) in a single dose appears to be able to act to bring an animal closer to a threshold for abnormal development than is the case in a homozygous normal individual. That the entire genotype may be involved in the demonstration has been suggested elsewhere[5], and is still being investigated[6]. Another phenomenon described in this communication is the ability of an animal to respond to the treatment with varying degrees of eye reduction on one or both sides; the extreme is complete bilateral anophthalmia. Both eyes are affected, and marked asymmetries in the same individual are not uncommon.

The animals used in this investigation were *ZRDCT-N* and *ZRDCT-An*; the former is normal-eyed, the latter an anophthalmic line. Both were derived by selection from a common stock, differ principally at the *ey* locus, and have been described in detail elsewhere[3]. The *ZRDCT-N* females were mated to their brothers or to *ZRDCT-An* males. Inseminations were detected by the copulation plug method[7]. Counting the day of insemination as day zero, females were injected intraperitoneally with 0·25 c.c. of a 0·3 per cent solution of trypan blue in 0·9 per cent sodium chloride during the seventh, eighth and ninth days *post coitus* (DPC). This was the régime found by Barber[2] to yield the highest frequency of eye defects, a low frequency of skeletal defects and low mortality. Mortality in the present material was very high; skeletal defects did not occur in the series reported here. There is some evidence (unpublished) that the

Table 1. ABNORMALITIES AND MORTALITY AT 18 DAYS *Post-coitus* AMONG TREATED AND UNTREATED LITTERS

Cross ♀ ♂	No. of litters	No. of implantations Living Normal	Abnormal	Dead	Description of abnormalities	Frequency (% ± *S.E.*) of abnormality	mortality
ZRDCT-N × *ZRDCT-An* Treated	15	17	14	81	*m*/+, +/*s*, *s*/+, 2 +/*an*, 2 *m*/*m* *m*/*s*, 2 *s*/*an*, 4 *an*/*an*	45·2 ± 8·9	72·3 ± 3·2
ZRDCT-N × *ZRDCT-N* Treated	16	19	1	127	*an*/*an*	5·0 ± 4·9	86·4 ± 2·8
ZRDCT-N × *ZRDCT-An* Untreated	6	46	0	14	—	0	23·5 ± 5·4
ZRDCT-N × *ZRDCT-N* Untreated	6	51	0	10	—	0	16·4 ± 4·7

In the "Description of abnormalities" column the character to the left of the stroke describes the left eye and the character to the right of the stroke describes the right eye. For an explanation of the symbols see text.

suspension medium of the dye may affect its teratogenic and toxic properties.

Experimentally treated females and untreated controls were killed at 18 DPC. The number of dead and resorbed fœtuses were noted and living fœtuses were examined for eye defects and other gross morphological abnormalities. The results (Table 1) were analysed with respect to the frequency and variety of defects and frequency of fœtal mortality. Although the degree of eye reduction may have been distributed continuously, distinction was made only between unaffected (+ = normal), slight reduction in size (*m* = medium eye), extreme reduction with maintenance of globular shape (*s* = small eye), and complete absence of an eyeball (*an* = anophthalmic). The tests of significance applied to the frequency data were the chi-square test of homogeneity and the Student–Fisher grouped-method *t*-test for the comparison of small samples[8].

Even in this small sample the higher frequency of eye defects occasioned by the teratogen among the group of fœtuses heterozygous for *ey* is highly significant ($t = 3\cdot3$; $df = 49$; $P < 0\cdot001$). This in spite of the slight heterotic vigour suggested by their decreased tendency towards fœtal death ($t = 2\cdot8$, $df = 257$, $P < 0\cdot005$). In most of the *ZRDCT-N* × *ZRDCT-An* matings earlier untreated litters were permitted to go to term as a test of fertility. All of the F_1 generation thus produced were completely normal-eyed. The single anophthalmic fœtus produced in the treated *ZRDCT-N* × *ZRDCT-N* matings was in fact occasioned by the teratogen, since several hundred *ZRDCT-N* progeny produced by routine brother × sister matings were all normal-eyed. Furthermore, in other series of treated *ZRDCT-N* × *ZRDCT-N* matings, additional abnormal young have been obtained both at birth and at 18 DPC.

Whereas the present material appears to allow the detection of *ey* in single dose, this is not the case in all crosses. I have reported[5] differences in response, in comparable crosses involving two sub-lines of the *C57BL* strain of mice. Sub-line 6 responds in much the same way as *ZRDCT-N*, but sub-line 10 does not. In crosses with *C57BL*/10 the effect of *ey* in single dose is masked or overridden by heterotic interaction. It would thus appear that if there were a marked difference in genetic modifiers between the eyeless and normal-eyed strains used in the cross one might not be able to detect the presence of *ey*. That there is a fair degree of variability in the system is also shown by the differences between my own findings and those of Barber. In the present material all degrees of eye reduction were noted. The phenomenon in Barber's material was all or none; either the eye was normal or it was absent.

Although the observations reported here and those of Barber do not constitute rigorous proof, both suggest that *ey* is in fact doing something in single dose, and that under appropriate environmental conditions it is possible to distinguish populations of animals which are heterozygous normal from those which are homozygous normal.

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SIDNEY L. BECK

Mammalian Genetics Centre,
Department of Zoology,
University of Michigan,
Ann Arbor.

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AGRICULTURE

Oversowing Pasture Legumes into the *Hyparrhenia* Grassland of Northern Rhodesia

BECAUSE nitrogen is the key nutrient in plant and animal nutrition in the tropics[1-3], the introduction of effective pasture legumes into the natural grassland is a simple method of raising herbage yield and animal production.

Two promising legumes, *Stylosanthes guayanensis* (*S. gracilis*) ('Stylo') and *Glycine javanica* L., were oversown into undisturbed *Hyparrhenia*-dominant grassland at the Central Research Station, Mazabuka, Northern Rhodesia. The average botanical composition by weight of the veld was: *Hyparrhenia dissoluta* Hubbard 54 per cent; *H. filipendula* Stapf 20 per cent; *Heteropogon contortus* Beauv ex R. and S. 15 per cent; other species 11 per cent. The veld was burnt at the end of the 1961 dry season and the legume seed was oversown in plots after the soil had been scratched with a hand rake to simulate a light cultivation. (Previous work had shown a light cultivation was necessary for the consistently reliable establishment of legumes oversown into the veld.) All plots were given a basal dressing of 200 lb./acre of superphosphate (8·3 per cent phosphorus). At this site, both legumes apparently nodulate effectively without inoculation.

The legume establishment was good. The average density of legumes was approximately 25 plants/sq. ft. on January 28, 1963 (range 14–46 legumes/sq. ft.). The herbage yield of veld alone, veld + 'Stylo' and veld + *Glycine* cut at a hay stage of growth on January 14, 1963, is shown in Table 1. The addition of 'Stylo' doubled the yield of herbage dry matter, increased the yield of crude protein five-fold and increased the calculated yield of digestible crude protein[4] ten-fold, compared with veld alone. The yield increase due to *Glycine* was approximately half that of 'Stylo'.

The usual assumption is that pastures of selected grasses will outyield the native grassland when the

Table 1. MEAN TREATMENT YIELD OF HERBAGE DRY MATTER (D.M.), CRUDE PROTEIN (C.P.) AND CALCULATED DIGESTIBLE CRUDE PROTEIN (DIG. C.P.)[4] (LB./ACRE)

	D.M.			C.P.			dig. C.P.
	Grass	Legume	Total	Grass	Legume	Total	
Hyparrhenia veld	2090 ± 144		2090 ± 144	85 ± 10		85 ± 10	25
Hyparrhenia veld + 'Stylo'	1094 ± 230	3539 ± 199	4630 ± 123	58 ± 10	404 ± 39	462 ± 33	255
Hyparrhenia veld + *Glycine*	1982 ± 428	1358 ± 489	3340 ± 220	92 ± 20	190 ± 64	282 ± 53	

Table 2. MEAN ANNUAL HERBAGE DRY MATTER YIELD OF *Hyparrhenia* VELD AND *Chloris gayana* KUNTH (GIANT RHODES GRASS) AT FOUR NITROGEN-LEVELS (10 LB./ACRE)

	No	*N*1	*N*2	*N*3	Mean ± 6·1
Rhodes grass	236	273	389	489	347
Hyparrhenia veld	314	356	422	597	422

Body of Table ± 16·6

Treatments: No = No fertilizer; *N*1 = 50 lb. N/ac.; *N*2 = 100 lb. N/ac.; *N*3 = 200 lb. N/ac. (nitrogen as urea).

nutrition and management are improved. But when *Hyparrhenia* veld at Mazabuka was directly compared at four levels of nutrition with Giant Rhodes grass (*Chloris gayana* Kunth), a standard pasture species, the yield of veld was greater than the Rhodes grass (Table 2). Thus there is no evidence as yet for the need to plough up and resow vigorous *Hyparrhenia* grassland, especially if pasture legumes can be fairly easily introduced into the veld.

Because cattle nutrition in this territory is generally limited by their protein intake[5], the ten-fold increase in digestible crude protein that resulted from sowing 'Stylo' into the veld should allow a large potential increase in animal production.

C. A. SMITH

Mount Makulu Research Station,
Chilanga, Northern Rhodesia.

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VIROLOGY

Use of Synergy in the Identification of Strains of Citrus Yellow Vein Virus

IN 1957 a virus-induced vein yellowing disease was found in four Eustis limequat (*Citrus aurantifolia* (Christm.) Swing. × *Fortunella japonica* (Thunb.) Swing.) trees in California[1]. Although the virus has been shown experimentally to have a wide host range within the citrus family, it has not been found to occur naturally in other limequat trees or any other citrus plants. Cross-protection examination revealed that although the yellow vein virus will interact in various ways with certain of the other citrus viruses, it is not related to them[2,3]. This communication presents evidence of the existence of strains of the yellow vein virus based on cross-protection tests and studies of the synergistic interaction between yellow vein and vein enation viruses.

Symptoms in plants singly infected with yellow vein virus are usually very mild and restricted mostly to yellowing of veins of an occasional leaf followed by recovery or lack of symptoms in subsequent growth. Grafts from recovered plants frequently fail to transmit yellow vein virus. When the symptomless plants are inoculated with the unrelated virus of vein enation, severe yellow vein symptoms reappear and severe dwarfing results. Because of the unusual and erratic behaviour of yellow vein virus when it occurs alone, making the identification of strains based on symptom expression and cross-protection reactions unreliable, the differentiation of strains of yellow vein virus was made by combining it with the vein enation virus and examining the variation in synergistic response.

The same grafting techniques described in another paper[4] were used in these investigations. Two of the four virus-infected Eustis limequat trees, designated Tulare and Coachella, were used as virus donors. The remaining two yellow-vein-virus-infected limequat trees were found to be unsuitable as donors because they also carried other citrus viruses. Vein enation virus came from a citrus plant that had been infected experimentally by *Myzus persicae* (Sulz.). Virus-free Mexican lime, *C. aurantifolia* (Christm.) Swing., and rough lemon, *C. jambhiri* Lushington, were used as indicator plants.

Virus-infected Mexican lime and rough lemon plants exhibit two distinct types of vein yellowing, depending on the source of the yellow vein virus interacting with the vein enation virus (Fig. 1). One (Tulare + vein enation) consists of severe yellowing of the veins of every leaf of the plant and severe dwarfing. In some cases plants infected with this virus combination are killed. The other (Coachella + vein enation) shows milder vein yellowing, with fewer leaves affected, and less dwarfing. These symptoms, referred to in this communication as 'mild' and 'severe', remain constant and unaltered. Transfers of buds from the mild and severe types to healthy indicator plants, in every case, produced the mild and severe symptoms. In no instance did the mild strain (Coachella) induce severe (Tulare) symptoms or vice versa.

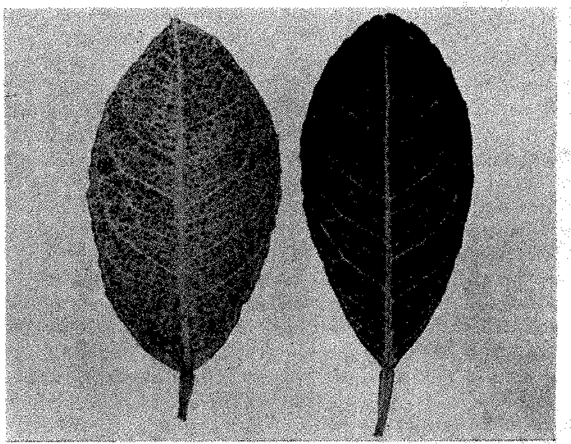

Fig. 1. Leaves of rough lemon infected with yellow vein and vein enation viruses. Left, severe yellow vein + vein enation viruses; right, mild yellow vein + vein enation viruses

In a series of cross-protection experiments, tissue from plants with the severe symptoms was grafted into plants with the mild type of symptoms to determine if the mild type would prevent or interfere with infection by the severe type. Protection was evident in all cases. The challenged plants with the mild symptoms remained unchanged. Transfers of tissue from the challenged plants to healthy indicator plants failed to induce the severe yellow vein symptoms or any evidence of a blend of the two types. These results indicate that cross-protection is effective and that the mild and severe symptoms are caused by strains of the same virus when each reacts synergistically with vein enation virus, rather than by different viruses.

From the results presented here it is concluded that the virus inducing yellow vein in citrus exists as strains. Two distinct strains are recognized. Strain Tulare is considered the severe strain which, in combination with vein enation virus, causes severe synergistic reactions in plants used in these tests. Strain Coachella causes milder synergistic responses in the indicator plants when combined with vein enation virus and is identified as the mild strain.

The discovery of strains in the yellow vein virus is interesting because of employment of the interactions between unrelated viruses to differentiate related strains of a virus. In this situation synergy appears to magnify the differences between related strains, making them more easily distinguishable. Attempts to separate strains of vein enation virus based on this synergistic reaction between it and yellow vein virus are now being made.

I thank Mr. M. K. Harjung for valuable assistance.

LEWIS G. WEATHERS

Department of Plant Pathology,
University of California,
Riverside, California.

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STATISTICS

Analysis of Spray-drop Data

THE spray-drop method of Williams and Backus[1,2] is a valuable technique in electron microscopy for the estimation of virus concentrations. A statistical analysis was described in the original papers[3]. In 1958 we showed[4] that this analysis was valid and gave a test to check that in any estimation the pairs of numbers used were unlikely to contain variations due to causes other than purely random ones. We pointed out that the ratio between the number of virus (V_i) and the number of latex (L_i) in the ith drop can be expected to vary between one drop and the next for two reasons. There is the variation due to random movements of the particles, both virus and latex, in suspensions at ordinary temperatures which introduces an uncertainty into whether a particle is in or not in a drop at the instant of formation. This introduces a variation in the numbers of particles expected in drops of the same size from a uniformly mixed suspension. It will have a Poisson distribution and has been termed by us inherent variation. The second reason is due to experimental factors—inadequate mixing, contamination during spraying, aggregation of particles, etc., and these have been termed induced variations. Our test shows whether the pairs of numbers obtained from a series of drops contain variations in excess of that which would be expected from the inherent variation alone. This test does not make any assumption about drop size and may be applied to a collection of droplets of any size distribution.

Opfell[5] has described a non-parametric test to discover any defects in the experimental technique. Such non-parametric tests (though usually less precise than parametric ones) are useful, but Opfell appears to have misunderstood the principles of our test when he justifies the need for his non-parametric one by implying that our test is insufficient by itself. Thus he says, "If the raw data satisfy the Williamson and Taylor tests and are free from induced variation arising from large variation in droplet size . . .", but he does not explain what induced variation he expects to get arising from "large variation in droplet size". Variation in droplet size does not itself add further induced variation, and our test checks for induced variation from all causes. Thus, if our test is satisfied, then it is reasonable to infer that the experimental data are satisfactory.

Watson[6] has published a statistical analysis based on correlation coefficients and regression lines. We have already pointed out[4] that correlation coefficients by themselves are misleading, and regression line analysis as presented by Watson is therefore unsatisfactory. He finds the regression line for V on L and compares the standard error of the estimate of V with the intercept of the line on the V axis. If the error is the greater, Watson considers that the regression is a straight line not significantly different from a line through the origin. If the standard error is less than half the intercept, then he considers the straight line is significantly different and the correlation (no matter how good) does not indicate consistency of the virus/latex ratio. In fitting the regression line as V on L, Watson tacitly assumes that the values of L are exact compared with V for each drop. This is not valid, as pointed out here, because inherent variations are present in both V and L. A regression line fitted so that it passed through the origin and the sum of the squares of the perpendiculars from each point to the line is a minimum would give the correct best estimate of the ratio from the slope of the line, but no estimate of the presence of induced variation would be possible since correlation coefficients as a test for consistency are of no value in this case.

Other authors[7,8] have used the χ^2 test. The values of χ^2 are very close to the values of $2F$ obtained from our test and which is itself distributed as χ^2. It is important to bear in mind the criteria which the tests are checking. Our likelihood test is an approximate test for the following two criteria. (*a*) If we could obtain many drops of exactly the same size the number of particles in them would form Poisson distributions about means ν_i and μ_i for the virus and latex respectively, (*b*) the ratio $\varkappa_i = \nu_i/\mu_i$ is constant for all i, that is, all drops.

The simple χ^2 test is an approximate test for the one criterion as expressed in (*b*). This test makes no recognition of any distribution such as (*a*).

It is also important to consider the approximations involved. The maximum likelihood test is approximately true for a large number of drops regardless of the counts within the drops. The ordinary χ^2 test is approximately true only when both the number of drops is large and the numbers within each drop are large (or made large by compounding drops). However, in most experiments the numbers involved are sufficiently large.

The values of χ^2 from the two tests usually differ. It can be shown that for a large number of drops

$$\chi^2_L = \chi^2_K\left(2 - \frac{R + 1/R}{r_i + 1}\right)$$

where χ^2_L is the component of χ^2 due to V_i and L_i counts in the ith drop in the likelihood test, χ^2_K is the component of χ^2 for the ith drop in the ordinary test,

$$r_i = V_i/L_i,\ R = \Sigma V_i/\Sigma L_i$$

Thus the contribution of the ith drop to the χ^2 of the likelihood test can be greater or less than the χ^2 of the ordinary test.

It appears that all authors determine the ratio of virus to latex in the valid way that is ($R = \Sigma V_i/\Sigma L_i$). We conclude, however, that not all authors use a valid test to check their data before computing this ratio. Any test based on correlation coefficients (and therefore including regression analysis) is in our opinion unreliable. The ordinary χ^2 test may be used, but being a general test for the equality of proportions may lead to the acceptance of data which on a more stringent test would be rejected. The method we have proposed represents the classical statistical approach applied to this problem and provides a solution in an easily workable form. The criteria tested are more stringent than the ordinary χ^2 test since a further requirement is added that the numbers of particles in drops of identical size would be Poisson distributed

K. I WILLIAMSON

Department of Scientific and
Industrial Research,
Plant Chemistry Division,
Palmerston North,
New Zealand

W B TAYLOR

Meat and Wool Boards,
Wellington,
New Zealand

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A Method of Calculating Net Assimilation Rate

A USEFUL measure of the photosynthetic efficiency of plants is 'net assimilation rate' (E) defined by Gregory[1] as the rate of increase of dry weight (W) per unit of leaf area (L), that is

$$E = \frac{1}{L}\,\frac{\mathrm{d}W}{\mathrm{d}t} \qquad (1)$$

In measuring W the plant is destroyed, so changes in W can be determined only by successive sampling from a population of plants, involving sampling errors In practice samples are commonly taken at intervals of 1–4 weeks for measuring both W and L The paired W and L sample means may then be used to calculate E_M, an estimate of the mean E for each time-interval $(t_2 - t_1)$, usually as proposed by Gregory[2]

$$E_M = \frac{(\bar{W}_2 - \bar{W}_1)(\log_e \bar{L}_2 - \log_e \bar{L}_1)}{(t_2 - t_1)(\bar{L}_2 - \bar{L}_1)} \qquad (2)$$

Application of this formula to data recorded by one of us (J.C S A) in an experiment on partial defoliation of maize gave the results shown in Table 1

Table 1 MEAN VALUES OF E_M (G/M²/WEEK)

	Period in weeks after flowering			
	0–2	2–4	4–6	6–8
Control	42 0	23 5	33 1	27 8
Defoliated	43 2	25 9	25 3	36 1
S E	±3 8	±4 2	±6 1	±9 3

There were 6 plots of partially defoliated plants and 6 of controls Samples of 9 plants were taken from each plot at fortnightly intervals 0–10 weeks from flowering The L values at week 10 and hence the E_M values for weeks 8–10 were unreliable because of the large number of senescent leaves, and have been omitted from Table 1 The standard errors are calculated from the variation between replicates

The errors of Table 1 are large and the values fluctuate erratically, particularly in the later periods This is because differences in W, which are subject to large errors, have to be taken and the errors of successive differences are negatively correlated

It occurred to us that these errors might be greatly reduced by fitting smooth curves to W and L, an approach which appears to have been overlooked by previous writers on this subject (see Watson[3] for a review and bibliography)

In this experiment quadratic curves gave a satisfactory fit, as is shown by Fig 1 The *S E* s of W were calculated from the variation between replicates; this tended to increase with time but was reasonably homogeneous over all dates after a log transformation The *S E* s shown in Fig 1 are the de-transformed values derived from the single pooled *S E* of log W In order to provide estimates of E and their errors quadratic curves $W = a + bt + ct^2$ and $L = a' + b't + c't^2$ were fitted to the W values (including week 10) and the L values (up to week 8) for each plot separately From (1) it follows that

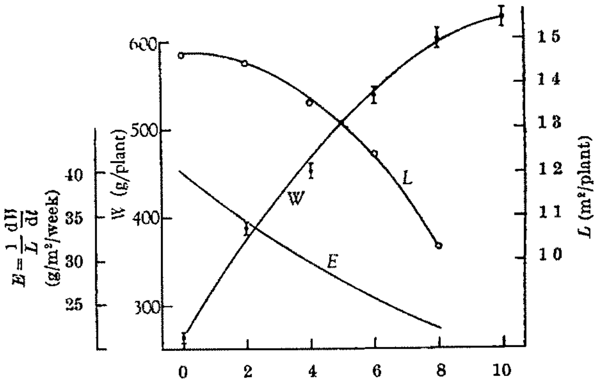

Fig 1 Changes in dry weight (W), leaf area (L) and net assimilation rate (E), of maize plants during 10 weeks following flowering

$$E = \frac{b + 2ct}{a' + b't + c't^2}$$

This was evaluated for each plot for $t = 0, 1, 2$ 8 weeks The treatment means of the values for $t = 1, 3, 5, 7$ (the mid-points of the intervals used in Table 1) are shown in Table 2, and a graph of the mean E of the control plots is included in Fig 1

Table 2 MEAN VALUES OF E (G/M²/WEEK)

	Period in weeks from flowering			
	1	3	5	7
Control	37 0	32 0	27 7	23 8
Defoliated	39 0	32 1	26 0	22 2
S E	±2 2	±1 6	±1 6	±2 2

The results of Table 2 are much more regular than those of Table 1, and the errors are much smaller. The values for the different times are not, of course, independent, but differences between treatments (for example, for average effect and rate of change) can be tested, in cases of doubt, by calculating the mean (m) and regression coefficient (b) of a suitable selection of calculated E values for each plot and comparing the treatment mean m and b values Thus we have

	$\bar{m}$		$\bar{b}$	
Control	30 1	(±1 5)	−2 2	(±0·5)
Defoliated	29 8	(±1 5)	−2 8	(±0 5)

The general falling trend of E with time is well established, and the absence of treatment effect is shown by the non-significance of m and b differences

Inherent in the curve-fitting method is a smoothing out of any deviations from the general trend of E with time. This loss is of no consequence here as the apparent deviations seen in the E_M figures are of little statistical significance and, even if to some extent genuine, are irrelevant to the main purpose of the investigation

The method is essentially empirical, and there is no necessity for the parameters of the curves to have biological significance, provided that the curves adequately describe the time changes of W and L

A J VERNON

Statistics Department,
Rothamsted Experimental Station

J C S. ALLISON

Agricultural Department,
University College of Rhodesia and Nyasaland.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 25

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C 2), at 2 30 p m and 5 30 p m —Colloquium on "Recent Advances in D C Amplifiers".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 4 p m —Prof A W Woodruff "Recent Advances in Tropical Medicine" *

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Lecture Theatre of the Science Museum, Exhibition Road, London, S W 7), at 5 30 p m —Mr S C Goddard "Louis Ducos du Hauron and the Early History of Colour Photography"

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Is Load Shedding Necessary and How Can Its Effects be Minimized?" opened by Mr A E Hawkins

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Dr E Schatzman (Paris) "Nuclear Reactions at the Surface of Stars and Stellar Evolution" *

BRITISH MEDICAL ASSOCIATION (at the National Hospital for Nervous Diseases, Queen Square, London, W C 1), at 6 p m —Dr Macdonald Critchley, F R C P "The Problem of Visual Agnosia" (Twelfth Victor Horsley Memorial Lecture)

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 6 p m —Dr T Vickerstaff "Scientific Aspects" (Second of three Cantor Lectures on "Colour in Modern Life")

Tuesday, November 26

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr W A Smeaton "Early Balloon Ascents, 1783–1795".*

UNIVERSITY OF LONDON (at Imperial College, London, S W 7), at 1 30 p m —Mr D E Broadbent "Adjusting to the Environment Learning". (Fourth of five lectures on "The Basis of Experimental Psychology")*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr R A Stephenson and Mr O T Barfod "Tanker Accommodation in the Mersey at Tranmere"

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College, Gower Street, London, W C 1), at 5 30 p m —Sir John Ritchie, C B "The Eradication of Bovine Tuberculosis and its Importance to Man and Beast" (Thirty-second Stephen Paget Memorial Lecture), followed by the Annual General Meeting

ROYAL INSTITUTION (at 21 Albemarle Street, London, W 1), at 5 30 p m —Prof R L F Boyd "Exploration of the Upper Atmosphere by Space Vehicles" (Afternoon Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties Further Lectures on November 27 and December 3 and 4)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr W E van Heyningen "The Bacterial Neurotoxins" (Twelfth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

INSTITUTION OF ELECTRICAL ENGINEERS, and the ROYAL AERONAUTICAL SOCIETY (at the Institution of Electrical Engineers, Savoy Place, London, W C 2), at 6 p m —Discussion on "Installation Aspects with Particular Reference to the Cooling of Electrical Equipment in Aircraft" opened by Mr P A Amer

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Symposium on "The Design of Small Mass-Produced Motor Car Engines"

UNIVERSITY OF LONDON, and the INSTITUTE OF BANKERS (in the Library of the Institute of Bankers, 10 Lombard Street, London, E C 3), at 6 p m —Dr T A Margerison "Britain To-morrow—a Consideration of How Best to Mobilise Her V Scientifically".

SOCIETY FOR ANALYTICAL CHEMISTRY, PHYSICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W 1), at 6 30 p m —Nineteenth Annual General Meeting, followed by Dr. W Cule Davies "Selection of a Physical Method"

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Dr J Idris Jones "Organo Aluminium Polymers"

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the SOUTH-EAST ESSEX TECHNICAL COLLEGE CHEMICAL SOCIETY, at the South-East Essex Technical College, Longbridge Road, Dagenham), at 7 p m —Mr. W G Busbridge "Isotopes in Industry"

SOCIETY OF INSTRUMENT TECHNOLOGY, SYSTEMS ENGINEERING SECTION (at Manson House, 26 Portland Place, London, W 1), at 7 p m.—Mr V A Gault "Lift Control Using Static Switches"

Tuesday, November 26—Wednesday, November 27

ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR, and the ZOOLOGICAL SOCIETY OF LONDON (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N W 1), at 10 30 a m daily—Symposium on "Social Organisation of Animal Communities"

IRON AND STEEL INSTITUTE (at Church House, Great Smith Street, London, S W 1), at 9 45 a m on Tuesday, and 10 a m on Wednesday—Autumn General Meeting Conference on "Fume Arrestment in Steelmaking Processes"

Wednesday, November 27

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 2 p m —Dr H Lehmann "Genes and Molecules".*

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S W 1), at 3 p m —Meeting on "The 1963 Food Group Summer Conference and Tour in the Netherlands".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "The Application of Electronic Building Bricks" opened by Mr. I V Idelson and Prof. J E B. Gray

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "High Voltage Generation and Distribution of A C in Ships" opened by Mr D Gray

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Annual General Meeting, followed by the Presidential Address of Mr J L Thompson

SOCIETY FOR VISITING SCIENTISTS (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W 1), at 7 30 p m —Discussion Meeting on "Science Centres". Chairman Prof A Haddow, F R S Speakers Mr J E Cummins and Sir Robert Robinson, O M , F R S

Thursday, November 28

PLASTICS INSTITUTE, REINFORCED PLASTICS GROUP (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 10 a m —Conference on "Sandwich Construction".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr J L Watling "Can There be Two Things Exactly Alike?"*

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W C 2), at 5 15 p m —Mr Tom Harrisson "100,000 Years of Stone Age Culture in Borneo".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Mr F J D Taylor, O B E , Mr W J Bray and Mr R W White "The Results of Tests at Goonhilly with the Experimental Earth Satellites Telstar and Relay"

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Consideration of the Practical Applications of Lasers" opened by Dr Denis Taylor and Dr R P Howson

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr A P Waterson "The Structure of Viruses" (Thirteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the BATTERSEA COLLEGE OF TECHNOLOGY CHEMICAL SOCIETY, at the Battersea College of Technology, Battersea Bridge Road, London, S W 11), at 6 30 p m —Prof J Lewis "Magnetic Criteria for Stereo-Chemistry of some Cobalt II Complexes"

Friday, November 29

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 10 a m —Dr Audrey Smith "Survival of Frozen Cells, Tissues, Organs and Animals" *

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W C 1), at 6 15 p m —Mr Robert Maxwell "The Economics of Publishing Scientific Material"

ROYAL INSTITUTION (at 21 Albemarle Street, London, W 1), at 9 p m —Sir Charles Percy Snow, C B E "The Two Cultures and the Sorcerer's Apprentice"

Saturday, November 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W 1), at 2 30 p m —Anniversary Meeting

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Mr Raymond Clausen "Some Aspects of Music and Life To-day in Malekula, New Hebrides" *

BOTANICAL SOCIETY OF THE BRITISH ISLES (in the Botany Department, British Museum (Natural History), Cromwell Road, London, S W 7), from 2 p m to 5 30 p m —Annual Exhibition Meeting

Monday, December 2

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, N W 1), at 4 p m —Prof John Francis (University of Queensland) "Veterinary Research and Education in Australia" *

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C 2), at 5 30 p m —Mr F Horner "Atmospheric Radio Noise as a Problem in Radio Communications"

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (joint meeting with the COMPUTER GROUP of the INSTITUTION OF ELECTRICAL ENGINEERS, at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Symposium on "Analogue Circuit Techniques using Transistors".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Some Experience on Projects in Courses for Diplomas in Technology"

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W C 2), at 6 p m —Mr Hulme Chadwick "Colour and Community". (Last of three Cantor Lectures on "Colour in Modern Life")

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Mr H R Hearn "Technical Developments in the Aerosol Field"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8 15 p m —Mr Norman G Dyhrenfurth "America on Everest".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Physicist (Basic grade), initially for training and routine work on the physical aspects of radiotherapy (including high energy X- and Gamma-radiations)—The Secretary, Mount Vernon Hospital, Northwood, Middlesex (November 29)

Experimental Officer or Senior Experimental Officer in the Department of the Mechanics of Fluids, for duties which will include the design and construction of electronic apparatus for use in fluid mechanics research—The Registrar, The University, Manchester 13 (November 30)

Lecturer/Assistant Lecturer (with a good honours degree and research experience) in Botany—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref 11/63/6 (November 30)

Research Assistant (with a good honours degree in physics, chemistry or metallurgy) in Applied Science, to work with Dr W G Townsend in the Electrical Engineering Laboratories on problems associated with the surface chemistry of semiconductor materials and other related effects—Dr W G Townsend, Engineering Department, University College of Swansea, South Wales (November 30)

Senior Lecturer in Mathematics—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C 1 (November 30)

Sub-librarian (with a good honours degree and preferably professional qualifications and appropriate experience) in the Faculties of Medicine and Veterinary Science—The Registrar, The University, Liverpool, quoting Ref. No CV/440/N (November 30).

Lecturer (with research interests in the field of organic molecular physics) in Physics—The Registrar, The University, Manchester 13 (December 1)

Lecturer (with a good honours degree and professional or research experience in civil or mechanical engineering) in the Department of Engineering—The Secretary, The University, Aberdeen (December 4)

Lecturer (with an honours degree in mathematics) in Mathematics in the Department of External Studies, University of Queensland, Brisbane, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (London and Brisbane, December 6)

Assistant Librarian (honours graduate with library qualifications)—The Secretary, The Queen's University, Belfast, Northern Ireland (December 10)

Lecturer/Senior Lecturer in Physical Chemistry at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London, December 10)

Senior Teaching Fellows and Teaching Fellows in the Department of Mathematics, Monash University, to assist in the teaching of one or more of the following subjects applied mathematics, pure mathematics and mathematical statistics—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, Monash University, P O Box 92, Clayton, Victoria, Australia (Australia and London, December 13)

Senior Teaching Fellows and Teaching Fellows in the Department of Physics, Monash University—The Secretary Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, Monash University, P O Box 92, Clayton, Victoria, Australia (Australia and London, December 13).

Senior Teaching Fellows and Teaching Fellows in the Department of Zoology and Comparative Physiology at Monash University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London S W 1, or The Registrar, Monash University, P O Box 92, Clayton, Victoria, Australia (Australia and London, December 13).

Lecturer, and an Assistant, in Mathematics—The Secretary of University Court, The University, Glasgow (December 31)

Chair of Applied Mechanics within the Department of Mechanical Engineering—The Registrar, The University, Liverpool, quoting Ref CV/453/N (January 11)

University Lecturer in Physiology (some preference may be given to a candidate with interests in the general field of body fluids and electrolytes)—The Administrator, Department of Physiology, The University, South Parks Road, Oxford (January 31)

Assistant Experimental Officer (with a pass degree or H N C or, if under 22, G.C E "A" level (preferably in chemistry and zoology), O N C, or equivalent), for a programme of research on the biochemistry of lipids and their possible significance in cardiovascular degeneration in experimental animals—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, near Reading, quoting Ref 63/N/13

Associate Professor in Pharmacology—Prof M F Murnaghan, Faculty of Medicine, University of Ottawa, Ottawa, Canada

Head (with a higher degree, an established field of research in electrical engineering, and a number of years of university teaching experience) of the Department of Electrical Engineering, Nova Scotia Technical College—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall London, S W 1, and President G W Holbrook, Nova Scotia Technical College, Halifax, Nova Scotia, Canada

Lecturer or Senior Lecturer (man or woman, preferably with teaching experience and a knowledge of modern methods) in Mathematics in the Training Department, to share in courses for the B A and B Sc (General) degrees and the three-year Teachers' Certificate—The Registrar, Goldsmith's College (University of London), New Cross, London, S E 14

Official Tutorial Fellowship in Physiology—The Master, St Catherine's College, Oxford

Post-doctoral Research Fellow, for work on a mass spectrometric study of gas reactions in shock waves—Prof J E Dove, Department of Chemistry, University of Toronto, Toronto 5, Ontario, Canada

Research Assistant (with an honours degree and research experience in the field of mechanical properties of materials), to take day-to-day charge of a research project on the fracture of metals and non-metals—Dr R D Hoyle, Mechanical Engineering Department, Imperial College of Science and Technology, London, S W 7

Research Associate (with a Ph D or honours B Sc in physiology or pharmacology), to investigate mechanism of action of sympathomimetic amines on the tissues of the eye, with special reference to their action on intraocular dynamics—Dr M E Langham, Department of Ophthalmological Research, Wilmer Institute, Johns Hopkins Hospital, Baltimore 5, Maryland, U S A.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ambassade de France, Service de Presse et d'Information Some Aspects of Scientific Research in France Pp 14 (London Ambassade de France, Service de Presse et d'Information, 1963.) [109

Royal Observatory Bulletins No 75 Time and Latitude Service 1962, July–September. Pp. B111–B130. (London H M. Stationery Office, 1963) 3s 6d net [109

Steel Castings Development Committee Data Sheet No 8 Electrical Resistivity of Steel Castings Pp 3. Data Sheet No 9 Torsional Properties of Steel Castings Pp 5 (Sheffield Steel Castings Development Committee, 1963) [109

Planning, Vol. 29, No 475 (9 September, 1963) French Planning—Some Lessons for Britain By Malcolm MacLennan Pp 323–402. (London Political and Economic Planning, 1963) 7s [109

General Register Office Census 1961 England and Wales County Report—Hertfordshire Pp xxiii+141. (London H M Stationery Office, 1963) 28s net [109

Royal Observatory Bulletins No 64 Photoelectric Magnitudes and Colours of Southern Stars By A. W J Cousins and R H. Stoy. Pp E103–E248 (London H M Stationery Office, 1963) 24s net [109

International Commission for Uniform Methods of Sugar Analysis Report of the Proceedings of the Thirteenth Session held at The University of Hamburg and at The Atlantic Hotel, Hamburg, Germany, from August 26th to August 31st, 1962 Pp vii+125 (Keston, Kent International Commission for Uniform Methods of Sugar Analysis, Paris Syndicat National des Fabricants de Sucre de France, New York Sugar Research Foundation, Inc, 1963) 28s, 19 francs, 4 dollars [109

Other Countries

Institut Royal Météorologique de Belgique. Publications, Série A, No 38 Les Anomalies Magnétiques de la Belgique Par Dr A de Vuyst Pp 31 Publications, Série A, No 40 Electricité Atmosphérique à la Base Roi Baudouin. Par A. van der Schueren et L Koenigsfeld. Pp 31 Observations Ionospheriques—Station de Dourbes Juin 1963 Pp 26. (Bruxelles Institut Royal Météorologique de Belgique, 1963) [189

Report of the Department of Agriculture, New South Wales, for the year ended 30th June, 1962 Pp 132 (Sydney Government Printer, 1963) [189

Organisation for Economic Co-operation and Development Ministerial Meeting on Science Agenda Item III, (Background Paper)—Science, Economic Growth and Government Policy Pp 6 (Paris Organisation for Economic Co-operation and Development, 1963) [209

Zoology Publications from Victoria University of Wellington No. 33 Anguillid Leptocephali in the Southwest Pacific By P H J Castle Pp 1–14 No 34 The Systematics, Development and Distribution of Two Eels of the Genus *Gnathophis* (Congridae) in Australasian Waters By P H J. Castle Pp 15–47 No 35 Studies on the Paua, *Haliotis iris* Martyn in the Wellington District, 1945–46 By Mary Sinclair Pp 16+4 plates No 36 The Holothurian Fauna of Cook Strait, New Zealand By D L Pawson Pp 38 (Wellington Victoria University of Wellington, 1963) [249

Canada Department of Northern Affairs and National Resources National Museum of Canada. Bulletin No 195 The Hunting Group—Hunting Territory Complex Among the Mistassini Indians By Edward S Rogers Pp iv+95 (Ottawa Queen's Printer, 1963) [249

New Zealand Report of the Department of Scientific and Industrial Research for the year ended 31 March 1963. Pp 87 (H 34) (Wellington Government Printer, 1963) 3s [249

Plants and the Migrations of Pacific Peoples a Symposium Edited by Jacques Barrau (Tenth Pacific Science Congress, Honolulu, Hawaii) Pp v+136 (Honolulu, Hawaii Bishop Museum Press, 1963) 4 dollars [249

Department of Scientific Affairs, Pan American Union, General Secretariat, Organization of American States, and Inter-American Press Association Technical Center, Inc Science Journalism Pp iii+52 (Washington, D C Department of Scientific Affairs, Pan American Union, 1963) [249

United States Department of the Interior Geological Survey. Bulletin 1133-C Geology and Hydrology of the Elk River, Minnesota, Nuclear-Reactor Site By R F Norvitch, Robert Schneider and R G Godfrey Pp iv+25+plates 1 and 2 Bulletin 1147-E Distribution of Elements in Colorado Plateau Uranium Deposits—a Preliminary Report. By A. T Miesch. Pp iv+57+plates 1–3. Bulletin 1156-A Geology of Brookhaven National Laboratory and Vicinity, Suffolk County, New York By Wallace de Laguna. Pp iii+35+plates 1 and 2. Bulletin 1162-E Diamond-Drilling Exploration of the Beecher No 3—Black Diamond Pegmatite, Custer County, South Dakota By J A Redden Pp iii+11+plate 1 Bulletin 1172 Measuring Geothermal Gradients in Drill Holes Less Than 60 Feet Deep, East Tintic District, Utah By T. S Lovering and H D Goode Pp iv+48+plate 1 Water-Supply Paper 1588 Ground-Water Geology of Bexar County, Texas By Ted Arnow Pp v+36+13 plates (Washington, D C Government Printing Office, 1963.) [249

Organisation for Economic Co-operation and Development, European Nuclear Energy Agency. Fifth Report on the Activities of the Agency. Pp iii+152 (Paris Organisation for Economic Co-operation and Development, European Nuclear Energy Agency, 1963) [249

Institutt for Atomenergi Kjeller Research Establishment Quarterly Progress Report, January–February–March, 1963 Pp ii+27+23 figures (Kjeller Institutt for Atomenergi, Kjeller Research Establishment 1963) [249

Golden Ties (Published on the occasion of the fifty years' jubilee of the Co-operative Condensed Milk Factory, "Friesland") Pp 8+60 photographs (Leeuwarden Co-operative Condensed Milk Factory, 1963) [249

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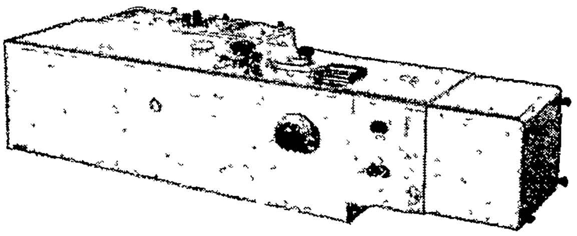

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Interested scientists are invited to write to Professor D G Catcheside, F R S, presently in the Department of Microbiology, University of Birmingham, Birmingham 15, who will move to Canberra in August, 1964

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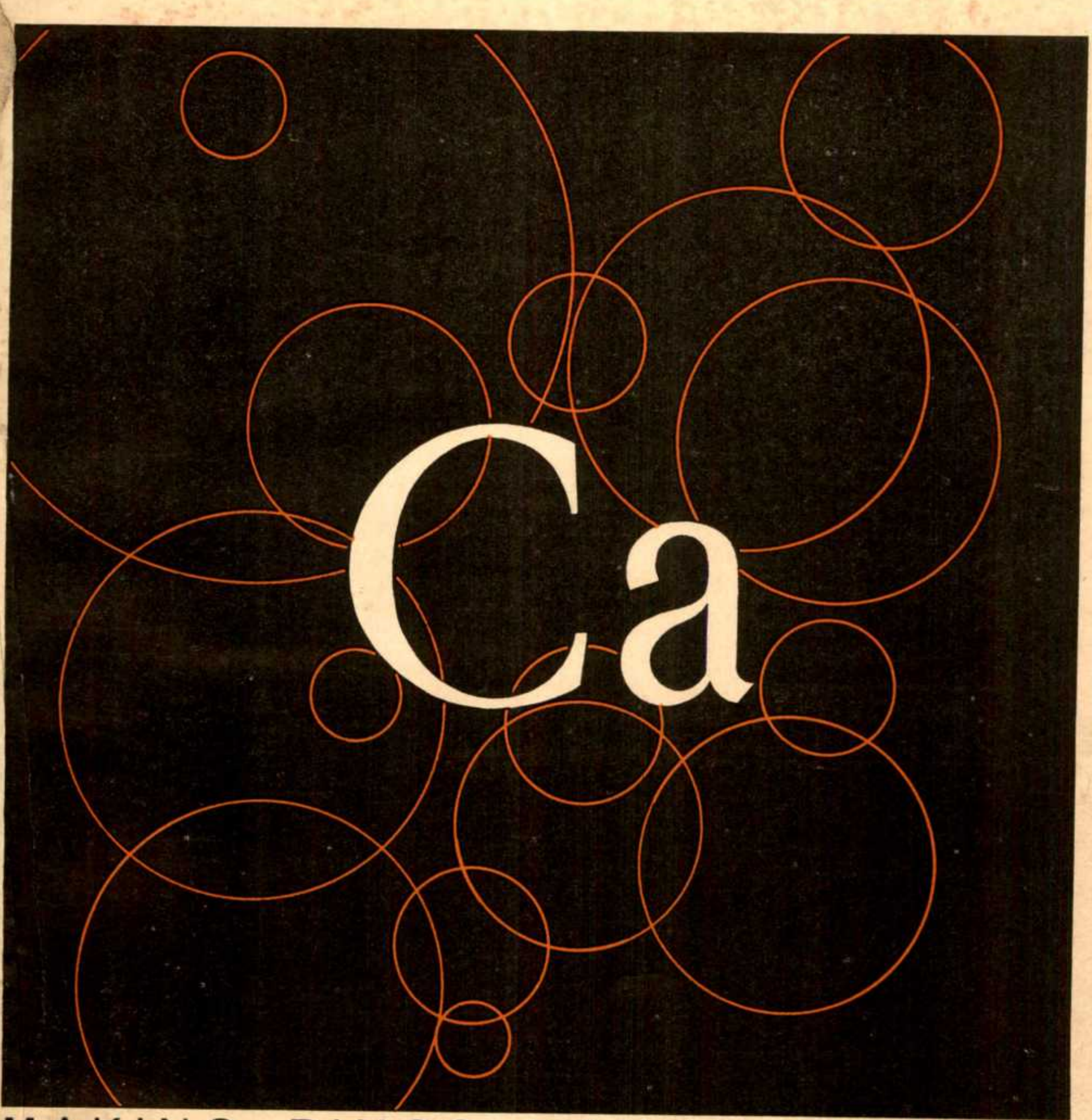

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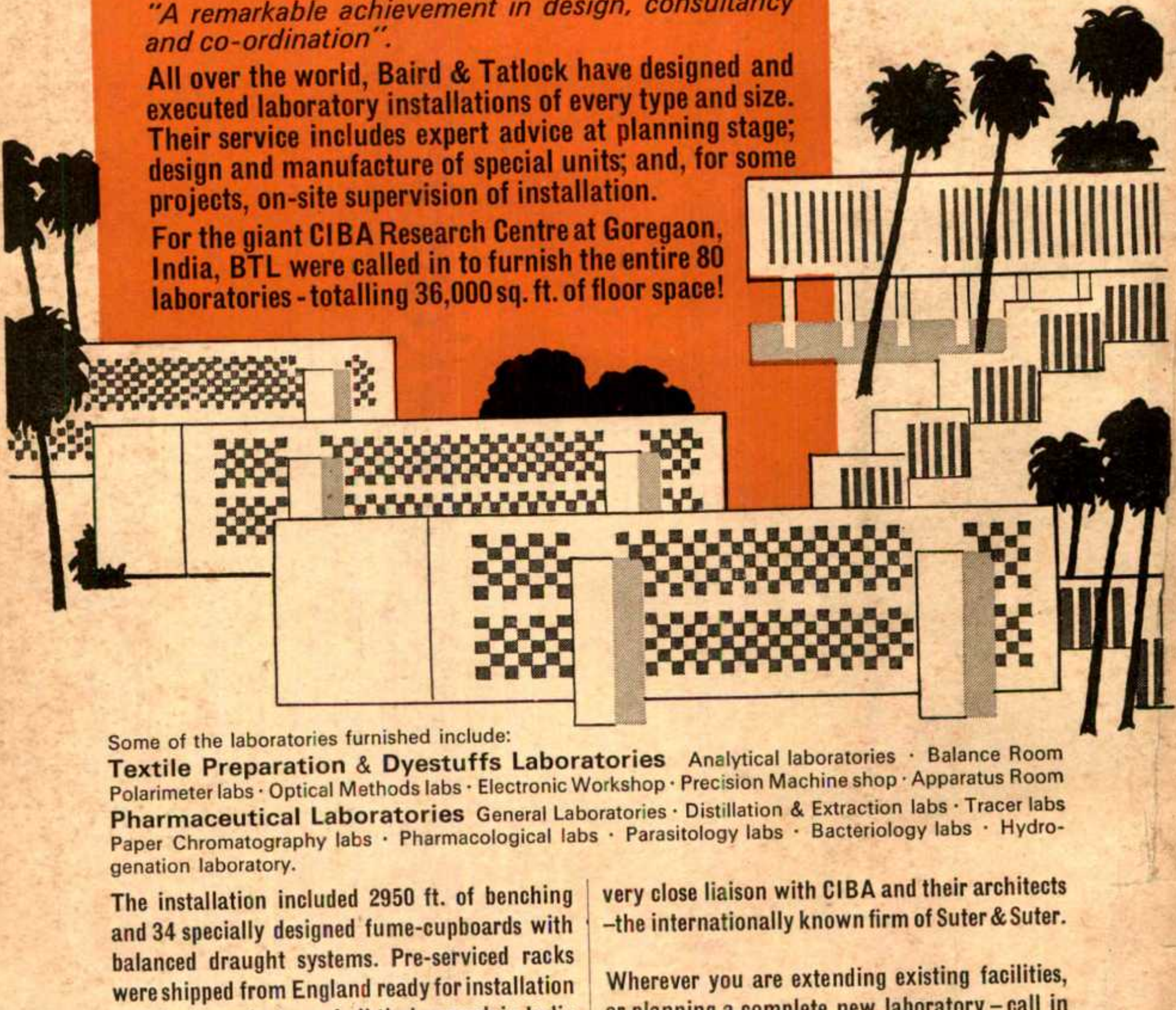

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PRESIDENT JOHN KENNEDY

News of the assassination, on November 22, 1963, of President Kennedy of the United States of America must by now have reached every part of the world.

Many columns of print, and numerous comments by radio and television, have already revealed the disgust and anguish at this senseless, indeed monstrous, act; moreover, from every country have gone messages of sincere sympathy with Mrs. Kennedy and all other members of the late President's family.

Nevertheless "Nature" feels that a special note of sympathy should be expressed, not only to the late President's family, but also to every American scientist from all other scientists throughout the world. Apart from the personal tragedy, we as scientists recognize that in more than one capacity the late President exerted a profound influence on scientific research and development, and its impact on world affairs—an influence which spread far beyond the frontiers of the U.S.A.

In our special field of human endeavour, therefore, we must remember this, and so deplore its loss.

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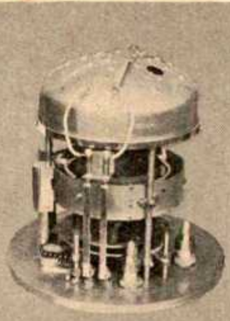

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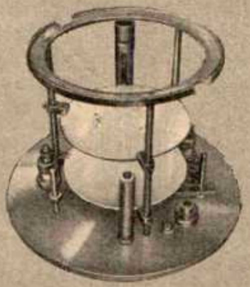

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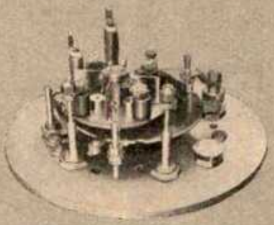

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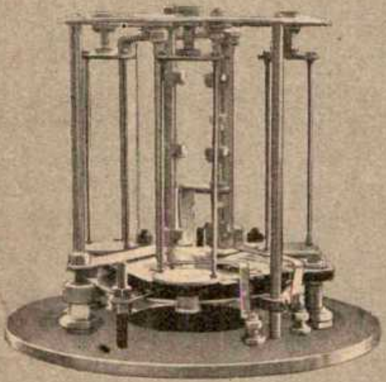

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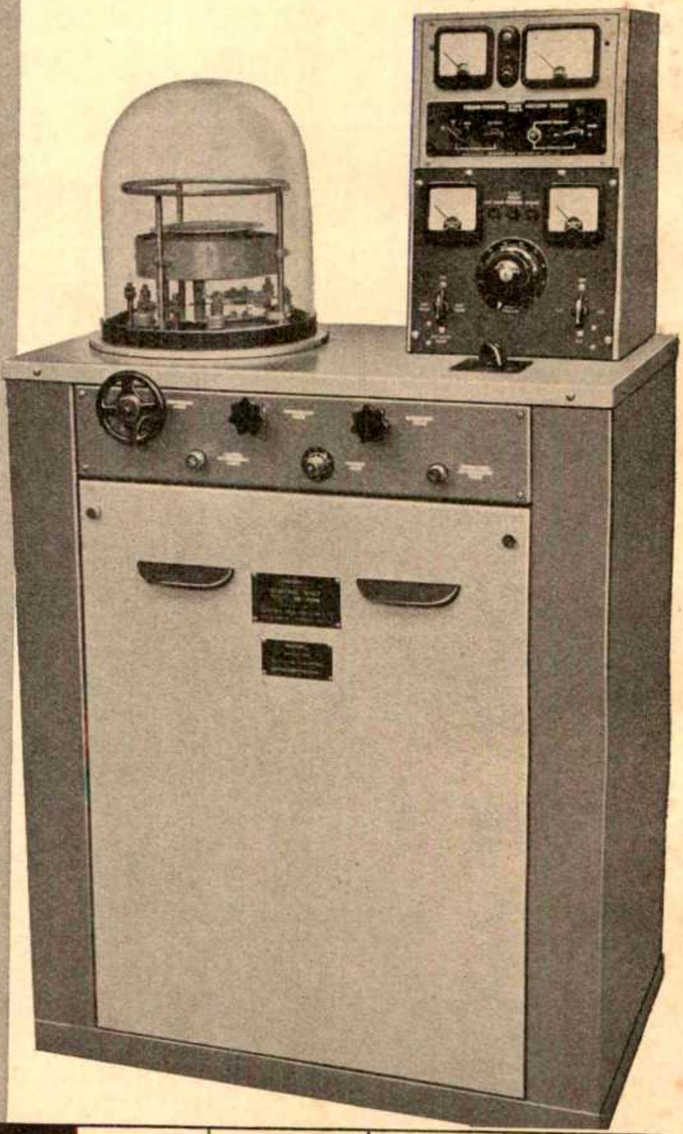

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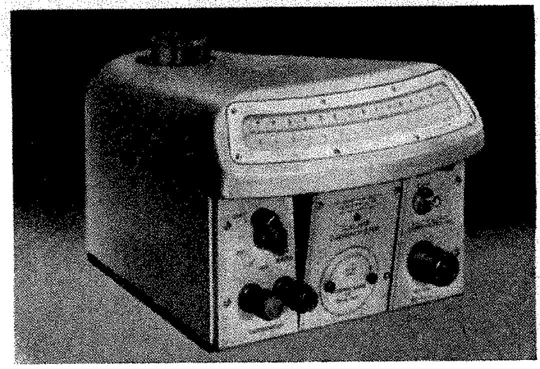

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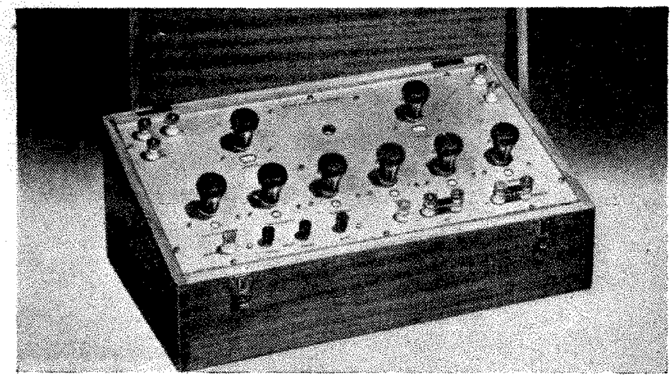

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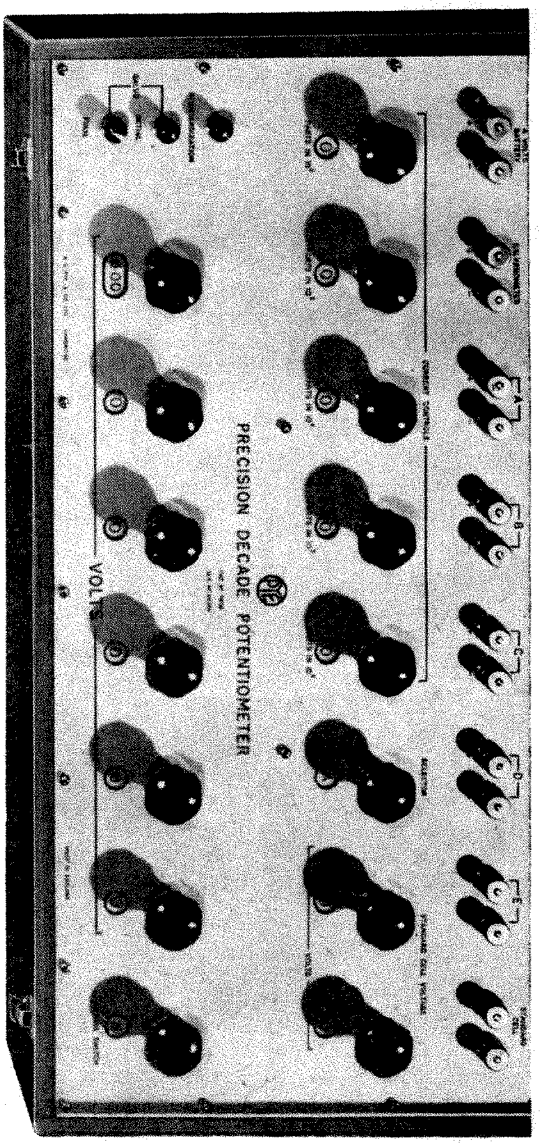

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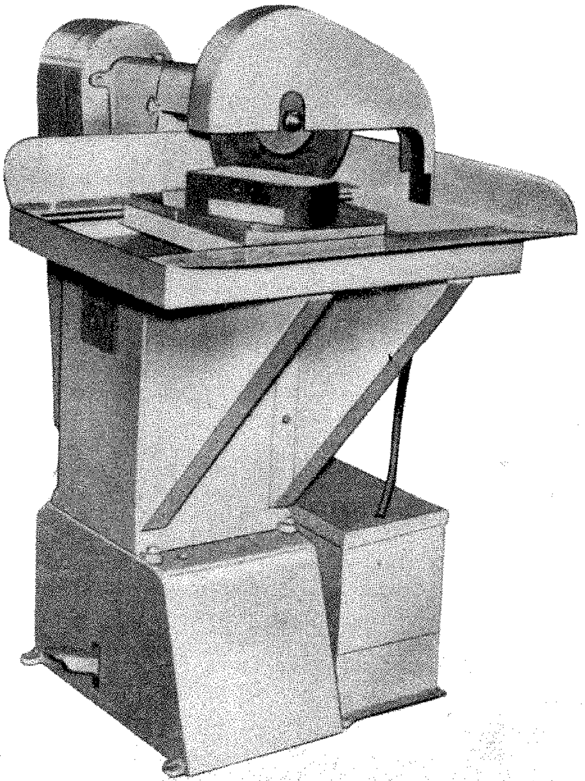

TRADE UNIONS: THE CALL TO REFORM

THE Broadsheet by B Donoghue, assisted by A. Oakley and Janet Alker, *Trade Unions in a Changing Society**, is part of a three-year study of the subject which is being carried out by Political and Economic Planning It was issued two months before the report on economic development and planning, which urged the Trade Unions to undertake the institutional changes required to enable them to function effectively in a world which increasingly requires economic planning, but although the report was adopted by the Trades Union Congress at Brighton in September, no positive proposals were advanced A further report on the British Trade Union structure was promised for next year, and, in the meantime, the Broadsheet should encourage the constructive thinking within the Unions that is essential as a first step to reforms that must come from the General Council if not from the Unions themselves

The Broadsheet follows an earlier study, issued in July 1962, of Trade Union membership, and shows even more clearly than the first Broadsheet the new functions which the Trade Unions must exercise in a changing society and the urgent need for an improvement in the organization and structure of the Unions, indeed, of the whole Trade Union situation in Britain It is obvious from the Broadsheet and from the debates at Brighton that some improvement in management would ameliorate the situation, if only by removing some of the excuses advanced by the Unions for not undertaking reforms, and this may well be the only material contribution that can come from outside Nevertheless, the Broadsheet displays dispassionately the realities of the situation and the danger to society as a whole if the Trade Unions continue to ignore these realities and fail to discover for themselves a new and relevant sense of purpose and fail to adjust their functions and organization accordingly

Some trade unionists may disagree with particular interpretations made in the Broadsheet, and there may well be a disposition to ignore some of the changes in our whole social and economic life and outlook, or at least to imagine that the responsibility for change and adaptation in institutions or relationships is solely a Government responsibility The changes, however, are too deep and fast for this to be a party political matter, and the Broadsheet is careful to avoid making them a party issue It points out, nevertheless, that the troubles which afflict industry and especially the Unions ultimately injure all members of the community and that there is a common advantage to be gained from ending them and a common responsibility to work towards ending them If, in fact, the Trade Union movement fails to ask itself honestly the basic questions about its position in the changed and still changing Britain, to answer them honestly and take the necessary action, in spite of difficult and even painful adjustments, the Unions must become increasingly anachronistic and will be unable to make positive contributions to the new society which is emerging in Britain

The Broadsheet begins by quoting from a speech by Mr George Woodcock to the Trades Union Congress in September 1962, in which he urged the Unions to do just this to enquire into Trade Union purpose, to ask themselves what they were here for and then to discuss the kind of structure which would enable them to serve that purpose After surveying broadly the industrial environment, including its size and complexity, the new techniques and changes in materials and processes and in the work force, the Broadsheet considers the striking changes in the organization, direction and climate of the British economy since before the Second World War The social environment is then considered and then the implications of these developments are analysed in a section which points out that basic objectives proclaimed by the Unions only twenty-five years ago have now been achieved to a considerable extent and for a majority of working people, and much of the social and economic framework within which these original objectives were pursued has been remodelled

Nevertheless, the mood of the Unions is almost wholly defensive and conditions their attitude to change of all kinds. More particularly, it inhibits them from dealing with the specific difficulties of individual Union practice, organization and recruitment, which with the changing conditions have sometimes become out of date or hindered the appropriate changes The pronounced growth of the white collar and female sections presents other problems, as does structural decline in industries and regions where membership has traditionally been high Nor have the implications of the extension of higher education for the recruitment of able officials been faced.

One result of the failure of the Trade Union movement to discover and proclaim for itself any dynamic objectives in society to-day and to adapt its practices and organization to contemporary conditions is a growing public hostility to the Unions This is unfortunate in itself, and it tends to encourage unbalanced hostile comment in the newspaper Press, with a further consequence that the defensive attitude of the Unions is intensified and those elements in the movement who wish to prolong class struggle and distrust any advice from outside are encouraged The Broadsheet urges the need for better public relations, though it admits that this will depend to some extent on an improvement in the public performance of the Unions

It should be clear enough that, like the early studies which Political and Economic Planning has made of the Trade Unions, this further investigation is thoroughly sympathetic It recognizes that the traditional role of the Unions is still necessary and valuable, and it points out that, besides the functions of bargaining, settlement of disputes, consultations and communications, the Unions' capacity to provide workers with a sense of protection and community in the work-place, and especially in the modern, highly capitalized plant, is of great importance. Nor should the services rendered by the Unions in the way of friendly benefits and legal advice, or as spokesmen on matters concerned with social security, occupational safety and health, be overlooked The very importance of these continuing functions accentuates the need to eradicate weaknesses and adapt the Unions to perform these functions with efficiency, unity and authority

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* *Planning*, 29, No 472, June 10, 1963 *Trade Unions in a Changing Society* By Bernard Donoghue, assisted by Janet Alker Pp 171-226 (London Political and Economic Planning, 1963) 5*s*

Even more important, perhaps, are the constructive suggestions made in the Broadsheet regarding the new functions and purpose of the Unions First, as regards fringe benefits negotiated voluntarily between Union representatives and employers, while British Trade Unions have hitherto shown less interest in these than their foreign counterparts, particularly in the United States, and in spite of some objections which can be raised to expanding fringe welfare, especially against benefits which supplement State provisions, it is suggested that there is a great opportunity for the Unions to adapt their practices and objectives The objections must be taken into account, but workers in general are highly conscious of a need for more security and higher standards of welfare

Next, the special situation and needs of women workers require attention, and here the Trades Union Congress is already taking a positive lead Again the provision of friendly benefits requires further study, and the Broadsheet makes several imaginative suggestions here To many workers a Union could offer a valuable service, for example, if it undertook to keep in touch with them after retirement, arranging home visits and providing social and holiday facilities This sphere of great and manifest need among old people has been comparatively neglected in contemporary society, the Broadsheet points to an opportunity which the Unions might well develop even if only among their own members

There are also opportunities for leisure work among the younger age groups, though these are perhaps overshadowed by the possibilities for Union provision in the field of education This is a subject in itself to which many people already consider the Trade Unions should give high priority, and devote fresh and original thought There is the question of special training for lay officers of the Union to equip them for the efficient execution of their duties In particular, the training of shop stewards is an issue of major importance and urgency, to which the Trades Union Congress and the British Employers' Federation have lately been paying close attention Plans for a joint campaign to stimulate co-operation between employers, Trade Unions and educational institutions to provide more courses for which stewards might be released from work with pay were announced in April

No less important is the larger question of education for the ordinary members as well as for the full-time officers It should be obvious that unless Union members are well informed regarding both the changing industrial and economic environments, positive attitudes and co-operation in planning and wage regulation, technological innovation, the abandonment of restrictive practices and so on, cannot reasonably be expected from them Such factors will be seen simply as a threat to their security and the underlying economic imperatives and the ultimate benefits to Union members will not be understood While it is now essential for the Unions to seek to protect their members in a constructive manner and without blindly opposing change, they must recognize that the only security of employment lies in the efficiency and competitiveness of British industry

Nor are these the only opportunities that confront the Unions They have responsibilities to minorities which are often overlooked, and at the national level there are economic objectives which can only be achieved with the co-operation of the Unions They could provide the institutional links between those who plan the policy and those who must ultimately work the policies, and harness the goodwill of the working community behind the objectives laid down as being in the national interest To do this they need not abandon any principles, though it would mean a change in the traditional conception of the role of Trade Unions and willingness to help promote, even to take the initiative in promoting, efficiency and economic growth

If the Unions are to do this, however, it is incumbent on the other parties, and especially the Government and the employers, to help by their own actions and examples in creating the conditions within which Trade Union reform can be facilitated Many people within the Trade Union movement recognize that changes in its structure and organization are desirable. It is important to strengthen their hand, to confront the movement with the fact that the reasons for change are related to its own future purpose and well-being and so to rally behind the reformers the large body of Trade Union members who are moderate and sensible but at present are sensitive to criticism, on the defensive and uncertain of what to do

Much of the analysis of the Broadsheet is supported by a section on the Trade Unions in a recent book, *Britain 1984 Unilever's Forecast*, in which Mr R Brech attempts to forecast the economic shape of the next twenty years He predicts a break-down of the big Unions into a number of small ones and the loss by the movement of much of the industrial and political power it has acquired over the past two generations Moreover, it is likely to be increasingly affected by the professionalization of the labour force and the growth of strong professional societies, covering engineers, scientists, mathematicians, statisticians and others While these may start as purely professional bodies they will increasingly take on protective functions

If Mr Brech's forecast is even approximately correct, it provides sufficient reason for the scientist and other professional workers to take a closer interest in the Trade Union movement in general Apart from the possibility just indicated of influencing that movement from within for the public good, the professional worker may sometimes be able to further that climate of opinion which the Broadsheet desiderates as essential for strengthening the progressive element within the Unions Nevertheless, the overriding reason for concern with the future of the Unions lies with the ordinary citizen

MASERS AND LASERS

Masers and Lasers

Molecular Amplification and Oscillation by Stimulated Emission By Gordon Troup Second edition (Methuen's Monographs on Physical Subjects) Pp viii+192 (London: Methuen and Co, Ltd ; New York John Wiley and Sons, Inc, 1963) 18s net

THE investigation of the interaction of radiation and matter received a significant stimulus about ten years ago with the practical demonstration of the first maser, a device which relies on the amplification of radiation by the process of stimulated emission. For some years the use of the maser principle was confined to the microwave region of the spectrum, where it has an important application in an amplifier with a very low noise temperature Recently the optical maser, or laser, has provided a source of radiation in the infra-red and visible regions

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with a degree of spectral purity, spatial coherence and power hitherto unachievable Apart from its numerous applications which are now being explored, the laser shows promise of giving a source of high power and narrow frequency range in the sub-millimetre or far infra-red where no such source at present exists

The first edition of Dr Troup's book was published in 1959 under the title *Masers*, and dealt exclusively with the microwave device The author was faced with the difficulty of writing on a rapidly developing subject, and with the necessity of keeping his treatment of the subject brief enough to fall within the compass of one of Messrs Methuen's well-established series of monographs The second edition, *Masers and Lasers*, contains essentially all the material of the first edition, in slightly revised and corrected form, together with additions describing the laser and its development up to January 1962 Apart from one chapter, which deals with principles common to all regions of the electromagnetic spectrum, only 16 pages are concerned with lasers

The theory of stimulated emission and related effects is approached both thermodynamically, following Einstein, and through quantum mechanics, maser action requires an inversion of population between two energy-levels, and the feasibility of producing such an inversion is later discussed The rest of the book is concerned mainly with a review of experimental methods and progress

As a treatment of the microwave maser, the book forms a most useful summary, which has benefited from the revision Although it was clearly the author's intention to cover both the microwave and optical regions of the spectrum he has confined himself largely to adding material to the first edition and has provided a work which is not so balanced a treatment as the title suggests Relaxation processes, for example, are discussed only in terms of crystalline solids, and there is no mention of equivalent effects in the gas discharge laser The only example of the relative magnitude of line broadening processes is, as in the first edition, the microwave transition in the ammonia molecule There are a number of other cases in which the original material has been retained unchanged in spite of the necessity for a broader treatment to include points relevant to lasers

The monograph is one of a series widely used by students who on meeting the subject for the first time might not detect some of the erroneous or misleading points which appear in this edition Among these are the use of the term "*Q*-factor *per unit length* of waveguide", an energy-level diagram of ruby, without further qualification, which is in fact only that of ruby at room temperature containing 0 05 per cent molar of chromium oxide, and the statement that the diameter of the discharge tube of the first helium–neon laser was 17 cm, instead of 17 mm There are also a few cases of confusing notation and inconsistencies which have arisen as a result of the revision of the first edition

The book can be thoroughly recommended as an introduction to maser principles and as a review of microwave achievements in this field The prospective reader should not, however, expect an equally full or lucid treatment of lasers J H Sanders

THE STATE OF PHYSICS

Physics in the Sixties

Edited by Prof S K Runcorn Pp vii + 112 (Edinburgh and London Oliver and Boyd, Ltd , 1963) 21*s*

To mark the completion of the new physics block at King's College, Newcastle upon Tyne, opened by Prof P M S Blackett, a series of lectures on aspects of physics was given Prof Blackett's speech touched on the development of physics in British universities

"Consider a moderately large physics department with an average of 100 undergraduates taking honours physics in each of three years, giving an undergraduate department of 300 Taking the present staff/student ratio as 1 . 7 or 8, as seems accepted as reasonable, there will be about 40 staff in the department Accepting further that 1 in 5 of the staff is a professor, there will be 8 professors. What should be the relation of these professors to each other and what part should they play in the three main functions of the staff—undergraduate teaching, postgraduate teaching and direction of research ?

"My own preference at present is on one hand for a completely unitary undergraduate teaching for a single unspecialized B Sc degree in which every member of the staff, including professors, takes part in the teaching And, on the other hand, for a number of research units with the maximum autonomy at postgraduate level In general, I think that every professor, if he wants to, should direct such a unit in our imaginary department there will then be about eight such independent research units with an average total of five academic staff each "

Each of the other chapters in the little book is the script of a lecture intended to be understood by the layman with some scientific knowledge or by scientists working in other fields The book should appeal to good sixth-form students who wish to obtain a background to their scholarship work in physics Prof L Rosenfeld, in a beautifully written chapter, traces the development of matter-force relationships from the days of the mythical Hermann Stoffkraft, a scientist less up to date, but as real as the mathematician N Bourbaki ! Sir John Cockcroft discourses on controlled thermonuclear fission, and gives warning of the tremendous difficulties, not realized in a premature over-optimism, which attend research work on this matter

Prof F Hoyle has a lecture on the origin of the solar system, in terms of recent work on nucleosynthesis, the relative abundance of a group of elements and modern knowledge of the origin of deuterons A D Garland writes on research on the Earth's interior, known as the 'Upper Mantle Project', C J Gorter gives an excellent short history of superconductivity and some possible future applications of low-temperature physics, and Prof R E Peierls rounds off the series with an essay on the state of physics, in which he deals, in particular, with the fundamental particles, the number of which increases in a manner which challenges the use of the word fundamental

The book, a readable and exciting little work, has been edited by S K Runcorn, professor and director of the Department of Physics, University of Newcastle upon Tyne W L Sumner

ELECTRONS AND ENGINEERS

An Introduction to Advanced Electrical Engineering

By C Jones (The Higher Technical Series) Pp x + 452 (London The English Universities Press, Ltd , 1962) 35*s* net

The number of books providing a link between elementary text-books on electrical engineering and advanced books on specialist subjects is somewhat limited, and this book is to be welcomed as an addition at a very modest price to the range available No claim is made for originality of material and no references are given to original papers There is, however, a list of books from which, it is to be presumed, most of the material for the present work was drawn

The book is mainly mathematical in its approach, and the standard is claimed to be that of a final-year for a first-degree course A student who had mastered its contents would compare favourably with the average student at the time of graduation The book can be safely recommended for the advanced student and is also likely to be of value to many lecturers for reference purposes The absence of numerical calculations and problems is a disadvantage which the author hopes to remove by the issue of a companion volume

The first chapter contains a useful summary of the mathematics required extending from elementary trigonometry to differential equations, Laplace transforms, conformal transformations and matrices It is too brief for any purpose other than to serve as a reminder of work learnt elsewhere Electrostatics, magnetism and electromagnetism occupy about one quarter of the book, and there follow separate chapters on electron ballistics, circuit theory, transients, symmetrical components, measurements and control systems The last chapter occupies more space than the three chapters given to electrodynamics and includes a useful section on magnetic amplifiers

The material is thus somewhat selective Machines are only mentioned incidentally in the treatment of servomechanisms Transformers are treated as a circuit element, and power transmission and distribution are treated as problems for circuit theory and symmetrical components Measurements are allotted only 40 pages, while valves are treated as a component for servo-mechanisms Transistors and rectifiers are not considered and although contact E M F is discussed there appears to be no reference to Hall, Seebeck, Peltier and Thomson effects

Selection is, however, essential in order to keep the size of the book within reasonable limits, and it might well be considered better to omit the chapter on "Measurements" altogether, since it is too short to do justice to the subject, in order to amplify the other sections A more serious criticism of the book is that the fundamental starting points of the subjects are too heavily condensed, sometimes to the point of inaccuracy For example, in paragraph (2) of Chapter 2 it is stated that a charge q produces an influence in space which can be observed experimentally This is not true Experimental observation can only be made of the reactions between charges Electric intensity is a mathematical conception which is convenient, but is not subject to observation The use of a unit charge for exploring the field is also unsound There is a similar confusion between experimental observation and mathematical conception in discussing magnetic fields The student would be well advised to obtain his ideas about the experimental basis of his subject from other books Maxwell is unbeatable in this respect, but for those who cannot bring themselves to look back earlier than this century, Jeans is to be recommended

One further omission must be referred to and that is the lack of a list of symbols or of adequate definitions of symbols Thus the symbol Z is introduced on p 188 for impedance without any definition Impedance is such a fundamental concept for circuit theory that this omission is difficult to understand

Once past the start, however the author's treatment is entirely sound and some of his mathematical solutions have an elegance which is not always found elsewhere

A H M Arnold

SKIN FRICTION AND HEAT TRANSFER FOR ROTARY FLOW

Hydrodynamic Resistance and the Heat Loss of Rotating Solids

By L A Dorfman Translated by N Kemmer Pp ix + 244 (Edinburgh and London Oliver and Boyd, Ltd , 1963) 63*s*.

THE engineer or scientist concerned with fluid mechanics and its applications is on the whole well served by existing text-books and monographs if his problems are those in which the motion of any solid boundaries can be regarded as rectilinear and the flow does not contain a major rotary component about an axis. However, for problems involving rotary velocity components and where the effects of viscosity are important the available books have generally provided little more guidance than a few references to scattered articles and research reports This is surprising, for these problems are not always particularly difficult and they can be of great practical importance They arise, for example, at various stages in the design and performance estimation of compressors and turbines, in the flow over propellers, in the cooling of electric motors and in the flight of rotating bodies To the theoretician they offer a rich field in which to find those rare gold nuggets of fluid dynamics—exact solutions of the Navier–Stokes equations

It is, therefore, fortunate and timely that Dr Dorfman has prepared this monograph on such topics (stimulated it seems by Prof Loitsyanskii), and this English translation by Prof Kemmer is particularly welcome

The book begins with a presentation without proofs of the basic Navier–Stokes equations and equation of energy for axially-symmetric flow of an incompressible, viscous fluid in laminar and turbulent motion The discussion continues with exact and approximate solutions for the laminar flow over an infinite rotating disk and includes the effects of a flow normal to the surface of the disk, of suction over the disk and some non-steady problems such as arise when the rotation of the disk is suddenly started or stopped A brief chapter follows on transition from laminar to turbulent flow on the rotating disk, this is based on the work of Gregory, Stuart and Walker, but the discussion of the theory is too scanty to be very helpful to the reader unfamiliar with this work. The disk with turbulent flow in its boundary layer, including the effects of surface roughness, is next considered, and this is followed by a discussion of the heat transfer at the disk surface with both laminar and turbulent boundary layers It is with these topics that the author's own research has been largely concerned Then follows a long chapter dealing with various problems of the flow around a disk rotating in a confined space, and it includes such topics as the effects of a parallel stationary disk, the effects of a cylindrical casing, the influence of roughness, leak effects and the flow between two rotating disks For such problems, the theories are almost always approximate (always so with turbulent boundary layers) and the comparisons with experiment that are presented are very welcome The following chapter is concerned with the flow and temperature distribution between rotating coaxial cylinders with the fluid both compressible and incompressible, and here in addition to the classical work of Taylor we find the results of some Russian work The final chapter deals with a rotating body of revolution in axial flow with particular reference to a rotating sphere

It will be seen that the author has confined himself to the basic topics that can be readily culled from existing sources and he has not attempted to make the book exhaustive It is largely but not entirely concerned with incompressible flow, and the rotating bodies considered are always simple such as disks, cylinders and solids of revolution This has given the author the opportunity to assemble his material in a logical sequence and the development is sensible and pleasing He has primarily aimed at presenting the essentials of the theories involved and he has not unduly stressed points of mathematical nicety and rigour The physical assumptions are generally clearly stated and he has presented comparisons of theory with experiment wherever the experimental data are available Any graduate who has already had a full undergraduate course in fluid mechanics or aerodynamics should have an adequate background for studying this monograph

Inevitably in a book of this kind with its emphasis on what the engineer calls 'working methods', the theories for turbulent boundary layers are semi-empirical and based on the simplest mixing-length concepts, however, a criticism which can be made is that these concepts are presented with no attempt at a critical assessment of their validity There are very few misprints and no obvious errors apart from a brief confused and confusing reference to the way in which the 'china-clay' method for observing transition works (p 43) A welcome feature of the book is the fact that the author has made use of and acknowledged the available important literature on his subject irrespective of the country of origin The printing, lay-out and diagrams are all very good, and Prof Kemmer is to be congratulated on the clarity and readability of the text, which betrays no evidence of being a translation This is a book that the engineer, scientist or research worker concerned with fluid mechanics and heat transfer will be glad to have ready to hand

A D YOUNG

WAVES AND SHOCKS IN PLASMAS

Waves in Anisotropic Plasmas

By Prof William P Allis, Dr Solomon J. Buchsbaum, and Prof Abraham Bers Pp xi + 280 (Cambridge, Mass The Massachusetts Institute of Technology Press, 1963) 57*s*

Magnetohydrodynamic Shock Waves

By Dr J Edward Anderson Pp xiv + 226 (Cambridge, Mass. The Massachusetts Institute of Technology Press, 1963) 6 50 dollars, 49*s*

THESE two books are respectively the seventeenth and sixteenth publications in the series of the Massachusetts Institute of Technology Press Research Monographs In each the many different types of wave possible within the framework of the analysis are investigated in detail, so that in one we find a two-dimensional diagram divided into 13 regions and, in the other, reference is made to a division into 52 regions of a five-dimensional space Both books are liberally illustrated with helpful diagrams and make excellent reference volumes

Waves in Anisotropic Plasmas is divided into two parts Part 1 on "Free Waves", by W. P Allis and S J Buchsbaum, and Part 2 on "Energy Power Theorems and Guided Waves", by A Bers Wave propagation is assumed to be of the form $\exp i\,(\omega t - \mathbf{k}\ \mathbf{r})$ in the presence of a static magnetic field, and linear equations are used throughout with the collision terms neglected soon after their introduction For much of the analysis the plasma is assumed to be temperate (thermal pressure gradients are neglected) so that waves of acoustic type are excluded, acoustic waves are, however, treated for the case of a scalar pressure in a separate chapter The most distinctive feature of Part 1 lies in the use made of (α^2, β^2) diagrams to discuss the different forms of propagation In these diagrams α is the ratio of the plasma frequency to the applied frequency, ω, and β is the ratio of the cyclotron frequency to ω, so that there are two values of β, one for the ions and one for the electrons If the product of these two values of β is now plotted against α^2, a diagram results in which various limiting conditions of resonance (where the phase velocity of the waves tends to zero) and cut-off (where the phase velocity of the waves tends to infinity) can be plotted as single distinct curves Thus the cyclotron resonances occur at two constant values of the ordinate and the plasma cut-off at a constant value of the abscissa ($\alpha^2 = 1$), while the cyclotron cut-offs and the plasma resonance occur as two smooth curves The set of limiting curves obtained by this means divide the diagram into 13 regions throughout each of which the propagation characteristics are broadly the same, and these the authors illustrate by means of small phase velocity diagrams inserted in each of the regions For further clarity coloured lines are used in some of these sub-diagrams A tribute to the informative nature of these diagrams is that Spitzer uses very similar illustrations (which he refers to as Allis diagrams) in the latest edition of his well-known *Physics of Fully Ionized Gases*

Part 2 deals with the propagation of power and gives a detailed field analysis of plasma wave-guides for the special cases of a static magnetic field imposed along and normal to the direction of the wave-guide The relations needed to transform the equations from the longitudinally magnetized to the transversely magnetized wave-guide are conveniently given in tabular form, and the rather complicated equations themselves are given by means of clearly laid out matrices This is in fact typical of the whole book in that emphasis is everywhere laid on the physics of the situation, and cumbersome algebra is avoided so far as possible

In *Magnetohydrodynamic Shock Waves* the author restricts himself to plane shocks which are collision dominated such that Hall currents are negligible and that the Navier–Stokes approximations are valid, relativistic effects are also neglected An investigation of possible discontinuities consistent with the relevant conservation laws (that is, the modified Rankine–Hugoniot relations) leads quickly to a set of equations connecting upstream and downstream parameters These are very effectively illustrated by means of three-dimensional plots for the three parameters, temperature, component of magnetic field parallel to the shock. and specific volume which by continuity is directly related to flow velocity In terms of these variables the equations are reduced to three in number which can be represented by surfaces of constant normal momentum flux, constant transverse momentum flux and constant stagnation enthalpy. two of these surfaces intersect in a curve which cuts the third surface in up to four points, any two of which (giving six possibilities in all) could represent conditions on either side of a shock These three-dimensional intersections can surprisingly be visualized quite easily with the help of the diagrams, perhaps this is just as well, because the reader has to accomplish a good deal of visualization before coming to the end of the book, and it comes as a relief at one point, much later in the book, to read "Because of the fact that this problem is five-dimensional, and hence impossible to visualize *in toto*, the discussion of slow shocks will be dropped at this point "

The principal aim of this monograph is at discovering whether magnetohydrodynamic shocks can be expected to occur in Nature, and this possibility is explored in detail by two methods The first of these, following Russian workers, is to investigate the stability of shocks with respect to small disturbances In this section a small amplitude wave is supposed to disturb the shock, and the resulting reflected and transmitted waves are investigated with regard to their energy content Boundary conditions are available at the shock for determining the strengths of the diverging waves, but for some types of shock they are insufficient in number so that infinitely many solutions to the small-wave problem are possible The interesting conclusion is drawn that these shocks are non-evolutionary, and if they were to exist at any instant they would rapidly break up by spontaneous emission of waves Switch-on and switch-off shocks (where the magnetic field component parallel to the shock is zero before and behind the shock respectively) do not belong to this category but are nevertheless found to be limiting cases where the energy carried away by the diverging waves tends to infinity, so that it is concluded that these shocks are unstable to certain wave disturbances This conclusion will no doubt lead to much controversy as indicated in the short appendix by W H Heiser, which describes an apparently successful attempt to observe switch-on shocks in the laboratory, although this of itself does not contradict Dr Anderson's conclusion since there is no reason to suppose that the shock would meet the required destabilizing disturbance during its short life-time in a shock tube In later chapters of the book, the shock layer is investigated directly from the differential equations of flow through the shock, and again the treatment is of great interest, although rather involved, with results that support those of the stability investigations Apart from the existence investigations which occupy much of this part of the book, close attention is given to the order of magnitude of the terms in the differential equations and it is shown that some terms, such as the electron pressure terms, which have been neglected by previous authors, can in fact be important

Dr Anderson keeps the physics of the problem well to the fore, and for the most part the mathematics is clearly presented E G BROADBENT

NATURAL FIBRES

Vegetable Fibres

Botany, Cultivation and Utilization By R H Kirby (World Crops Books) Pp xvi+464+54 plates (London Leonard Hill (Books) Ltd , New York Interscience Publishers, Inc , 1963) 84*s*

DESPITE the fact that vegetable fibres have been, and undoubtedly will continue to be, of the utmost importance to mankind, few comprehensive general reference books have been published on their botany, cultivation, and production Apart from Matthews (Mauersberger), sixth edition, 1956, which deals, necessarily briefly, with this subject as one section of textile fibres, in general, one has either had to search the literature, or else to consult more narrow specialist texts (such as Haarer, 1952) or older publications (for example, Goulding, 1919)

The appearance of Dr Kirby's excellent book comes at a time when large masses of the world's population are arriving at self-government and must realize, inevitably, the importance of husbanding their natural resources in the production of wealth Among these, vegetable fibres of all types can play a valuable part Not only does this book provide an easy means of acquiring technical information its recognition of the importance of economic factors is also most commendable Apart, therefore, from students of textiles and agriculture, etc , it will be consulted by textile interests at executive policy-level and also by those entrusted by Governments with the economic development of this aspect of agriculture

The subject-matter is well classified into botanical families and deals mainly with plant cultivation and fibre production, and to a lesser extent with botanical matters In addition to flax, hemp, jute, *Hibiscus* sp , ramie, sunn and Mauritius hemp, sisal, New Zealand flax, manilla, coir, etc , a large number of lesser-known fibres are discussed in detail The chapter on the mechanical production of stem fibres is a stimulating introduction to modern practices which may have to play a vital part in the future The very great difficulties of handling, expeditiously and economically, large masses of fibre-bearing material, in which the valuable fibre content may only be small, the difficulties of imitating, mechanically, actions which appear to be so easily, albeit laboriously, done by hand, and the delicate economic balance between mechanization on one hand, and peasant production on the other, are all realistically assessed It is obvious that the author, who has visited fibre-producing areas all over the world, writes very much from first-hand knowledge

It is to be expected, however, that a publication of this type, in which one author deals with so large a field, is unlikely to be free from criticism, more especially in those sections where information is probably second hand The comparatively scant attention paid to 'utilization' suggests that this term might have been inferred rather than meriting inclusion in the title Why, for example, is jute, second only in bulk importance to cotton, and also flax, given only a few lines compared with two pages for ramie, a comparatively unimportant fibre ?

It is unfortunate also that clear line-drawings could not have been included of the bast fibre lattice work and its positioning within the stem and also drawings or clear photomicrographs of the ultimates within the fibre bundles of most of the fibres instead of only one or two In general, also, the many photographs of decorticating, ribboning and other machinery are not good, except possibly in a pictorial sense, for unlike line drawings they do not clearly illustrate how the machines work In a reference book, clear tabular presentation of statistics is to be expected It is to be regretted that many useful data are scattered, not very systematically, throughout the text, a drawback which will not appeal to the business executive It would have been helpful if the admirable costing flow-sheets, presented on p 189 for sunn hemp and on p 214 for kenaf, could have been repeated for some other fibres

In Chapter 1, and elsewhere, some erroneous generalizations exist Thus, tensile strength is not necessarily the most common test to which fibres are submitted (p 8), not all fibres are affected by moisture in their physical properties (p 8), the cementing matter holding the ultimates together in the fibre bundle is by no means always pectinous (p 11), nor are all fibres sprayed with lubricants or oil during processing (p 13) It is not necessarily true, in all aspects of vegetable fibre usage, to claim that the higher the cellulose content the more valuable the fibre (p 10) Retting is not the most important operation for all fibres (p 21), many are won from their natural setting by mechanical means only During the Second World War, so-called natural or mechanically decorticated-unretted flax was used without de-gumming by the dry-spinning industry, which did not find it stronger or superior to retted fibre (p 35) What are 'strong' chemicals (p 10) ?

Greater reference might also have been expected to be made to the considerable experience gained and research done by the U K Flax Advisory Committee in all phases of flax cultivation, retting and production etc , during the Second World War, some of which was published in *Quality in Flax* by A J Turner (1954)

Apart from these minor criticisms, however, this book is of undoubted merit well printed, well-bound, and carefully indexed, with an extensive bibliography, it is assured of wide demand H L PARSONS

A HAND-BOOK OF THE LARGER FUNGI

Collins Guide to Mushrooms and Toadstools

By Morten Lange and F Bayard Hora Pp 257 (96 plates) (London Wm Collins, Sons and Co , Ltd 1963) 30*s*

JAKOB LANGE'S magnificent coloured plates of toadstools in his *Flora Agaricina Danica* have long been among those most valued by specialists in European Agaricales It was, therefore, a happy thought on the part of his son to make a selection of them available on a reduced scale, two-thirds the size of the originals, to a much wider audience, in the form of a cheap popular hand-book dealing with the larger fungi A few plates of the more conspicuous Ascomycetes, Gasteromycetes, Boletes and Aphyllophorales have been supplied by other hands, but it must be admitted that some of those depicting polypores are much less successful than those of agarics by J Lange Sometimes, even with the latter and with Boletes the colours as reproduced scarcely bear out the brief descriptions printed opposite each plate Oddly enough, the figure of *Laccaria proxima*, duly shown with elliptical spores in the Flora, is here reproduced as *L laccata* and said to have globose spores In the Flora, essential microscopic details, especially of spores and cystidia, were indicated on the plates by line-drawings beside the coloured illustrations One wonders why these have been omitted from the present work The authors must know that a considerable proportion of the species depicted cannot be identified with certainty by macroscopic characters alone, even the generic key provided cannot be worked without reference to some microscopic details To overcome this difficulty spore dimensions and other microscopic features are quoted briefly after the entry of each specific epithet in the index. Some characteristic basidiospore shapes are drawn in Figs 11 and 12 on p 230, while some rather crude sketches of cystidia appear in Fig 9 on p 228 This appears to be a valiant effort by the English editor to overcome a radical defect of the original Danish edition

Because there was no such cheap and reliable hand-book covering the same field available in English, one must be grateful to the authors and publishers for their enterprise The colour reproduction is reasonably faithful on the whole, and such blemishes as do occur have probably to be accepted as inevitable if so many coloured plates are to be offered so cheaply in these days of expensive book production Anyone tempted to use the book for identification of species in serious ecological or floristic investigations should remember, however, that it figures scarcely a quarter of the species of Basidiomycetes known to occur in the British Isles and that many of the omitted species are superficially not so very different from some of those illustrated Identifications to be placed on record should, therefore, be checked by reference to the more critical works listed in the bibliography or be verified by a specialist while the collections are in a fresh condition

R W G Dennis

LEECHES

Leeches (Hirudinea)

Their Structure, Physiology, Ecology and Embryology
By K H Mann With an appendix on the Systematics of Marine Leeches by Prof E W Knight-Jones (International Series of Monographs on Pure and Applied Biology Division Zoology, Vol 11) Pp viii+201 (London and New York Pergamon Press, 1962) 45s ; 7 50 dollars

DR K H MANN is to be congratulated on this excellent book The leeches or Hirudinea are not a well-known group of animals indeed, biology students normally come out of university knowing very little about them This regrettable state of affairs can now be remedied for here is presented, for the first time, a comprehensive survey of this small, though important, order of the phylum Annelida designed for use by students, teachers and research workers

The medicinal leech *Hirudo medicinalis*, is taken as a type and, with meaningful text and a selection of first-class diagrams, its morphology and histology explained with exceptional clarity Then the latest knowledge of leeches is analysed to delight the physiologist, behaviourist, ecologist, and systematist alike A survey of the group introduces the reader to the wide range of form encompassed in the Hirudinea, and chapters on nutrition, excretion and water balance, circulation and respiration; muscle, nerve and locomotion; sense organs and behaviour, reproduction and development, and ecology, continue a delightful zoological story

Finally, there are two appendixes on the systematics of the group The first of these, by K H Mann, on fresh-water and terrestrial leeches includes diagnoses from sub-orders to families and illustrated keys to species from Central Europe, Britain and North America The second, by E W Knight-Jones, provides a key to marine genera of the world, diagnoses of North Atlantic genera and species, with a host list

Extensive use will be made of this gem of a book by all zoologists interested in the invertebrates It is especially recommended to teachers as a source for new adventures by their pupils, for "leeches are fascinating animals, full of strange zoological paradoxes" Norman Tebble

TAXONOMY OF TIMBER BEETLE LARVÆ

A Monograph of the Immature Stages of Australasian Timber Beetles (Cerambycidae)

By E A J Duffy Pp vii+235+11 plates (London Printed by order of the Trustees of the British Museum (Natural History), 1963) 105s

TWO major difficulties beset workers in the field of larval taxonomy, especially those who, like the author of *A Monograph of the Immature Stages of Australasian Timber Beetles*, deal with large groups on a regional basis. First, museum collections of identified larvæ are usually poor or non-existent, and the worker must depend heavily on the assistance of resident entomologists in those countries which he himself often cannot visit Secondly, few such investigations are likely to be complete, and taxa must be defined on the basis of less-extensive material than is available of the adult stage The industry of Mr Duffy—and his ability to elicit co-operation—are made evident by the excellent coverage of this first review of the immature stages of the Australasian Cerambycidae.

All sub-families represented in the region are characterized in terms of both larval and pupal structures Descriptions of, and keys to, the late-stage larvæ of 100 species are provided, these are representative of 77 genera contained in 43 tribes, more than half the tribes known to occur in the region The pupæ of 44 species are similarly treated Records of host plant are given for some 250 species There are also excellent summaries of bionomic data (including much previously unpublished), and of the control methods used against species of known economic significance in forestry The stated aim of the monograph is to provide a means of identifying the immature stages of these insects The lucid keys and descriptions, supplemented by very good drawings and photographs, should fulfil this purpose admirably

In his earlier monographs of the immature Cerambycidae of the Palæarctic and Ethiopian Regions, Duffy was able to select "single fundamental characters" that defined the higher taxa, and to arrange keys that placed these taxa in "the generally-accepted sequence of adult classification". In his review of the Neotropical fauna, and in the present work, neither of these aims was realized fully Some taxa could be defined only in terms of different combinations of the same or similar characters Larvæ often exhibited such morphological heterogeneity that portions of the same taxon had to be brought out in remote parts of the key Such results inevitably place in question the validity of phylogenies deduced from morphological characters of the adult stage alone It is interesting to note that, due to divergences of larval characters, Duffy found it appropriate to elevate to sub-family rank three taxa that are traditionally given tribal status

The *Monograph* will prove of very great practical value to forest entomologists in Australasia, and represents a notable addition to Duffy's series of publications in this field His next volume, on the Cerambycid fauna of the oriental region, will be awaited with interest

The printing, format and binding are of that most pleasing quality one has come to associate with the British Museum (Natural History) monograph series

P. B Carne

The Annual of the British School at Athens

No 57, 1962 Pp xii+242+53 plates (London The British School at Athens, 1963) 105s net

THE 1962 *Annual of the British School at Athens* is dedicated to the great British epigraphist, Marcus Neibuhr Tod, thus it is that of twelve articles on Greek archæology five are concerned with inscriptions The most substantial, by L H Jeffery, is on "The inscribed gravestones of archaic Attica" Miss Jeffery provides a detailed catalogue of these inscriptions, establishes between six and ten masons operating in the sixth century B C and briefly puts together what is known of the named sculptors of this period Of the other epigraphical contributions, Daphne Hereward publishes ten Greek inscriptions from the Khersonese and P M Fraser "Two Dedications from Cyrenaica", the first showing religious links between Cyrenaica and Crete, the second between Cyrenaica and Samothrace The other two articles use inscriptions to demonstrate historical theses, that by D M Lewis to show that the Island of Keos possessed a federal constitution in the first part of the fourth century B C, that by A M Woodward to show connexions between the famous oracle of Ammon in the Libyan desert and the establishment of a sanctuary of Ammon at Athens, also in the fourth century B C

There are two site publications Jones, Sackett and Graham publish their excavation of the Classical house lying near the Aigaleos-Parnes wall, north-west of Athens and used for a very short period, about 420–410 B C The article contains much meticulous information on Classical Greek houses, and relates this house to the standard publications of those at Olynthus and Athens The other site is the Byzantine basilica church at Knossos in Crete, built early in the sixth century A D on top of an earlier Christian cemetery extending back perhaps as far as 200 A D This excellent publication of Frend and Johnston (with fine photographs of the mosaics) throws light on a little-known period of Cretan history and relates the Knossos basilica church to others in the Island

Of the other contributions, D M Metcalf gives a study of Byzantine history in the Aegean from coin hoards of Heraclius I (610-641), J Boardman publishes miscellaneous finds of the seventh and sixth centuries B C from Knossos, and D M Bailey the Greek and Punic Lamps from Tharros in Sardinia The only prehistoric articles this year are by John Chadwick with "Further Linear B tablets from Knossos" and by Hope Simpson and Lazenby with another of their topographical studies, this time of various islands in the Dodecanese PETER WARREN

Neutron Diffraction

By G E Bacon Second edition. (Monographs on the Physics and Chemistry of Materials) Pp xii+426 (Oxford· Clarendon Press, London Oxford University Press, 1962) 55s net

THE use in research in solid-state physics of the powerful method of neutron diffraction dates from about 1950, when, with the advent of nuclear reactors, intense beams of neutrons became available Neutron diffraction complements and supplements X-ray diffraction, and has particular applications in the study of magnetic and molecular structures and the lattice dynamics of crystals

Prof Bacon ranks as a pioneer and as a leading authority in the construction and use of neutron diffraction apparatus When the first edition of his monograph appeared in 1955 it was the first comprehensive text-book on the subject The wealth of unique practical experience described in it and the important information that the use of the method had obtained or was capable of obtaining soon spurred others to make use of the technique, and it is therefore not surprising that the second edition quotes some 500 instead of 300 references to published work

The main structure of the first edition has not been altered. The monograph is still divided into two parts The first chapters—now with the addition of a chapter on inelastic scattering—deal as before with the physical principles of neutron diffraction, the experimental methods, and the determination of fundamental data for structural investigations The remaining nine of the sixteen chapters in the second edition discuss the increasing number of applications of neutron diffraction, in the determination of the atomic positions of light elements, the study of molecular structure, the investigation of magnetic materials, and the examination of diffraction by gases, liquids and amorphous solids The sections in the first part have been revised and brought up-to-date, and much of the second part has been re-written to conform with the great increase in application of neutron diffraction during the seven years between the two editions

The monograph is excellently produced, and the text and diagrams are easy to read It is the standard and authoritative text-book on the subject of neutron diffraction, and it is warmly recommended to the honours physics undergraduate and to the research worker who are interested in the atomic architecture of materials, whether physicist, chemist or metallurgist.

S WEINTROUB

Encyclopedia of Chemical Technology

Vol 1 A to Aluminium By Kirk-Othmer Pp xix+990 (London and New York John Wiley and Sons, Ltd, 1963, second edition, completely revised) 260s per volume for subscribers to the complete set of 18 volumes.

THE first edition of "Kirk-Othmer" in fifteen volumes published between 1947 and 1956 has won international acclaim for the authority of its information and amply fulfilled the original editors' intention of presenting the entire field of chemical technology for professional chemists and chemical engineers who wish to know about specialized practices and materials, particularly outside their own immediate experience The editorial board has now embarked on the publication of the second edition, which, they claim, is more than a mere revision of the first All the technological articles have been rewritten, some by the same, some by a different, author Each author is a specialist and each article has been reviewed by one or more other specialists

The first edition concentrated on presenting United States technology, the second is to contain contributions from other countries This is most welcome, particularly if it leads to the inclusion of European trade names and chemical specifications, the lack of which was a drawback to British readers seeking comparisons between American and European products

The new volume contains forty-six articles They range from "Abherents", a more sophisticated term for release agents used in moulding, casting and baking, through "Ablation", which is concerned with the re-entry problems of missiles, to "Aluminium", where mention is made of the new carbothermic and reduction-distillation processes now being studied in France and Canada The article on "Alkyd resins" reflects the changes in the meaning of the term since the first edition was written "Acetate and triacetate fibres" have been separated from "Rayon", and a useful, though not over-extensive, survey of the aerosol industry is included He who seeks information on industrial alcohol must now wait for "Ethanol" to appear

Changes in format are minor An index is not provided, but those readers fortunate enough to have access to and experience of the first edition should be able, by judicious comparison of the two editions, to overcome this drawback to a large extent

The first edition appeared over a ten-year period The editors propose a similar schedule for this enlarged edition W D MANIECE

MONKS WOOD EXPERIMENTAL STATION

By Dr. KENNETH MELLANBY, C.B.E.

Director

MONKS Wood Experimental Station of the Nature Conservancy was officially opened by Lord Hailsham, Minister for Science, on October 28 [see following article]. It is the first research station in England specially built by the Conservancy for scientific research.

The buildings cover some 25,000 sq. ft. They are mostly single-storey laboratories, but there are also a library, lecture room, canteen and hostel block. The tower, which, though only about 50 ft. high, dominates the rather flat Huntingdonshire countryside, is essentially a water tower (needed to ensure adequate pressure in the laboratories), the space below the tanks being used to produce fourteen study-bedrooms for postgraduate students working at the Station.

The architect, Mr. K. A. Poole, of Messrs Cowper, Poole and Partners, has endeavoured to design a building which is functional and yet fits into the traditional pattern of the area. The brickwork is similar in colour—a pale yellow—to that found in many eighteenth- and nineteenth-century buildings in Cambridgeshire and Huntingdonshire. The laboratories are constructed from a series of units, 11 ft. × 22 ft., which has allowed flexibility in design and economy of construction. Unlike many recent buildings, the rooms have their main windows at the narrow end, and the benches are all at right angles to the windows. Additional illumination at what would have been the dark end of the room is obtained by a clerestory window, made possible by the lower level of the roof of the central corridor. The services run in the corridor roof and in the vertical members between the 11-ft. units. Laboratories of one or two units are provided, and folding doors in the library/lecture room allow meeting-rooms of various sizes, up to one which can seat nearly three hundred, to be made. Although the standard of the building work and of the laboratory fittings is good, this has proved to be, for to-day, an economical building, costing not more than about £4 per square foot of floor space.

The Station houses three research sections. They are all engaged in work concerned with the Conservancy's function of controlling and preserving the flora and fauna of Britain, and in giving scientific advice on conservation. Monks Wood is called an "Experimental Station" because it will tend to concentrate on research in applied ecology rather than on the long-term fundamental research carried out in other Conservancy stations, and is thus intended to serve as a link between the Research and Conservation Branches. However, rigid demarcation is not always possible or desirable, and fundamental aspects of conservation will obviously be tackled where necessary.

The Station has some 70 acres of arable land attached which will be used for experiments. Already some miles of hedges have been or are being planted, and some plots for work on soil fauna have been laid down. There is a meteorological station which keeps records of the temperature, including that at several depths in the soil down to 4 ft., rainfall, etc., and a 'potential evapotranspirometer' fits into the series maintained by the Conservancy in many parts of Britain, to contribute to the study of the country's water balance.

Work will be done where most appropriate in all parts of Britain; but when possible travelling will be kept to a minimum. The Station is now responsible for the management of the National Nature Reserves of Monks Wood (387 acres of mixed woodland actually adjacent to the laboratories) and Woodwalton Fen (514 acres of fen and marshland, about three miles away as the crow flies). Not all experiments can be performed on National Nature Reserves, where conservation interests must be paramount; but the programme should help rather than hinder the preservation and diversity of the flora and fauna.

The Conservation Research Section is under Dr. E. Duffey, who was previously regional officer for East Anglia, and is thus very familiar with the practical work of the Conservancy. This Section hopes to do research into Nature reserve management, and to build up a store of ecological knowledge which will be available to organizations responsible for wildlife conservation. Field work at present consists of investigations of the invertebrate fauna of open-ground habitats, mainly grass heaths on sand which include many important Nature reserves; the management of chalk grassland to preserve floristic richness, and a study of fen problems, particularly aquatic fauna, at Woodwalton Fen.

Dr. J. D. Ovington is head of the Woodland Research Section. He was formerly at the Conservancy's Station at Grange-over-Sands, where he investigated woodland productivity, work he also developed in the United States. This Section carries out research on the productivity of the woodland ecosystem, and is responsible for giving advice to the Conservancy regional staff on the management of the woodlands in some 56 National Nature Reserves. They produce working plans for the reserves when this is necessary. Woodland is the natural plant cover over most of the land surface of Britain, so woodland research has an important part to play in all reserves, even in those where trees have to be discouraged.

The Toxic Chemicals and Wildlife Section is under Dr. N. W. Moore, formerly regional officer in South-west England and a lecturer in the University of Bristol. This Section was set up to examine the effects of pesticides

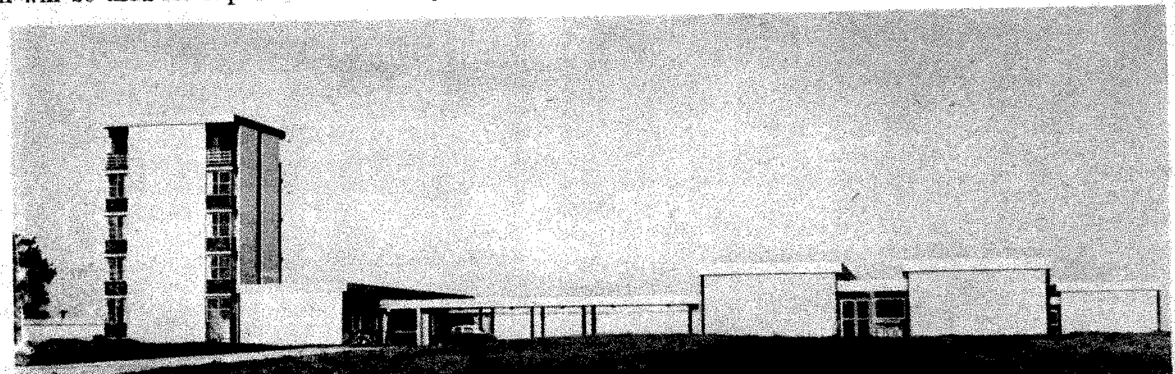

Fig. 1. Monks Wood Experimental Station, Huntingdonshire

on wildlife, and to advise governmental and other bodies on this subject. Its work has had the most publicity, owing to the widespread concern about the possible misuse of toxic substances. The Section, like the rest of the Station, is primarily interested in conservation, but particularly in the way new hazards (due to persistent and poisonous substances) may affect conservation. It is carrying out background investigations on wildlife habitats affected by pesticides, for example, examinations of the flora and fauna of hedges. Surveys of the distribution of persistent insecticidal residues in wild populations are in progress. These investigations are showing that residues of organic chlorine insecticides are widely distributed in terrestrial, freshwater and marine habitats, and that predators contain higher residues than other animals. Field experiments on the effects of pesticides on the flora and the invertebrate fauna have been started, particularly dealing with soil inhabiting species. The Section also includes an information service which acts for the Committee on Ecological Effects of Chemical Controls of the International Union for the Conservation of Nature and Natural Resources.

The Nature Conservancy has always believed that it has an important role in education, and that this must be exploited if conservation and the understanding of wildlife problems is to be successful. Monks Wood has been designed to play its part in education at several levels; in particular, it will be concerned with the postgraduate diploma course in conservation, run by University College, London. The residential accommodation was planned primarily to house these students, who will spend a substantial part of their year doing field work in the neighbourhood. A laboratory has been set aside for them and other visiting workers. Plans are well advanced for a residential course for some of those taking the diploma in technology in applied biology, and various other classes and seminars are under consideration. The recommendations of the Robbins Committee, that there should be close contacts between universities and research establishments, is welcomed by the Conservancy and the Station. Monks Wood will, I hope, make a contribution to many aspects of postgraduate and other training, and will thereby benefit from the inspiration which research can receive from teaching and from contact with students.

EFFECT OF TOXIC SUBSTANCES ON WILD LIFE*

By THE RIGHT HON. VISCOUNT HAILSHAM, Q.C.†

Lord President of the Council and Minister for Science

I FIND that most people are still unaware of what the Nature Conservancy is; rather vaguely they seem to associate it with the National Parks and seem to think that the Nature reserves we have established are primarily there for the purpose of recreation.

The Nature Conservancy is a scientific organization concerned with the scientific study of our environment—ecology, to use a more technical term. It can achieve its purpose only by acquiring and retaining the goodwill of all those who own, work in, enjoy, and otherwise use the countryside.

Monks Wood will be the centre for applied ecological research undertaken by the Conservancy. It will help to bridge the gap between the long-term fundamental work carried on at other establishments by the Conservancy and its practical application in land use problems. Monks Wood will also be used for the training of postgraduate students in ecological subjects. I have always attached great importance to close working contacts between Government research establishments and university departments. The staff and excellent facilities at Monks Wood enable it to provide systematic and properly supervised courses for postgraduates. Development of postgraduate studies along these lines is fully consistent with the Robbins Report.

Of the three scientific activities going on at Monks Wood—conservation research, woodlands research, and research into the effect of toxic chemicals on wild life—I shall refer to the third only, not necessarily because it is the most important, but because it is the most topical, and because there are things about it which the public should bear in mind.

The first is that this is a particular aspect of a general problem which concerns me as Minister for Science at several different points, and concerns several different Ministries in three or four different ways.

The second is that it tends to be a subject which arouses strong emotion, and strong emotion is bad for scientific judgement. The scientific evidence, which is voluminous, justifies almost any opinion other than extreme ones, and those who hold extreme positions on either side must get used to the view that they are not supported by the scientific evidence, although they may seek to express their opinions in scientific, or pseudoscientific, language.

The third is that, quite apart from the scientific evidence, the practical judgement in favour or against the use of toxic chemicals in agriculture must be one of balance and caution. I am not prepared to back scientific farming absolutely if it results in the widespread destruction of wild life, particularly if it threatens whole species. I am not prepared to favour the conservation of wild life absolutely against scientific farming.

There are also two particular facts to be borne in mind. The first is that people are apt to forget how much recent effects on the destruction of bird life were the result of the indiscriminate use of a particular group of chemicals, the dangers of which are now appreciated and which are now more effectively controlled. The second is that the defences mounted by the Government in that particular case are as much an example of the effectiveness of our defences—of which an important element is the Nature Conservancy—as of the dangers of the chemicals.

On one thing we can all agree and that is the importance of continuous research. This is the value of Monks Wood, and of various other stations and units of the Nature Conservancy, the Agricultural Research Council, the Medical Research Council, the Department of Scientific and Industrial Research and the Departments of Agriculture. It is not a question whether enough research is being done. The point is that the research will have to be continuous and ever changing precisely because the problem is a continuous and changing problem. This is why I have Prof. A. C. Frazer's Committee keeping research in this field under continuous review and advising me so that the Government can ensure that our research effort is meeting changing needs. The use of chemicals in agriculture is only a special case of a much wider question: the use by mankind in almost every field of his activity in this technological age of increasingly powerful substances the effects of which are sometimes unexpected, occasionally dramatic and often undesired.

This is a question which cannot fail to attract the attention of Government in a variety of different ways, and it is worth while admitting at the outset that no Government can claim to have discovered absolute answers to all the questions. Nor will it ever do so, because the nature of the dangers will continually change with the change in technology and science. What is wanted is not an absolute answer but a proper means of watching issues as they arise and a proper organization for ensuring that scientific opinion influences policy at a sufficiently early stage. By far the most dramatic and tragic recent case of this general question was the side-effects of thalidomide on the human fœtus; but it would not be difficult to find examples of many kinds in industrial medicine or public health or, for that matter, in nuclear physics.

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* Substance of an address delivered at the official opening of Monks Wood Experimental Station, Huntingdonshire, on October 28 (see *Nature*, 190, 488; 1961; and preceding article).

† Now the Right Hon. Mr. Quintin Hogg, Q.C.

There is, in fact, practically no way in which man has not altered his environment. It is one of the permanent and serious dangers of a scientific and technological society and one against which a scientific and technological society must learn to erect substantial defences. We cannot have the benefits of science and technology without running some of these risks; but in applying new knowledge we cannot do so responsibly unless we make some insurance against the risks. What we cannot hope to do, however, if we are to accept the benefits of a scientific and technological society—which I believe it is the overwhelming desire of the people of Britain and, indeed, of the whole modern world to do—is to guarantee that there will never be any unexpected or evil side-effects on wild life, human beings, and domestic animals and crops. In some cases these results will appear only after the greatest possible care has been taken, after they have been applied over a long period of time and over a wide population or considerable area.

This means, so far as wild life is concerned, the Nature Conservancy; so far as the interests of agriculture are concerned, the Agricultural Research Council; so far as human life is concerned, the Medical Research Council; and so far as industry is concerned, the Department of Scientific and Industrial Research; and finally, so far as the balance of public advantage is concerned, the Minister for Science must hold a watching brief to see that scientific opinion is continuously directed to the relevant questions, and that public opinion is constantly advised about the biological consequences of a technological age.

I wish to pay my own tribute to the staff of Monks Wood Experimental Station, who have been pushing ahead with the aid of a certain amount of improvization before the building was completed, and to the people and organizations who have shown their willingness to help. I must also thank, on behalf of the Nature Conservancy and my own behalf, the University of Cambridge, Lord Iveagh, Lord de Ramsey and other landowners who have generously provided facilities for research. A number of private firms have also helped with information and facilities. The work of Monks Wood, and indeed of many research establishments, depends for its success on good relations and close links with universities, landowners and industry.

NEW PERSPECTIVES IN BIOLOGY

By Prof. JOHN T. EDSALL
Biological Laboratories, Harvard University, Cambridge, Mass.

A NOTABLE symposium on new perspectives in biology was held at the Weizmann Institute of Science, Rehovoth, Israel, during June 10–17, to celebrate the inauguration of the Ullmann Institute of Life Sciences (Fig. 1). A distinguished group of biologists, biochemists and biophysicists, from Europe, from the United States, and from Israel, presented papers on the present state and future prospects of many fundamental problems in biology, and carried on informal discussions together for a week.

We may recall briefly the history of the Weizmann Institute. Its forerunner, the Daniel Sieff Research Institute, was dedicated in 1934, and formally opened by Dr. Chaim Weizmann, who played an active part in its development. Expansion of the Sieff Institute was conceived in 1944, when a group of Dr. Weizmann's friends wished to honour his seventieth birthday, and the Weizmann Institute was formally dedicated by Dr. Weizmann in November 1949. It has since grown to comprise eighteen research units, covering a wide range from applied mathematics and theoretical chemical physics to genetics and cell biology. It includes a graduate school, with about 115 students working for the Ph.D. degree. Its contributions in many areas have exerted a profound influence, and visiting scientists have gone to work there from all over the world.

The Ullmann Institute of Life Sciences, which was dedicated at the beginning of the symposium, on June 10, fills an urgent need of the Weizmann Institute. Workers in biochemistry and in many aspects of biology have been carrying on distinguished research in increasingly cramped surroundings, as numbers and activity grew. The Ullmann Institute, given by Siegfried and Irma Ullmann, of New York, will comprise research units of biochemistry, biophysics, virology, and genetics, in much ampler quarters and with exceptionally fine research facilities.

The dedication of the new Institute, held in the open air, close by the building itself, in the late afternoon light, was an impressive and moving ceremony. David Ben Gurion was there to offer greetings on behalf of the Government of Israel; it was one of his last official acts before his unexpected resignation a week later. Amos de Shalit, director of the Weizmann Institute, and Ephraim Katchalski, who will play a leading part in the Ullmann Institute, spoke briefly of what this new development will mean. Mr. Ullmann warmly replied, and Mrs. Ullmann then formally opened the new laboratories. An air of genuine dedication, not usually felt in such ceremonies, pervaded the whole event. Israel is a country where men have achieved much in a few years, against great odds. The growth of the Weizmann Institute into one of the world's great research centres, within so short a period, was one manifestation of this. Here was one further achievement, leading to still greater activity in the future.

Immediately thereafter, Jacques Monod (Pasteur Institute) delivered the opening lecture of the symposium on the role of allosteric effects in the control of cellular metabolism. These involve the transmission of inhibiting, or sometimes of activating, effects from one region of an enzyme molecule to another, the inhibiting agent representing generally the final product of a metabolic pathway, whereas the enzyme catalyses the initial reaction in that same pathway. The profound biological significance of these mechanisms is clear; Monod and his colleagues, J.-P. Changeux and F. Jacob, have recently provided an admirable review (*J. Mol. Biol.*, 6, 306; 1963).

The following morning session was devoted to proteins, beginning with a survey of important current trends in present-day protein research by J. T. Edsall (Harvard), followed by a discussion by J. C. Kendrew (Cambridge) of the detailed structural features of myoglobin and hæmoglobin, their implications for protein structure in general, and the prospects and difficulties of further explorations of protein structure by X-ray crystallography. H. Neurath (University of Washington) dealt with the structure and function of proteolytic enzymes, and particularly with

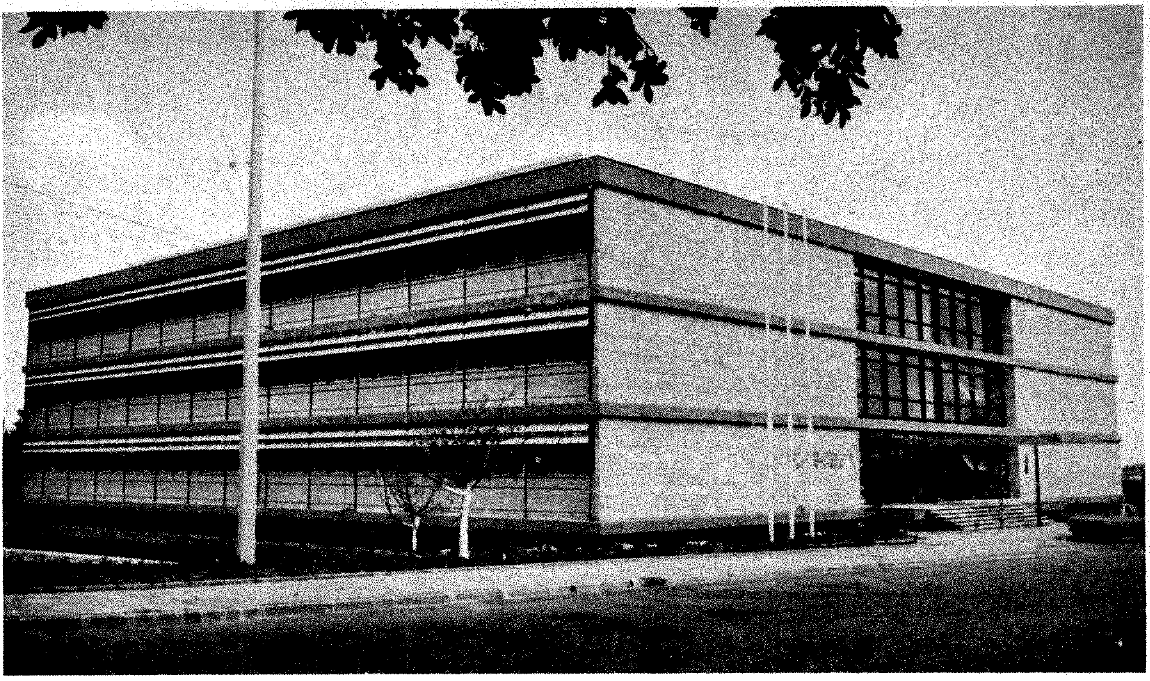

Fig. 1. The Ullmann Institute of Life Sciences, Weizmann Institute of Science, Rehovoth, Israel

the recent remarkable findings in his laboratory involving the activation of pancreatic procarboxypeptidase to give at least two distinct active enzymes.

In the afternoon session E. Katchalski (Weizmann Institute) surveyed his work on synthetic polyamino-acids and their many applications in biology, including the induction, in 'prolineless' mutants of *E. coli*, of an enzyme, proline imino-peptidase, which digests polyproline; the growing of 'whiskers' of polyamino-acid side-chains on the amino groups of natural proteins; the stabilization of enzymes by binding them to water-insoluble carriers; and the use of polypeptidyl derivatives of aminoacyl compounds of soluble ribonucleic acids to pick out the molecules of *S*-RNA that are specific for particular amino-acids. F. A. Lipmann (Rockefeller Institute) gave a thoughtful and searching discussion of the problems of protein biosynthesis. F. Lynen (Munich) considered the co-ordination of metabolic processes by multi-enzyme complexes, with definite morphological structure, but at a far simpler level than that of the mitochondrion. The protein components possess both catalytic sites and binding sites that hold the components of the complex together. The pyruvate dehydrogenase system (Koike, Reed and Carroll, *J. Biol. Chem.*, **238**, 30; 1963) and the multi-enzyme complex concerned with converting malonyl coenzyme *A* to higher fatty acids—the latter has been investigated intensively in Lynen's laboratory—are outstanding examples of such systems.

On the following day all participants in the symposium journeyed to Jerusalem, where a morning session was held at the Hebrew University, in one of the buildings of the beautiful new campus on Givat Ram. Paul Doty (Harvard) discussed his work, and that of others, on hybrid molecule formation in nucleic acids, and George Klein (Stockholm) considered fundamental problems in the genetic aspects of neoplasia. The afternoon was spent in visiting Jerusalem and its surroundings in Israel, and in the evening the President of Israel gave a delightful and most informal reception for the whole group.

In Rehovoth again, the symposium continued the next day with discussion of nucleic acids. Erwin Chargaff (Columbia University) presented, with clarity and elegance, the problem of nucleotide sequences in nucleic acids, and his own approach to the problem by the use of reagents that selectively attack the purine or the pyrimidine rings—the latter offering far the more difficult chemical problem. David Elson (Weizmann Institute) considered the structure of ribosomes, with particular reference to certain enzymes found in ribosomes. When 70 *S* ribosomes dissociate into 50 and 30 *S* components, at very low Mg^{++} ion concentration, ribonuclease is found exclusively in the 30 *S* component. Deoxyribonuclease is found in neither, but passes into solution, the process being reversible; whereas β-galactosidase is found in both components, and does not leave the ribosomes when they dissociate. He described how transfer (soluble) ribonucleic acid can be obtained from ribosomes exposed to high salt concentration. Alfred Gierer (Tübingen) discussed the mechanism of protein synthesis in reticulocytes, with particular emphasis on the role of polyribosomes, the great importance of which has become manifest only during the past year or so, as a result of work in his and several other laboratories. Severo Ochoa (New York University) discussed the present state of work on the genetic coding problem—a subject so widely known that I omit further comment on it here, in spite of its supreme importance. C. B. Anfinsen (National Institutes of Health, Bethesda) considered the problems involved in predicting three-dimensional structure of proteins from the amino-acid sequence. There is strong experimental evidence from his work on ribonuclease and other proteins that, given the sequence, the peptide chain will fold spontaneously into the correct steric structure, but the problems of predicting this structure are formidable.

That evening, in Tel Aviv, E. B. Chain (Rome, and, eventually, Imperial College of Science and Technology, London) gave a dramatic and very interesting lecture on the newly developed penicillins, which have so significantly enlarged the therapeutic uses of penicillin.

The symposium then turned to problems of development and differentiation. E. Kellenberger (Geneva) reported on the use of facultative lethal mutants for the investigations on the assembly (morphogenesis) of phage particles. These mutants are distributed all over the genetic map, so that they affect numerous genes: 8 genes affect the formation of the phage head. Mutants in some genes produce abnormal assembly of sub-units, as, for example, into long tubes, the 'polyheads'. The sub-units are not self-assembling, but need supplementary information (morphogenetic principles) to be shaped correctly.

The micrographs of 'polysheath', however, suggest that the sub-units of the tailsheath are of the self-assembling type. Leo Sachs (Weizmann Institute) considered problems of cell differentiation and the immune mechanism. Lymphoid cell precursors, in tissue culture, can form essentially pure cultures of either mast cells or antibody-producing cells, provided a suitable layer of other cell types is present in the medium. Lymphoid precursors from lymph nodes of a rat, exposed to mouse cells, differentiate to give cells releasing antibodies, which destroy the mouse cells, that is, this is a heterograft reaction *in vitro*. Both Sachs, and independently Dulbecco, have examined the transformation of normal into tumour cells by the polyoma virus; the virus acts directly on the cells to induce the change. Michael Feldman (Weizmann Institute) considered the role of the thymus in promoting antibody formation in the adult organism, making use of the histocompatibility antigens which are determined by the *Y*-chromosome of male animals. Transfer of such antigens from a male to a female of the same species, in a tissue graft, leads to rejection of the graft by the female, due to an anti-*Y* immune response. Some tumours induced in males cannot grow when transplanted into females, because they evoke a similar response. However, animals previously irradiated with X-rays fail to develop the immune response until after several weeks, and the tumour in the female continues to grow. If the animal has been thymectomized, the immune mechanism does not recover at all after X-radiation; grafting a thymus back into such animals, however, does lead to recovery of the immune response. This recovery is an inductive effect of the thymus; it is not due to production of immunologically competent cells by the thymus itself.

H. H. Weber (Heidelberg) discussed the role of ATP in the active transport of ions, with especial reference to the work of W. Hasselbach in his institute on the vesicles of the sarcoplasmic reticulum, which accumulate calcium ions. There is a very close correlation between the Ca^{++} ions transported and the ATP hydrolysed (2 Ca^{++} per ATP). He concludes that the ATP donates an energy-rich bond to phosphorylate a carrier in the membrane, and that the phosphorylated carrier has an affinity for Ca^{++} several hundred times as great as the unphosphorylated carrier. David Nachmansohn (Columbia University) considered chemical control of movements of ions across conducting membranes, with special reference to nerve and electric organs. Hugo Theorell (Stockholm) set forth, with beautiful clarity, his recent work on complexes of liver alcohol dehydrogenase with coenzymes and inhibitors or substrates.

The last session was devoted to immunochemistry. Michael Heidelberger (Rutgers University) described his recent work on the immunological properties of the capsular material of pneumococci of various types. The structure of the carbohydrates in these capsules is now becoming known in far more detail than ever before—in type *SV*, for example, recent work of Barker in Birmingham, on material supplied by Heidelberger, has identified *N*-acetyl-L-fucosamine and *N*-acetyl-6-deoxytalosamine, among other constituents. These two sugars were never before known in natural products. The chemical identification of antigens by immunochemical techniques is now being refined, and in many cases furnishes a short cut to determination of the structure of the antigen. Michael Sela (Weizmann Institute) described his research work on the development of polyamino-acids and their derivatives as synthetic antigens. One good antigen can be made from polylysine, by first growing alanyl chains on the amino groups of the lysyl side-chains, and then attaching tyrosyl chains to the alanyl chains. If the attachments are made in reverse order—first tyrosine, then alanine—the resulting product is not antigenic. The tyrosyl groups must be on the outside to make a good antigen. The optical specificity of the amino-acids is important—compounds made from combinations of D-amino-acids are generally non-antigenic, even when the corresponding L-compounds are strongly antigenic, as recent work of Gill and Doty has shown (*Nature*, 197, 746; 1963). Dr. Sela's recent work has shown that electric charge is not necessary for antigenicity; they have recently synthesized a water-soluble uncharged polymer that is strongly antigenic (Sela, M., and Fuchs, S., *Biochim. Biophys. Acta*, 74, 797; 1963). When galactose is attached to a synthetic polymer, the galactose grouping becomes a powerful antigenic determinant. Immunological tolerance may be established to synthetic antigens, as shown by experiments on new-born rabbits. The aim of all this research work is to throw light on the nature of the immune mechanisms found in living organisms; the synthetic chemicals are merely a means to this end.

J. C. Kendrew concluded the final session with brief, graceful and humorous comments on some of the major points of the symposium.

One non-scientific interlude deserves mention. Midway in the week we left Rehovoth for a two-day trip along the coast, to Caesarea, Haifa and Acre, then to the Galilee mountains and the Lake of Galilee. We stopped overnight at a kibbutz which, in addition to the usual farming and other community activities, ran a small and very pleasant hotel for visitors. That evening all of us had the opportunity to talk with members of the kibbutz and learn directly about their way of life, its values and its problems. We returned with renewed zest to the scientific conference after this fascinating interlude. In addition to this thoughtfully arranged pause in the symposium, all of us will remember the warm and generous hospitality of our Israeli hosts, during and after the conference, which combined with the high level of the scientific discourse at the meetings to make this a most memorable occasion.

RADIO OBSERVATIONS OF OH IN THE INTERSTELLAR MEDIUM

llege of Science Calcutta

By Dr. S. WEINREB
Lincoln Laboratory, Massachusetts Institute of Technology
Prof. A. H. BARRETT
Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Mass.
AND
Dr. M. L. MEEKS and J. C. HENRY
Lincoln Laboratory, Massachusetts Institute of Technology, Lexington, Mass.

In this article we wish to report the detection of 18-cm absorption lines of the hydroxyl (OH) radical in the radio absorption spectrum of Cassiopeia *A*, thereby providing positive evidence for the existence of OH in the interstellar medium. The microwave transitions of OH in the ground-state, $^2\pi_{3/2}$, $J = 3/2$, arise from two Λ-type doublet-levels, each of which is split by hyperfine interactions with the hydrogen nucleus, so that four transitions result. The two strongest lines have been previously measured in the laboratory at 1,667·34 ± 0·03 Mc/s ($F = 2 \rightarrow 2$) and 1,665·46 ± 0·10 Mc/s ($F = 1 \rightarrow 1$) with relative intensities of 9 and 5, respectively[1], these results are in agreement with theory The suggestion that these lines might be detected in the radio spectrum of the interstellar medium has been made by Shklovsky[2] and Townes[3] A previous search by Barrett and Lilley[4], in 1956, was unsuccessful, primarily because the laboratory measurements of the frequencies had not been made A recent search for OH emission also yielded negative results[5]

Our observations were conducted on 10 days between October 15 and October 29, 1963, using the 84-ft parabolic antenna of the Millstone Hill Observatory of Lincoln Laboratory, Massachusetts Institute of Technology, and the spectral-line autocorrelation radiometer designed by Weinreb[6] The receiver uses digital techniques to determine the autocorrelation function of the received signal. The resulting autocorrelation function is then coupled directly into a digital computer that performs a Fourier transformation and displays the resulting spectrum on a cathode-ray tube or a precision x–y plotter During one integration time-interval of 2,000 sec, a 100-kc/s portion of the spectrum is determined with a frequency resolution of 7 5 kc/s The ability to see immediately a calibrated visual display of the measured spectrum and average this result with others greatly facilitated the conduct of the experiment and eliminated almost all post-observation data handling The system noise temperature was 420° K, of which 110° K was due to Cassiopeia *A* System tests were performed by observing the hydrogen line

The results obtained during the first evening of our observations showed strong evidence of the 1,667 Mc/s line in Cassiopeia *A*, the signal is visible after 2,000 sec of integration We decided that positive identification of OH absorption lines of Cassiopeia *A* would be secured before proceeding to observations of other regions Our results indicate that two of the three clouds showing strong H absorption[7], namely, those at radial velocities of − 0 8 km/sec and − 48 2 km/sec, also give rise to OH absorption lines that we have detected at both 1,667 Mc/s and 1,665 Mc/s The strong H absorption line at − 38 1 km/sec appears to be composed of two lines at − 37 4 km/sec and − 42 1 km/sec when observed at the OH frequency It is to be expected that a one-to-one correspondence between OH absorption and H absorption will not be observed because of (*a*) greater thermal broadening of H lines, (*b*) larger optical depth of the H lines, (*c*) possible OH/H abundance variations from cloud to cloud A typical record showing the 1,667-Mc/s line in the − 0 8 km/sec cloud is shown in Fig 1 A summary of all of our observations is presented in Table 1

Table 1 SUMMARY OF OH LINE ABSORPTION MEASUREMENTS IN THE CASSIOPEIA *A* RADIO SOURCE

Radial velocity (km/sec)	1,420 405 Mc/s H-line optical depth (ref 7)	1,667 357 Mc/s OH-line optical depth	Observed line width (kc/s)	Number of OH radicals per cm^2	Abundance ratio relative to H
−0 8	1 85	0 016±0 005*	13	~2×10^{14}	~1 5×10^{-7}
−37 2	—	0 010±0 005	13	~1 5×10^{14}	—
−42 1	—	0 012±0 005	20	~3×10^{14}	—
−48 2	4 0	0 016±0 008	25	~5×10^{14}	~1×10^{-7}

* An optical depth of 0 010±0 003 with line width of 16 kc/s was observed for the 1,665 402 Mc/s line

The evidence that we are indeed detecting interstellar OH in these observations may be summarized as follows

(1) Lines at both 1,667 Mc/s and 1,665 Mc/s have been detected with frequencies and intensity ratios that are in good agreement with the expected values

(2) The OH absorption spectra at both frequencies show general agreement with the H absorption spectra

(3) The absorption lines disappear when the antenna is positioned off Cassiopeia *A* by one degree in both azimuth and elevation.

(4) The lines shifted 20 kc/s between October 17 and October 29, this is the shift expected from the orbital velocity of the Earth during this time-interval

A quantity of immediate astrophysical interest which follows from our observations is the abundance ratio of OH to H This can be obtained in the following way the spectral change in antenna temperature ΔT_{OH} owing to the OH absorption is given by

$$\Delta T_{\text{OH}} = \tau_{\text{OH}} T_{\text{AC}}$$

where τ_{OH} is the OH optical depth in the direction of Cassiopeia *A* and T_{AC} is the antenna temperature attributable only to Cassiopeia *A* The maximum optical depth is given by

$$\tau_{\text{OH}} = \frac{hc^2 A N_{\text{OH}}}{8\pi k T_s \nu_0 \Delta\nu} \frac{g_i}{\Sigma g_i}$$

where A is the spontaneous transition probability, T_s is the excitation temperature analogous to the H spin temperature, ν_0 is the line frequency, $\Delta\nu$ is the line width, g_i is the statistical weight of level i, and N_{OH} is the total number of OH radicals per unit cross-section The statistical weight g_1 of the upper level of the 1,667-Mc/s transition is 5, and for the 1,665-Mc/s transition it is 3, the sum of the statistical weights for all levels is 16 The spontaneous transition probability A must be evaluated by using the matrix element derived from a quantum-mechanical treatment of Λ-type doubling[8], and has the value 2 86 × 10^{-11} sec^{-1} for the 1,667-Mc/s transition The OH dipole moment used in these calculations[9] is (1 60 ± 0 12) × 10^{-18} E S U

The excitation temperature T_s is the subject of considerable uncertainty because it cannot be assumed that it will be

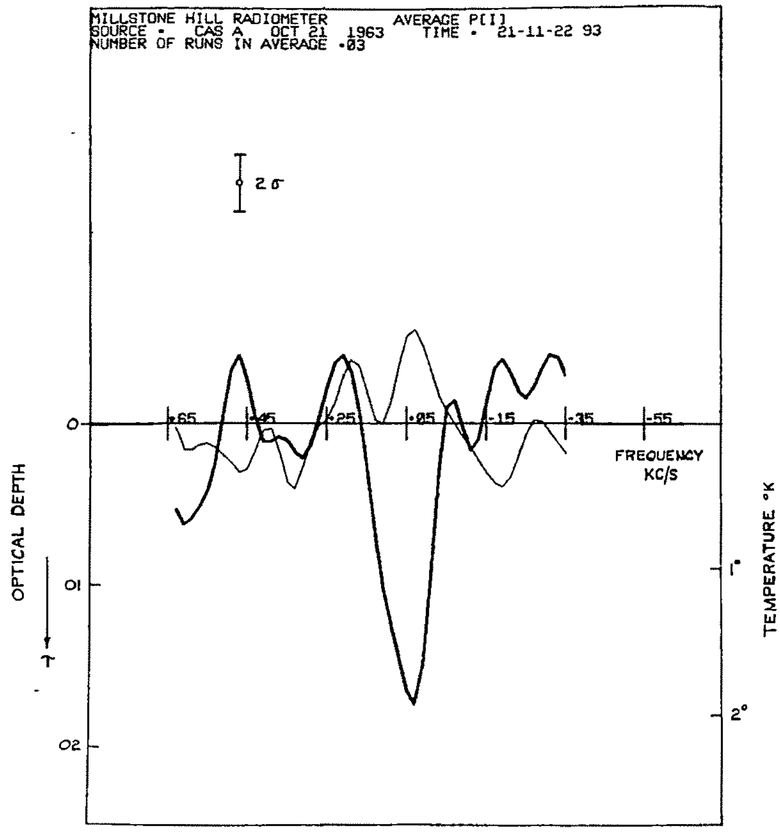

Fig 1 Observed 1,667 Mc/s OH absorption spectrum in Cassiopeia *A* The heavy line shows 8,000 sec of data taken with the antenna beam directed at Cassiopeia *A*, and the light line shows 6,000 sec of data taken with the beam displaced slightly from Cassiopeia *A* The frequency scale is specified in kc/s with respect to the local standard of rest assuming the line rest frequency to be 1,667,357 kc/s

the same as that for H The excitation temperature is defined by the relation

$$\frac{n_i}{n_0} = \frac{g_i}{g_0} e^{(-h\nu)/(kT_s)}$$

where n_i and n_0 are the densities of radicals in the upper and lower states of the transition, respectively For H it has been shown that T_s equals the kinetic temperature in a typical galactic gas cloud[10] because radiative transitions are relatively rare as compared with collisional transitions For OH, however, the spontaneous transition probability is 10^4 times larger than for H, so radiative transitions play a more dominant part in establishing the equilibrium population distribution A detailed evaluation of the processes that will be important in determining the OH excitation temperature has not been made, but a preliminary investigation shows that slow-moving positive ions may be very effective in inducing transitions between the two states, in spite of their low abundance in a H cloud[11] This situation arises because the OH transition is of an electric dipole type, and therefore can be induced by the Coulomb field of electrons and ions which leads to large interaction radii A similar result has been obtained by Purcell when considering the population of the fine-structure states of the $n = 2$ level of H in the solar atmosphere[12] An upper limit on T_s can be set by our observations off Cassiopeia *A* from which one would expect to detect OH emission From the preliminary observations we conclude that any OH emission adjacent to Cassiopeia *A* is less than 1° K, this result implies a T_s less than 50° K for an optical depth of 0 02 For purposes of computing the total number of OH radicals from the foregoing equations we have assumed a T_s of 10° K More extensive observations for OH emission will enable a better estimate of this quantity to be made

The values of the number of OH radicals per cm² in the direction of Cassiopeia *A* are shown in Table 1 The OH/H abundance ratio can be calculated from the results of the H absorption on Cassiopeia *A*[7], and gives typical ratios of 1×10^{-7} (see Table 1) This ratio can be compared with estimates of the CH/H abundance ratio 10^{-6} by Stromgren[13] and 2×10^{-8} by Bates and Spitzer[14].

Our observations have enabled a more accurate determination of the frequencies of the two strongest Λ-type doublet lines of OH The laboratory and astronomical values are shown in Table 2 It is possible that these values will be of interest to the molecular spectroscopist for a more accurate evaluation of the hyperfine coupling constants.

Table 2 REST FREQUENCY OF OBSERVED LINES

Transition	Laboratory measurement	Astronomical measurement
$F=2\rightarrow2$	1,667,340±30 kc/s	1,667,357±7 kc/s
$F=1\rightarrow1$	1,665,460±100 kc/s	1,665,402±7 kc/s

We thank many people for their co-operation in this experiment We also thank R E Gay, P J Conrad, C Blake, R H Erickson, J F MacLeod, and the entire personnel of the Millstone Hill Observatory for their assistance

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OBITUARIES

Dr. A. A. Griffith, C B E., F.R.S.

ALTHOUGH the name of Dr A A Griffith, who died on October 11, was not well known by the general public, he was a creative and original thinker who had considerable influence on the development of jet propulsion systems A champion of the high-efficiency axial compressor while Whittle was experimenting with the more robust centrifugal engines, Dr Griffith continued to be ahead of his time in urging, in turn, the by-pass system, small engines for jet lift, the use of hydrogen as a fuel, and had still other novel projects under consideration at the time of his death

Dr Griffith was born on June 13, 1893, and attended secondary school at Douglas in the Isle of Man A Tate technical science scholarship took him to the University of Liverpool in 1911, where he obtained successively a B Eng (first-class honours in mechanical engineering), M Eng (1917) and D Eng (1921)

His first interest was in mechanics, and his joint paper with G I Taylor on the use of soap films in solving torsion problems gained the Hawksley Gold Medal from the Institution of Mechanical Engineers in 1917 His famous paper on "Theory of Rupture", in which he used surface energy considerations to determine the stress conditions under which a crack will propagate and lead to failure of a material, was published in 1920, and the concept of the 'Griffith Crack' was discussed at the first International Congress on Applied Mechanics at Delft in 1924

He had no regular apprenticeship, but received general workshop training between July 1915 and November 1916 at the Royal Aircraft Factory Following this training, he was successively draughtsman, technical assistant and senior technical assistant in the Physics and Instrument Department at the Royal Aircraft Establishment During April 1920–April 1928 he was a senior scientific officer in the Physics and Instrument Department, Royal Aircraft Establishment, and then principal scientific officer in charge of the Air Ministry Laboratory, South Kensington, until April 1931, dealing chiefly with engine and instrument work

During this period he became a firm advocate of the gas turbine for aircraft propulsion, and in 1926 wrote a Royal Aircraft Establishment report entitled "An Aerodynamic Theory of Turbine Design", in which he formulated a number of new ideas Under his supervision, work on a turbo-compressor test rig was started at the Establishment in 1927

In 1931, Dr Griffith was made principal scientific officer in charge of engine research, Engine Department, Royal Aircraft Establishment, in November 1938, the acting head of the Department, and later head of the Department at the Royal Aircraft Establishment until May 1939

During 1921–31, he was an official member of the Elasticity and Fatigue Sub-Committee of the Aeronautical Research Council and then official member of the Engine Sub-Committee, Aeronautical Research Council, until 1939 His connexion with the Council continued until his retirement in 1960 Indeed, in his later years he used the Committee as his main vehicle of communication with the technical world, he published little elsewhere, largely because of the relevance of his work to defence projects

He was a founder member of the Gas Turbine Collaboration Committee

On June 1. 1939, Dr Griffith took up the position of research engineer of Rolls-Royce, Ltd , at Derby, working on the initiation and prosecution of aero engine research, responsible to Mr E W Hives, general manager (later Lord Hives chairman) He was given a pleasant room at Duffield Bank House, a quiet haven on the banks of the Derwent, five miles from Derby

Griffith's first work, completed in 1941, was an axial flow jet engine including twenty contra-rotating compressor stages, each driven independently by turbine blades at the tips of the compressor blades, ducted fan propulsion, centrifugal fuel supply and other items that were far in advance of contemporary practice The high-pressure part of the system was, in fact, built and tested Throughout his work on jet propulsion, Dr Griffith believed in the superior efficiency of the axial type compressor, and the contra-flow engine was followed by a series of design investigations of axial types, parallel with work on many component rig test investigations of combustion, blade form and rotor design

A technical memorandum prepared by Dr Griffith in 1945, together with a preliminary design scheme created under his supervision, were the basis of the Rolls-Royce Avon engine, this was followed in 1946 by his proposals for a by-pass engine Preliminary design schemes for the latter type were completed early in 1947 and led to the Rolls-Royce Conway engine

Early in 1941, Dr Griffith realized that the thrust potential of a turbine engine, combined with its low weight, provided a basis for the design of an aircraft which could take off and land vertically by virtue of engine thrust alone and thus only need sufficient wing area for its cruise condition He made a detailed investigation of engine weight relative to size and showed that the 'Square-Cube' law, by which an engine's weight decreases as the cube of its linear dimensions, whereas the thrust only decreases as the square, would hold good down to quite small unit sizes This convinced him of the advisability of using a number of small engines in order to obtain the optimum lift/weight ratio for vertical take-off applications His first design studies of vertical take-off aircraft incorporated a multiplicity of small engines with means for deflecting their thrust downwards for taking-off and landing, control of the aircraft in the jet-borne phase being by variation of the thrust of engines situated fore and aft for pitch and separated laterally for roll The possibility, with this scheme, of difficulty due to insufficiently rapid thrust-response rate led Dr Griffith to the idea of using compressed-air jets fed from engine compressor air bleed

He conceived the scheme for testing this system, now well known as the 'Flying Bedstead', and supervised the preliminary design scheme, which incorporated two Rolls-Royce Nene turbine engines arranged back to back with their thrust deflected downwards in a central position Control air was tapped from the engine compressors and fed to control nozzles positioned fore and aft and on outriggers laterally On August 3 1954, this jet-lift test rig achieved its first free flight for 8½ min Many subsequent flights clearly demonstrated the success of Dr Griffith's vertical take-off proposals Parallel with these tests and until his retirement in 1960, Dr Griffith initiated many aircraft design studies using specially designed lightweight lift engines and separate engines for forward thrust Late in 1953 he commenced a detailed investigation of a supersonic jet-lift transport designed for a Mach No of 2 6 at 60,000-ft altitude

Dr Griffith's work was recognized by fellowship of the Royal Society in 1941, a C B E in 1948, the award of the Silver Medal of the Royal Aeronautical Society in 1955 and, in 1962, the Bleriot Medal

Although Dr Griffith was very little known to most people within Rolls-Royce, due partly to the secluded location of his office, I was privileged to be his personal assistant and designer for twenty-one years, sharing his office for sixteen years at Duffield Bank House and later having an adjoining design office at Littleover Old Hall for the five years prior to his retirement

Alan Arnold Griffith was truly a man of vision, quiet and retiring, resisting publicity yet fascinating to know intimately by daily contact His brain provided new lines of thought for development in a continuous flow and our discussions were often enlivened by his ready wit and good humour DONALD EYRE

Prof E P. Sharpey-Schafer

EDWARD PETER SHARPEY-SCHAFER, professor of medicine in the University of London at St Thomas's Hospital Medical School, died on October 23 at the age of fifty-five

His father was killed in the First World War and consequently he was brought up in the house of his famous grandfather, Sir Edward Sharpey-Schafer He went to school at Winchester and entered King's College, Cambridge, as an exhibitioner and Kitchener Scholar His clinical studies were completed at University College Hospital where he obtained the Fellowes Gold and Silver Medals After qualifying, he held the post of house physician to Sir Thomas Lewis, a position which he spoke of with great pride in later years After serving as resident medical officer at the National Heart Hospital, he joined Sir Francis Fraser as first assistant in the Department of Medicine in the early days of the Postgraduate Medical School He was appointed professor of medicine at St Thomas's Hospital Medical School in 1948

It is impossible to assess the enormity of the loss which St Thomas's Hospital has suffered in the death of Prof Sharpey-Schafer He is the third member of their consultant staff to die in recent months, and though they were all men of high intellect and profound wisdom, he was undoubtedly the greatest of the three Only those who were close to him had the opportunity to know him He was essentially a shy man, which led people to think his manner brusque and off-hand, but his great human qualities showed themselves when he considered that someone was being treated unjustly The same consideration for others was manifest in his investigations on patients if ever it became apparent that one was suffering discomfort he immediately abandoned the procedure

Prof Sharpey-Schafer was an outstanding research worker who is known throughout the world for his work on the circulation However, he entered the field of original work in endocrinology and made important contributions in investigations of the thyroid, acromegaly and ovarian function Although he forsook this sphere during the War, he still taught the younger endocrinologist some fundamental facts years later

With Prof J McMichael he introduced cardiac catheterization as a method of examination of the cardiovascular system From then onwards he continued to be fascinated by the control mechanisms in this system in health and disease Every week he continued to make simple but fundamental observations on his patients, and a steady stream of original work flowed from his pen He investigated hæmorrhage and heart-failure and remarked on the aspects of similarity in these two conditions One of his particular achievements was the demonstration of vasodilatation in fainting subjects and the part played by complete immobility when soldiers fainted on parade. He demonstrated the vascular reflex abnormalities in patients with tabes dorsalis, diabetes and barbiturate intoxication. This led to the simple treatment of the latter group of patients by blood volume expansion when they lapsed into a hypotensive state. Of recent years his dominant interest has been in the control of venous tone, and this led him on to the vascular changes during exercise. For months on end he would talk to all and sundry about this problem until he found at least a partial answer in the realm of chemistry. This was rather unfamiliar territory to him and he soon enlisted the assistance of one of his staff in a series of conclusive investigations.

When Prof. Sharpey-Schafer first arrived at St. Thomas's Hospital he and his staff were thought of as experimentalists. He had an uphill task in earning the esteem and respect of the clinically orientated members of the nursing and medical staff. As the years passed he became more and more valued for his judgement both of individuals and of problems. Of the many people who sought his help and advice, not one was let down. The references he wrote for the junior members of the hospital staff sparkled with wit and impressed themselves on the reader by their originality. No idle clichés were ever found in these or any other of his writings.

Perhaps one of the most striking aspects of the personality of Prof. Sharpey-Schafer was his attitude to orthodoxy. He declared that he had an abhorrence for the whole of the pharmacopœia, and on one occasion to stress the point he crossed off all the drugs on his patients' treatment cards. He delighted in poking fun at the Establishment and in *Who's Who* stated that his recreation was "childish pursuits". In fact, he was a keen and accomplished naturalist and photographer, and when he produced a new batch of his colour photographs of birds or insects he was armed also with all the fascinating details of their life-habits, such as the speed of their wing beats, time and place of emergence, etc.

Those who knew "Sharpey" well appreciated the warmth of his character, his unfailing loyalty and the high standards which he demanded of everyone engaged in research. He was a man who always kept to his word and who could be relied on to help anyone who was in need. He will be an inspiration to all those who worked with him, but few will reach the standards which he achieved.

Prof. Sharpey-Schafer leaves a son and daughter by his first marriage. He married Dr. Sheila Howarth and they had two daughters. IVOR H. MILLS

NEWS and VIEWS

School of Science: Massachusetts Institute of Technology

DR. JEROME B. WIESNER has been appointed Dean of the School of Science in the Massachusetts Institute of Technology. Dr. Wiesner, now special assistant for science and technology and director of the U.S. Office of Science and Technology, will succeed Dr. George R. Harrison, who will retire at the end of this year.

Dr. G. R. Harrison

DR. HARRISON graduated from Stanford University and received his Ph.D. degree there in 1922 while an instructor in physics. After two years as a National Research Fellow at Harvard University he returned to the Stanford faculty and remained there until his appointment at the Massachusetts Institute of Technology in 1930. He was director of the Research Laboratory of Experimental Physics for twelve years before becoming dean of the School of Science in 1942. In spectroscopy, his field of specialization, Dr. Harrison invented several instruments of great value and compiled the *M.I.T. Wavelength Tables*, an encyclopædic work used by spectroscopists throughout the world. He was editor of the *Journal of the Optical Society of America*, co-author of *Practical Spectroscopy* and author of *Atoms In Action*, *What Man May Be*, and other books. During the Second World War, Dr. Harrison was chairman of the Instruments Section, and later chief of the Optics Division of the National Defense Research Committee, Office of Scientific Research and Development, and, for a time, chief of the Research Division at General MacArthur's headquarters in the south-west Pacific. He received the War Department Medal of Freedom and Presidential Medal for Merit. He was awarded the Rumford Medal of the American Academy of Arts and Sciences, the Frederic Ives Medal of the Optical Society of America, the Elliott Cresson Medal of the Franklin Institute and the Medal of the Society of Applied Spectroscopy.

Dr. J. B. Wiesner

DR. WIESNER received bachelor of science, master of science and doctor of philosophy degrees from the University of Michigan. In 1940 he was appointed chief engineer of the Acoustical and Record Laboratory of the Library of Congress and in 1942 went to the Radiation Laboratory at Massachusetts Institute of Technology, where he was a member of the Microwave Components Division and then group leader of 'Project Cadillac', assigned to devise an airborne radar system. After the War, Dr. Wiesner spent a year at Los Alamos, returning to the Institute as assistant professor of electrical engineering. He was made an associate professor in 1947, professor in 1950 and Institute professor in 1962. In 1947 he became assistant director of the Research Laboratory of Electronics, in 1949, associate director, and in 1952, director. He was appointed acting head of the Department of Electrical Engineering in 1959. Dr. Wiesner's scientific contributions, particularly in the field of microwave theory, have been notable. He was chairman of the Institute's steering committee for a Center for Communication Sciences established in 1958. Dr. Wiesner was staff director of the American delegation to the Geneva Conference for the Prevention of a Surprise Attack in 1958, and for a number of years he has devoted much effort to the search for methods of arms control. Already a member of the President's Science Advisory Committee, he went on leave from the Institute in 1960 to become special assistant for science and technology to President Kennedy. He was also made director of the Office of Science and Technology when that agency was established in 1962. He has received the President's Certificate of Merit and is a member of the National Academy of Sciences.

"Isotopes": the Fiftieth Anniversary

ON December 4 the Chemical Society (president, Prof. J. Monteath Robertson) will meet in the Department of Chemistry, University of Glasgow, to commemorate Frederick Soddy's communication in *Nature* of exactly fifty years ago (92, 399, December 4, 1913). Soddy was then working in the Physical Chemistry Laboratory of the University of Glasgow. This was a time of intense research and rapid advance in all fields of radioactivity. Although priority issues are inevitably debatable, it remains evident that the University of Glasgow made an important contribution by creating a lectureship in "Physical Chemistry including Radio-activity" (1904) and appointing the youthful Soddy His work there on isotopes resulted in the communication in *Nature*, where, from the various explanations of "chemically inseparable" substances, he came down firmly for "isotopes"—for which he there first adopted the name—and also provided a valid interpretation of atomic number in terms of nuclear charges This work on isotopes is referred to in his Nobel prize citation (1921) Soddy's Glasgow colleagues, Lord Fleck and John A Cranston, will speak on these researches, H J Emeleus will review the present field of radiochemistry, and Dr Andrew Kent will present a historic note

Basic Research at the Royal Armament Research and Development Establishment: Dr J W Maccoll

ON September 30, Dr. J W Maccoll retired from the post of principal superintendent of the Basic Research Division of the Royal Armament Research and Development Establishment, Fort Halstead Dr Maccoll, who was born in Aberdeenshire, graduated B Sc at the University of Glasgow in 1924, where he studied mechanical engineering and pure science, and afterwards pursued aerodynamic researches under Thom at Glasgow, Bairstow at the Imperial College of Science and Technology, where he took his Ph D in 1927, Prandtl at Gottingen, and von Kármán at Aachen He joined the Ordnance Board as ballistic research officer in 1929, to apply the principles of aerodynamics to ballistics, and it was in the 1930's that his fruitful collaboration with Sir Geoffrey Taylor led to their classic paper on 'Taylor–Maccoll' flow, and other papers Following the report of the Guy Committee in 1942, and the creation of the Armament Research Department with Prof (later Sir) John Lennard-Jones as its first chief superintendent, Dr Maccoll was first appointed deputy superintendent and, later, superintendent of the newly formed branch for theoretical research Since 1945 he has been in charge of basic research at Fort Halstead and has built up, over the years, a substantial group, with extensive experimental facilities for work on hypersonic flow, hypervelocity projection and flow in the upper atmosphere, and with up-to-date facilities for high-speed computation (see *Nature*, 185, 346, 1960) Dr Maccoll, who has always been a well-known figure at scientific meetings, will be remembered, particularly in the Scientific Civil Service, for the importance he has always attached to sound fundamental research, for his scientific foresight and his shrewd judgment Although officially retired, he has not severed his connexion with the Royal Armament Research and Development Establishment, but is maintaining his interest in the work of the Establishment by acting as a part-time consultant

Genetics in the University of Leicester: Prof R. H. Pritchard

DR R H PRITCHARD, who has been appointed to the newly established chair of genetics in the University of Leicester, has distinguished himself especially by his studies on the properties of the genetic material of micro-organisms Using a selective technique which permitted a high degree of resolution, he showed that the processes of recombination between sites very close to each other on the chromosome of *Aspergillus nidulans* obeyed rules different from those governing recombinations between more distant sites His present work, with *Escherichia coli*, is contributing greatly to the elucidation of the factors underlying thymine-less death and induction in lysogenic bacteria Dr Pritchard, who was born in 1930, gained the Carter Gold Medal and first-class honours in botany from King's College, London, prior to joining the Department of Genetics in the University of Glasgow in 1951 After completing his Ph D work, he spent a year as Rask-Ørsted Fellow in the University of Copenhagen He returned to Glasgow in 1955 and remained there as, successively, Nuffield research assistant and lecturer until his appointment to the staff of the Medical Research Council's Microbial Genetics Research Unit at the Hammersmith Hospital in 1959 The selection of Dr Pritchard to head a new Department in the University augurs well for the continued development of biological studies at Leicester

New Department of Microbiology in the Queen's University of Belfast

LORD HARDING OF PETHERTON, chairman of the National Fund for Research into Poliomyelitis and other Crippling Diseases, laid a plaque to mark the foundation of a new building for the Department of Microbiology, Queen's University of Belfast, on November 12 The National Polio Fund made a grant of £70,000 towards the cost, the Northern Ireland Hospitals Authority is contributing £50,000 and the balance of cost of approximately £350,000 is to be met by the Government of Northern Ireland Sir Hugh Casson is the architect of the new building, which is being erected near the Institutes of Pathology and of Clinical Science on the hospitals site at Grosvenor Road It is linked to these Institutes and to the Royal Victoria Hospital It provides in a podium extensive animal houses and in five floors teaching and research laboratories and a virus reference laboratory for the Hospitals Authority The building is expected to be completed by October 1964

Science and Adult Education

A REPORT entitled *Science and Adult Education*, issued by the Association of Scientific Workers, recommends that in view of the importance of science and technology to the nation, the number of science classes in adult education should be increased to 20 per cent of the total within the present quinquennium, and especially in the longer courses (Pp 11 London Association of Scientific Workers, 1963 2*s*) This extension should be directed particularly at students with little or no previous education in science, and additional funds for this extension should be made available by the Ministry of Education. Some additional full-time appointments of science tutors with responsibility for both teaching and expanding the provision of science classes should be made by extra-mural departments and by the Workers' Educational Association An immediate and substantial increase in the fees of part-time tutors is proposed as well as funds for extending special experimental schemes Special attention should be directed to including science courses in summer schools and week-end schools to create an atmosphere in which science is regarded as a normal feature of adult education, to ways of integrating science into courses in social studies and the arts, and to ways of adapting science courses for the needs of special groups More books suited for adult teaching are required and the publication of such books by successful tutors should be encouraged Branches of the Workers' Educational Association should be encouraged to discuss ways of recruiting students for science classes and of conducting publicity for such classes

Shortage of Science Teachers

THE report of a recent survey carried out by the National Union of Teachers, by means of a questionnaire, to which 22,000 schools responded, throws further light on the shortage of specialist teachers in England and Wales (*The State of Our Schools a Report of the Findings of the National Survey of Schools, 1962* Part 3 *An Analysis of the Findings Relating to Grammar Schools, Comprehensive Schools and Special Schools* Pp 19 London National Union of Teachers, 1963 2*s* 6*d*) In grammar schools, among all subjects, the greatest shortages are in mathematics, in which 28 additional teachers are required per 100 schools, and in science, for which the deficiency is 24 After these, the next most serious shortages are in English and foreign languages In comprehensive schools, which as a whole are of more recent construction, the shortages are still greater, the figures being 68 for mathematics, and 59 for science—along with 32 for English, 27 for geography, 23 for foreign languages and 22 for history Once again this survey brings out the meagre provision in both types of school for the teaching of engineering, mechanical and geometrical drawing, and rural science Most of the larger grammar schools and three-quarters of the smaller ones obtain some assistance with laboratory work Among other deficiencies of various kinds which the survey reveals, one striking fact is the acute shortage in comprehensive schools of teachers specially trained to teach backward children These deficiencies are not merely matters of school administration, for it is clear that, to a certain extent, among young people, a scientific attitude can be fostered by appropriate upbringing and training, while in varying degree there is scope for scientific ability at all levels and in all sectors of society The deficiencies, therefore, are symptomatic of weaknesses in Britain's national culture

Research in South African National Parks

THE varied subjects of the papers published in *Koedoe*, the journal for scientific research in the national parks of the Republic of South Africa (No 6, 1963), show the scale of the work being carried out under the auspices of the National Parks Board of Trustees A number of systematic and distributional investigations of both vertebrates and invertebrates is included as well as several very interesting contributions on the use of different immobilizing drugs in the capture of large mammals The use of such drugs is rapidly becoming widespread not only for bringing wild animals into captivity, but also in the scientific study of their biology, for it enables close examination to be made and marking by ear-tags or other means to be carried out An interesting collection of photographs illustrates the technique by which a syringe containing the drug is incorporated in a dart fired from a special gun or crossbow A drug for use with all species of mammals has not yet been made, but excellent results are being obtained by combining the immobilizing and tranquillizing drugs in varying doses according to species A paper by Pienaar and Van Niekerk describes the use of a tranquillizer on a number of oribi captured by driving them into nets in order to introduce them into the Kruger National Park, where the species became extinct in 1943

The National Art Gallery and Dominion Museum, New Zealand

As with so many museums and art galleries, both in Britain and abroad, the real bottleneck at the National Art Gallery and Dominion Museum, New Zealand, is not finance but space for exhibits, for storage and for working While plans under consideration for the transfer of the Art Gallery to a new building may provide an ultimate solution, the Museum must seek some immediate relief within a much shorter time than it will take to build a new Art Gallery The annual report for the year ended March 31, 1963, states that efforts have been made to persuade the Police Department to vacate the building on the Board's property which is still used as a clothing store (Pp 26 Wellington, New Zealand Government Printer, 1963 1*s* 6*d*) Public relations are given constant attention, and in recognition of a considerable degree of municipal support the Museum tries to make its contribution as a civic community

The Yale Peabody Museum of Natural History, New Haven, Connecticut

ANNUAL Report No 4–1963 of the Yale Peabody Museum of Natural History, New Haven, Connecticut, is the record of a progressive institution with a relatively large staff engaged on research and teaching as well as normal museum activities (Pp 60+13 photographs New Haven, Conn Yale Peabody Museum of Natural History, 1963) Dr S Dillon Ripley, the director, stresses the fact that research leads to the development and refinement of the exhibits Sarawak Museum in Borneo presented four specimens of *Lanthanotus borneensis*, one of the rarest and most primitive of living reptiles It is an earless animal, of which only eighteen specimens are known in the world, and figures significantly in the hypothesis that snakes were derived from some monitor-like lizard in that it possesses many of the structures to be expected in an ancestor It was discovered in 1878, and so little is known of its habits that the first observation of its feeding occurred in New Haven in 1963

The Smithsonian Institution, Washington

THE annual report of the Smithsonian Institution for 1962 is, as usual, a large volume of several hundred pages recording the strengthening of the academic staff, and progress in the renovation of the exhibits (Pp x+610+106 plates Washington, D C Government Printing Office, 1963 4 75 dollars) Attendances reached the high figure of 8,923,131—an indication of the popularity of the Smithsonian Institution The great study collections are utilized by hundreds of research workers from other Government bureaux and from universities all over the United States The total number of catalogued objects now in the Smithsonian is nearly 56 million When the east and west wings of the Natural History Building are completed and opened for use and when the great new Museum of History and Technology is open, the effectiveness of the whole pattern of public display and of the use of study collections will be greatly appreciated.

Register of Research in the Human Sciences (1960–63)

THE second edition of the *Register of Research in the Human Sciences* (*1960–63*), compiled by the Warren Spring Laboratory of the Department of Scientific and Industrial Research, has recently been published (Pp. vi+226 London H M S O, 1963 20*s* net) The new edition presents a much more comprehensive account of research in this field and in fact contains twice as many entries as its predecessor Included within this title of "Human Sciences" are abnormal psychology, demography, developmental psychology, experimental psychology, industrial psychology, industrial sociology, physiological psychology, public administration, rural sociology, social anthropology, social disorganization, social economics, social geography, social history, social medicine social psychology, social stratification, and urban sociology The entries are arranged in broad subject headings and underneath are grouped in alphabetical order the names of the research institutions Throughout, the research projects are numbered Then follows a brief description of the projects, the person (or committee) responsible for the research, research workers, approximate starting date, approximate date of completion, interim results, final results, parties concerned with enquiries and other information Two indexes are provided, the first deals with institutions giving address, telephone number, name of person in charge, etc , the second is a subject index covering all fields from "absenteeism" through "crime", "housing", "management", "rural communities", "smoking", "under-developed countries", to "youth groups", etc

Family Planning

FACED with a world population which could possibly be double that of the present 3,000,000,000 within forty years, the Family Planning Association has decided to launch a massive international campaign The fact that the Association is embarking on this campaign does not mean that it wants to urge developing nations to adopt birth control or population limitation as such Countries must decide their own population policies, but an increasing number are adopting family planning some because they have a serious abortion problem, others because their economic development is greatly hampered—indeed sometimes prevented—by population increases which rapidly absorb the results of all attempts to raise the standard of living Lord McCorquodale of Newton has agreed to become chairman of the Campaign Committee, which is composed of a number of prominent social, industrial and scientific workers Details of its work are set out in the thirty-second annual report, which also describes the remarkable increase in clinics and services which the Association is providing throughout Britain (Pp 22 London The Family Planning Association, 1963) At the end of April, 1963, 163 clinics were prescribing oral contraceptives, 51 clinics are able to offer facilities for taking cervical smears

Prehistoric Cultures of North-east Angola

Prehistoric Cultures of North-east Angola and their Significance in Tropical Africa, by Prof J Desmond Clark, is the title of a monograph published through the Dundo Museum by the Companhia de Diamantes de Angola (Diamang) (*Publicacoes Culturais*, No 62 Part 1 Pp 222, Part 2 Pp 225–385 (80 plates) Lisboa Companhia de Diamantes de Angola, 1963). It is a serious study of the prehistory of North-east Angola by one of our foremost prehistorians For many years, Prof Clark was the curator of the Museum at Livingstone and is now professor at Berkeley University in California The volumes start with an interesting table giving the radiocarbon dates and pollen analyses for the various cultures and the climatic stages during which they occur Thus, the Sangoan of the early Gamblian is dated as far back as 38,000 years, while the Makalian (Tshitolian culture) can be fixed at about 6,830 years ago In the preface, Prof Clark pays tribute to the late J Janmart, formerly head of the prospecting department, whose intense interest in the cultures of the area largely started these cultural studies He also mentions, among other colleagues, Mrs Clark, who has made the excellent line drawings of the implements which illustrate the work A short outline of the relevant geology is given, and this is followed by a note on the raw materials used by prehistoric man The various climates then come under review Chapter 3 describes the stratigraphical and cultural succession in Lunda. the terminology in general use in the Congo being used There are numerous maps and sections given, which illustrate the sequence Chapter 4 continues the story given in Chapter 3, but is concerned with more recent times There follow chapters describing in greater detail the industries produced by the various cultures present These are, of course, of very great interest to the specialist Angola has from time to time been a refuge area when climate changes elsewhere have become such that human existence became difficult As a result, the typological studies of the various industries compared with those from other localities are a matter of great interest Volume 2 is devoted to illustrations of tools and photographs of a number of sites having a separate volume for these illustrations makes close study of the different cultures very much easier The two volumes will be of great value to anyone wishing to know about the prehistory of this interesting region of Africa Prof and Mrs Clark are to be congratulated on the result, and the Companhia de Diamantes de Angola for financing these publications

Barnard's Star

BARNARD'S STAR is the second nearest known stellar system, with apparent visual magnitude 9 5, a parallax of 0″ 55 and a proper motion of 10″ 3 per annum It has been investigated at the Sproul Observatory of Swarthmore College by P van de Kamp (*Astronomical J*, 68, 515; 1963) on photographs taken over a period of forty-five years, 2,413 plates with 8,260 exposures obtained on 609 nights by many observers Barnard's Star has the largest known secular perspective acceleration in proper motion This was determined (−0″ 00014 in R A, +0″ 00118 in declination) The residuals of the observations from the predicted positions allowing for proper motion, secular acceleration and parallax were investigated The residuals showed a periodicity of about 24 years, and indicate the presence of a companion A semi-axis major of 0″ 0245 and an eccentricity of 0 6 for the main star fit the observations If the mass of Barnard's Star is 0 15 solar mass, the companion has an orbit with semi-axis major of 2″ 4, or 4 4 astronomical units, and from the displacements of the main star it can be shown that the mass of the companion is 0 0015 solar masses, or 1 6 times the mass of Jupiter Its apparent visual magnitude would be perhaps 30, far below the reach of existing telescopes

Modern Instrumentation in Science and Industry

ON January 17, 1964, the London Sections of the Institute of Biology, the Institute of Physics and Physical Society, and the Royal Institute of Chemistry will hold a one-day symposium on "Modern Instrumentation in Science and Industry" at the Institution of Electrical Engineers, Savoy Place This is the latest of a series of successful joint symposia held by the London members of three Institutes, and will deal with some general (morning session) and some specific (afternoon session) aspects of the use of instruments and automated plant in biology, chemistry and physics The chairman of the symposium will be Sir John Cockcroft Of special common interest will be a lecture by G Malcolm Dyson, research director of the Chemical Abstracts Service, on "The Correlation of Chemical, Physical and Biological Properties of Substances by Mechanized Programmes" Other speakers are W Grey Walter (Burden Neurological Institute, Bristol) on "Some Aspects of Instrumentation in Experimental Physiology", R S Medlock (George Kent, Ltd, Luton) on "Automatic Control in Chemical Industries", P H Hammond (Autonomics Division, National Physical Laboratory) on "Self-Optimizing Control Systems", J M Hirst (Rothamsted Experimental Station) on "Measurements of Foliage Infection by Fungal Pathogens", V S Griffiths (Battersea College of Technology) on "Automatic Control in the Chemical Laboratory", and S Arnott (Medical Research Council Unit, King's College, London) on "Recent Developments in Molecular Biology" The lectures will be followed by discussion There is a registration fee of £1 for members of the participating societies and £3 for non-members, which will include refreshments and pre-prints Tickets can be obtained from the Finance Officer, Royal Institute of Chemistry, 30 Russell Square, London, W C 1

The American Geographical Society. Elections

THE following have been elected Honorary Fellows of the American Geographical Society· Dr George Wust director of the Kiel Oceanographic Institute, Kiel, Germany, now visiting professor of oceanography at Lamont Geological Observatory, Columbia University, Dr John Quincy Stewart, associate professor emeritus of astrophysical sciences at Princeton University, and Dr Gilbert F White, former president of Haverford College, now professor of geography at the University of Chicago

Gairdner Foundation Awards

THIS year's Gairdner Foundation awards have recently been announced The Foundation was established in 1957 by J A Gairdner, the Toronto industrialist Annual

Awards of 5,000 dollars and Awards of Merit of 25,000 dollars are bestowed on those who "have made contributions to the conquest of disease and the relief of human suffering" The following are the most recently announced

Award of Merit to Dr Murray L Barr, head of the Department of Anatomy, University of Western Ontario, for his discovery of the fundamental difference between male and female cells, thus contributing to greater knowledge of factors governing heredity

Annual Awards to Dr Jacques Genest, director of clinical research, Hotel Dieu Hospital, Montreal, for his work on high blood-pressure, Prof C Walton Lillehei, professor of surgery, University of Minnesota, Minneapolis, for development of several pioneering techniques in open heart surgery, Dr Irvine H Page, director of research, Cleveland Clinic Foundation, Cleveland, Ohio, for discoveries linking the kidney to high blood-pressure, Prof E G L Bywaters, professor of rheumatology, University of London, for his work on the clinical features of rheumatoid arthritis which have delineated the special features of the course of the disease, Dr Pierre Grabar, director of the French National Cancer Research Institute, Paris, for contributions to the knowledge of the identity of proteins which have aided research in immunology and leukæmia

NATO Advanced Study Institutes Grants

The North Atlantic Treaty Organization is offering grants towards the organization of Advanced Study Institutes during 1964 The meetings, which may cover any scientific topic, should last for two weeks or longer and may take the form of practical experimental courses as well as the more usual type with lectures and discussions Grants may be used to cover, in whole or in part, the travelling and living expenses of visitors, costs of publication, etc Applications, which must be made before December 15, should be addressed to the Scientific Affairs Division, North Atlantic Treaty Organization, Place du Maréchal de Lattre de Tassigny, Paris 16, from which further information can also be obtained

University News

Aberdeen

N M Levy and Dr M A Lappin have been appointed to lectureships in electrical engineering and geology, respectively Dr W L Cunningham has been appointed to a research fellowship in biological chemistry

Bristol

Dr P J Randle, lecturer in biochemistry in the University of Cambridge, has been appointed to the newly established chair of biochemistry, and Mr J C Shepherdson has been appointed to a chair of pure mathematics in respect of his post as reader in mathematics in the University Both appointments will take effect as from April 1, 1964 The following appointments have also been made. *Lectureships*, Mr G A. Battersby (electrical engineering), Dr G I Fray (organic chemistry), Mr P M Keen (pharmacology), Dr J S Littler (organic chemistry), Dr T M Sutherland (chemical physiology) *Research fellowships*, Dr R R Hillier (physics), Dr J Malos (physics)

East Anglia

Mr M B Glauert, at present senior lecturer in the Department of Applied Mathematics in the University of Manchester, has been appointed professor of mathematics in the School of Mathematical and Physical Sciences Dr. N Sheppard, assistant director of research in spectroscopy and lecturer in chemistry in the University of Cambridge, has been appointed professor of chemistry in the School of Chemical Sciences Dr I Gibson has been appointed lecturer in biology in the School of Biological Sciences

London

Prof. C A Fisher, professor of geography in the University of Sheffield, has been appointed to the chair of geography (with reference to Asia), tenable at the School of Oriental and African Studies, from October 1, 1964 The title of professor of industrial and management engineering has been conferred on Prof S Eilon, in respect of his post at Imperial College of Science and Technology

Announcements

Mr E W Madge, of the Dunlop Rubber Co , Ltd , has been awarded the Colwyn Medal for 1963 of the Institution of the Rubber Industry, for his scientific and technological services to the industry Dr F H Cotton, head of the National College of Rubber Technology, London, has been awarded the Hancock Medal, for his services to the Institution and the rubber industry

The *Year Book of the Royal Society of Edinburgh, 1963*, covering the session 1961–62, includes, besides the obituary notices of Fellows, the proceedings of ordinary meetings, lists of recent awards, the accounts of the Society and the list of Fellows at October 22, 1962 (Pp. 144 Edinburgh Royal Society of Edinburgh, 1963 20*s* , 3 50 dollars)

Mr G L'E Turner, Museum of the History of Science, Broad Street, Oxford, writes "I am investigating the development of British microscopes in the nineteenth century For the investigation of the resolving power of some historical instruments I wish to have the use of a Nobert test plate and a Grayson rulings I am wondering if there is a reader in possession of one or other of these probe plates who would be prepared to lend it to me for this work If unable to lend such a plate, I should nevertheless be glad to hear of its whereabouts and any details of its construction and history "

A symposium on "Productivity in Research", arranged by the Institution of Chemical Engineers, will be held in London during December 11–12 The programme will include sessions on· the policy of higher industrial management, organization and interaction in research success, the effect of environment Further information can be obtained from the Institution of Chemical Engineers, 16 Belgrave Square, London, S W 1.

The third international congress on catalysis will be held in Amsterdam during July 20–25, 1964 The congress will be devoted to a discussion of the mechanism of heterogeneous catalysis, and papers will be presented on molecular description of the catalytic reaction and its intermediate states and selectivity in heterogeneous catalysis Further information can be obtained from the secretary of the congress, Dr D M Brouwer, P O Box 3003, Amsterdam

A symposium on "Treatment of Pain in Incurable Disease" will be held at Addenbrooke's Hospital, Cambridge on January 18 The programme will include researches on the action of phenol on nerve fibres, films of the treatment of spastic conditions with phenol, recent advances in the treatment of cancer pain, analgesic and other drugs in the treatment of chronic pain; the surgery of pain The fee for the symposium will be 1 guinea Further information can be obtained from the Secretary of the Medical School, Tennis Court Road, Cambridge

Erratum In an advertisement (*Nature*, 199, diii, 1963) of books published by the International Society of Plant Morphologists, Department of Botany, University of Delhi, Delhi 6, one title was erroneously given as *Plant Tissue and Organic Culture—A Symposium* For *Organic* read *Organ*

PARTICLE SIZE ANALYSIS AND FLOW OF POWDERS

THE second regional meeting on "Particle Size Analysis" was held on October 2 at the University of Sheffield under the auspices of the Whiting and Industrial Powders Research Council Like the first meeting at Hatfield, it attracted an audience of about 200 people from all parts of the country, and on this occasion one person from as far away as Norway There was also an opportunity of visiting a specially arranged exhibition of particle size analysis equipment, at which sixteen exhibitors displayed twenty-four instruments The day was divided into two sessions, particle size analysis being discussed in the morning and the flow of powders in the afternoon

The chairman of the morning session was Mr D C Soul, director of research of the Whiting and Industrial Powders Research Council The first lecture, by Dr B H Kaye, also of the Whiting and Industrial Powders Research Council, entitled "A Random Number Technique for Investigating the Accuracy of Microscope Methods of Particle Size Analysis", gave, as he himself said, the mathematically less-expert particle size analyst a 'do-it-yourself kit' for establishing the confidence limits to be placed in a given microscope particle count, or to compute the number of counts necessary to give a definite confidence The method depends on assigning numbers corresponding to those in the size classes of particles found or anticipated, then to obtain random numbers from tables or otherwise, and interpret each number as a particle Repetition of this process, involving no mathematics at all, allows a number of curves to be drawn, which will be enclosed by a 'scatterband' indicative of the purely statistical variations to be anticipated, but stripped of all experimental or operators' errors The technique was illustrated by discussions of several typical analyses A technique of 'stratified' sampling, in which the particles under test are divided into overlapping groups, which are counted separately and later recombined, was shown to give narrower confidence limits at no extra effort The questions dealt with the need for preliminary counts of unknown samples, 'homogenizing the results of the stratified groups to a unified whole, and the fact that the confidence limits of the whole, the average size, will be better than that of any particular group, which might be of interest, for example, for unwanted coarse material

The second lecture on "The Use of the Optical Microscope in Particle Size Analysis", by Dr V Timbrell, was read in his absence by three of his co-workers at the Pneumoconiosis Research Unit of the Medical Research Council and the Welsh School of Pharmacy Mr Reynolds described the electro-optical arrangements of the 'double image' microscope designed by Dr Timbrell, in which two images of the same particle are produced by a vibrating mirror The amplitude of vibration is adjusted until the images are just contiguous, when the particle diameter in the direction of vibration can be read from a calibrated meter Mr Cook discussed the advantages to be gained by this method, which lends itself to a certain degree of automation by comparison with the graticule method Dr Barnett amplified this theme and supported it with figures showing the vastly increased reproducibility in inter- and intra-operator comparisons, because the operator has only to find the position when two images precisely join, rather than estimate the sizes of irregular areas Up to 400 particles can be counted in 10 min The questions were concerned with the price and availability of the microscope, the need or otherwise to make sure of random orientation of the particles, the possibility of using a projection method or examining photomicrographs rather than slides, and methods of dispersing certain particles for mounting on slides Interesting answers to these latter questions suggested thixotropic liquids and, for completely three-dimensional random distribution, dispersing the particles in a thermosetting resin and eventually preparing a section

Dr J A Kitchener, of the Royal School of Mines, took the chair for the afternoon session During the first lecture, "The Flow of Powders", Dr J C Williams, principal lecturer in powder technology at the Bradford Institute of Technology, gave a series of demonstrations to show the segregation of powders by size when being filled into a hopper Fertilizer fractions differing 5 · 1 in diameter, the smaller particles being dyed green or black, segregated distinctly in the 'Perspex' containers and hoppers The effect is caused by the fact that the smaller particles cannot flow down the slope of the heap, which is dotted with excessively large 'potholes'. Hoppers with more nearly vertical walls exhibit this phenomenon less Similar demonstrations and slides showed the absence of flow at or near the hopper walls as they were emptied This means that all except monodisperse powders will unmix when being charged into or discharged from a hopper, a phenomenon serious enough to merit close attention The questions elicited a number of interesting facts—to name but one, that density and shape factors may also promote segregation, but in a conflict of factors, size is always predominant Some desegregation becomes noticeable at particle diameter ratios 1 1 2.

Mr R L Brown, deputy director of the British Coal Utilization Research Association, discussed "Angles of Friction and Sliding of Non-Cohesive Powders" The angle of repose is the steepest angle against the horizontal of a heap of material at rest, the angle of sliding is the angle to the horizontal of the interface between stationary and moving material of a bed of powder discharging through an aperture These angles depend not only on the material itself, but also on the geometry of the container and the manner of forming the heap, stress distributions are all-important, so that reproducible but mutually exclusive results may be obtained Departure of the particles from sphericity and the presence of fines tend to increase the angles In order to relate them to a more fundamental property, the angle of internal friction observed when a powder is sheared, is introduced; except against very rough surfaces, the angle of external friction is usually smaller Internal friction and sliding as shown by the crater above the circular discharge aperture appear to be related There are considerable differences in results derived from two- and three-dimensional surfaces, for example, in long, rectangular hoppers as against circular ones Questions concerned the coating of powders, which may aid flow by destroying aggregates, different forms of 'bridging' in hoppers, and the advantages (starting a movement) and disadvantages (compaction) of vibrators in hoppers

The last speaker of the day, Dr L Cohen, of Simon-Carves, Ltd , discussed "The Flow of Cohesive Bulk Solids" Rather than see how particle properties can be made to explain bulk properties, the lecturer described Prof Jenike's approach of considering the heap as a homogenous, isotropic, plastic material developing strength under its own compacting weight, a conception that has also been found fruitful in soil mechanics The principles of the Jenike shear cell, in which a compacted powder is sheared (*a*) across its bulk and (*b*) against a solid surface were explained The powder breaks down and begins to flow when its cohesive shear strength is exceeded The practical history of the powder (dampness, temperature, residence in the hopper) is of crucial importance, but given the right conditions of flow function can be derived which is an invaluable aid to hopper design, and which is capable of forecasting differences in flow behaviour of otherwise seemingly identical powders The questions dealt with the design of bunkers, the degree of standardization of Jenike's procedure and its applicability to damp and wet materials, the treatment of powders of very low bulk density (magnesium carbonate at 8–9 lb /ft ^{3}), mechanical aids to hopper discharge and the effects of flow-promoters

The meeting was closed with a vote of thanks to speakers, audience and organizers by Dr Kitchener, who also announced that preparations for a third regional meeting on powder technology topics in the spring were under way

TRANSPORT AND TRAFFIC NOISE

A SYMPOSIUM on "Transport and Traffic Noise" was held in the University of Southampton during September 27–28 The symposium, which was sponsored by the Society of Acoustic Technology and the Institute of Sound and Vibration Research of the University of Southampton, was attended by about 130 people representing various firms related to the motor industry, local government authorities, universities and colleges of advanced technology

The symposium was opened with a lecture by Prof E J Richards, the director of the Institute of Sound and Vibration, on the impact of the report of the Committee on the Problem of Noise, headed by Sir Alan Wilson Prof Richards, who was a member of the Committee, directed attention to the startling increase, which occurred between the 1948 and 1961 noise surveys, in the number of people who notice noise outside their homes—42 per cent in 1948 and 91 per cent in 1961 The statistics given in the report suggest that road traffic noise is largely to blame for this increase In fact, of the various external noises, 36 per cent of people questioned in 1961 found road traffic noise disturbing, compared with 9 per cent who were disturbed by aircraft noise Prof Richards discussed the findings of the Wilson Committee in general terms and described some of the recommendations which it had made

The second lecture was given by Dr B W Robinson (National Physical Laboratory) on the measurement of noise Dr Robinson outlined the various units which have been introduced for the measurement of noise and discussed the relation between the objective (db), the frequency weighted decibel (dbA), the unit of subjective assessment (phon), and the attempts which had been made by Stevens, Zwicker and others, to compute a single loudness figure which agrees closely with the phon Dr Robinson also discussed the standard practices which have been suggested for rating the noisiness of motor vehicles and which may soon be implemented by law

The afternoon session was devoted to two technical lectures, by Dr T Priede (C A V, Ltd, Warple Way, London, W 3) and Dr P O A L Davies (Institute of Sound and Vibration Research) Dr Priede discussed the subject of engine noise, with particular reference to Diesel engine noise, illustrating his talk not only with slides, but also with tape recordings, which proved to be a much more efficient means of showing the result of decreasing a noise by a few decibels Dr Priede discussed the mechanics by which noise was created in a Diesel engine and described some of the extensive investigations which have been carried out at C A V, Ltd, to reduce it Special mention was made of air-intake noise, the reduction in noise which may be obtained by smoothing the cylinder pressure, and the noise produced by resonance of the engine casing

In his lecture on silencer design, Dr Davies compared the results which may be obtained from the traditional acoustic theory of silencers, and the new shock wave theory which has been investigated by him and his co-workers at Southampton Single-pulse shock wave theory can be used in discussing an exhaust system, because for a typical four-stroke engine running at 3,000 r p m the primary pulses travel down the exhaust pipe at intervals of about 18 ft Dr Davies showed that the single pulse theory may be used to give a picture of the gas flow through the exhaust system, to calculate the back pressure, and to determine the attenuation which will be produced by the system The acoustic filter theory still has its place in the field of harmonic response, but the single pulse theory describes the transient response in a way the older theory could never do

The evening session of the conference consisted of an open lecture by Mr H Purkis, of the Building Research Station, on the subject of transport noise and town planning. Mr Purkis first directed attention to the way in which noise nuisance has been growing and is likely to grow in the future, owing to the tremendous increase in vehicle ownership and industrialization He then discussed some of the proposals which had been suggested for combating the nuisance in new towns Motor-vehicles could not be prohibited from a town without killing all commerce, and it could not be expected that the noise-levels which were to be laid down for cars would be able to counteract the increase in overall noise-level which would be produced by the increasing number of vehicles

Since overall noise-level must be expected to increase, means must be found to prevent the noise reaching houses, shops and offices Mr Purkis discussed sunken roads, smooth flowing motor-ways lined with specially insulated buildings to prevent the noise permeating to other dwellings, as well as some of the unsuccessful (that is, noise-producing) devices which were being used by traffic engineers to-day—one-way streets, which lead to faster, more noisy, traffic, and the use of side streets which distributes the noise to parts of the town which were previously quiet

On the Saturday morning, consideration was given to the problem of noise as it affects the passengers in a car The first lecturer was Dr G M L Gladwell (Institute of Sound and Vibration Research), who presented a review of the literature on the subject He paid particular attention to the noise caused by the passage of the tyres over the road, to the way in which this was transmitted through the car structure, and to the mechanical/acoustic properties of the passenger cabin He also stressed the need for more fundamental investigation into the vibration characteristics of a car, and the use of electronic analogue computers to make the investigation of the vibration characteristics of a projected design more logical and less a matter of guesswork In the extended discussion period following this review, representatives from Peugeot, Ford, Humber, Pirelli and Dunlop contributed from their own experience The discussion centred on the road noise problem M J Genestier, director of the Peugeot Research Laboratory, explained how they had devised the suspension system of their 404 model specifically for the Michelin *X* tyre, a tyre which excites vibration in a frequency-range much narrower than others, and had managed so to tune the suspension system that the vibrations that were excited were not transmitted to the car Other representatives stressed the need for car and tyre manufacturers to co-operate, and consider when designing a car the characteristics of the tyres which were to be fitted

Dr A Chiesa, from the Pirelli rubber laboratory in Milan, gave the next lecture on some experimental investigations which they had undertaken on the noise inside cars After a general discussion on the various origins of the noise, Dr Chiesa described the basic experimental method which is used The noise produced in a running car varies in intensity and in frequency content It must therefore be investigated by using statistical methods Most of the important information about the noise could be obtained from its frequency spectrum, and Dr Chiesa discussed the instruments which were used in the Pirelli laboratories to obtain this spectrum completely automatically He then showed how the various kinds of vibration could be distinguished by an inspection of the spectrum, illustrating his remarks with slides of typical spectra obtained by driving over various kinds of road Dr Chiesa again emphasized the importance of co-operation between car and tyre manufacturers, and described the kind of research programme they carry out when a certain manufacturer considers fitting one of their tyres

The final lecture of the symposium was on the subject of hovercraft noise and was given by Prof Richards He began by pointing out that hovercraft present an extremely difficult noise problem, much greater than that of conventional aircraft or helicopters They operate at ground-level, very often near to dwellings, their most likely means of propulsion is an airscrew, which is basically noisy, and at present they develop very high horse-power in a relatively flimsy structure Prof Richards went on to define the various noise sources—engines, propellers, fans, air jets, etc All these sources can generate noise, which in turn can produce resonances in the aircraft structure He then stressed that a fair estimate of the likely noise production of a hovercraft could not be obtained from prototypes, which inevitably were constructed from components already in use in aircraft, motor-cars, etc , and which were not designed specifically for hovercraft Provided that proper attention was directed to the components, the design of the hovercraft could undoubtedly be modified to minimize the noise that was generated Prof Richards directed attention to the fact that the noise problem for conventional aircraft was realized only when they had been in use for many years, but that, provided sufficient pressure was exerted on designers, it could and should be dealt with at an early stage of the development of hovercraft

Reports on the research work on which some of the lectures were based are to be published in forthcoming issues of the new *Journal of Sound and Vibration* (Academic Press, Inc , London, Ltd)

G M L GLADWELL

FUNDAMENTAL ASPECTS OF COMBUSTION

THE annual Coal Science Lecture of the British Coal Utilization Research Association was established in 1951 in the belief—to use the words of Sir Charles Ellis, then its president—"that a Research Association should in its own particular field play a part analogous to that of the great learned societies and should, in addition to applying knowledge and attempting to produce immediate results, also take its share of responsibility in the progress of the fundamental work of its subject" The previous Lectures, which have proved eminently successful, were given by eight leading British scientists and three from overseas Prof D. W van Krevelen (University of Delft), Monsieur R Cheradame (Centre d'Etudes et Recherches des Charbonnages de France). and in 1962, Mr H Seidl (Deutsche Babcock and Wilcox Dampfkesselwerke)

The twelfth of the series was delivered by Dr D T A. Townend at the Institution of Civil Engineers on October 16 Dr Townend, who was until 1946 Livesey professor of coal gas and fuel industries in the University of Leeds, retired as director general of the British Coal Utilization Research Association in September 1962 and, when inviting him to give the lecture, the Association's Council suggested that he should speak on a subject to which his earlier work on the fundamental aspects of combustion was relevant hence his title "Some Reflexions on Combustion"

Dr Townend said that, on reflecting on a suitable theme, he had been led more and more to appreciate how dominating an influence the combustion of the elementary combustibles, hydrogen and carbon monoxide, must play in the overall combustion and gasification of all solid fuels For coal was first pyrolysed to yield combustible gases, volatile hydrocarbons and coke, the primary combustible gases being hydrogen and methane, and at temperatures above 700° C, hydrogen was almost the sole product. Carbon monoxide was the primary product of the oxidation of coke and the volatile hydrocarbons yielded on pyrolysis ultimately hydrogen and carbon Finally, even hydrocarbons were first transformed essentially into carbon monoxide and hydrogen before their overall combustion was completed Some 30 per cent of the potential heat in coal was released during the production of carbon monoxide by the direct oxidation of carbonaceous material, and the remainder was released from the combustion of carbon monoxide and hydrogen The presence of hydrogen and carbon monoxide in the combustion gases within a pulverized-fuel-fired furnace was illustrated from the present work of the International Flame Foundation at Ijmuiden

Dr Townend next directed attention to the important part played by hydrogen and oxygen atoms in the chain mechanisms inherent in the combustion of hydrogen and carbon monoxide, in flames their concentrations were greater than would be expected from theoretical considerations The presence of hydrogen atoms was readily demonstrated by the phenomenon of candoluminescence whereby certain activators (for example, oxides of bismuth, manganese and lead) added to the oxides of host materials (for example, calcium, strontium and barium) cause a characteristic light emission (fluorescent spectra) when brought in close proximity to the flames of hydrogen-containing combustibles The hydrogen atoms recombine, however, in the presence of diluent gases and at critical diluent concentrations the light emission is repressed Moreover, at the point of the repression (*a*) the flames of hydrocarbons are rendered non-luminous, and (*b*) in the presence of sodium salts the yellow *D*-line emission is also quenched In further illustration of the part played by hydrogen atoms and their recombination by molecular collisions or on surfaces, it was shown how the heat conduction from a hydrogen flame to a surface was 20 per cent greater than that from a fairly dry carbon monoxide flame, although the calorific values are much the same

The presence of oxygen atoms in carbon monoxide flames had been established some years ago in connexion with work on boiler availability, and there was little doubt that they were mainly responsible for the oxidation of sulphur dioxide to sulphur trioxide with the ultimate formation of sulphuric acid

Another important problem concerned with the combustion of carbonaceous fuels was the presence of chemisorbed layers on the fuel surface At low temperatures, an unstable carbon–oxygen–water-complex (peroxygen) controlled the oxidation of the coal substance, at about 70°–80° C its breakdown could lead to rapid oxidation and with certain fuels to spontaneous ignition At combustion temperatures the presence of a layer, defined as oxycarbon, was an important factor in the combustion process

Reviewing the overall picture, Dr Townend pointed out that in the practical field a matter of importance was the control of the aerodynamics of a combustion system to provide adequate mixing of solid particles with the reactant gases, including hydrogen, carbon dioxide, water and atoms and radicals, for gasification and oxidation were mutually involved He also considered the likely advantages to be gained by combustion under pressure, having regard to the chemical and physical factors involved

In conclusion, Dr Townend emphasized that many combustion phenomena accepted to-day as commonplace would not have been predicted forty years ago, and as an illustration he mentioned the manner of his discovery of the two-stage process of the ignition of hydrocarbons which had proved the key to the elucidation of the problem of 'knock' in internal combustion engines

After the lecture a dinner in honour of Dr Townend was held at the Savoy Hotel, at this function the Coal Science Medal was presented to Dr Townend by Mr E H. Browne, president of the British Coal Utilization Research Association

A CHANNEL LINK

ALTHOUGH the report of the working group of British and French officials on *Proposals for a Fixed Channel Link*[*] was published two months later than the Broadsheet *Transport in the Common Market*, issued by Political and Economic Planning in July[†], it is significant that in the Broadsheet Mrs Trench makes no reference at all to either a tunnel or a bridge for the English Channel After indicating the three objectives of a common transport—removal of obstacles which transport would offer to the establishment of the general Common Market, creation of healthy competition of the widest possible scope, provision of means of transport meeting in quantity and in quality the requirements of the European Economic Community—the Broadsheet reviews the measures proposed These include a tariff policy for freight, and after considering the general proposals the Broadsheet considers international transport by road and the domestic road transport of goods

The investigation finds that there is still no sign of an agreement between member States on the proposals or even of the policy being implemented in 1963–65, and it is unlikely that there will be any substantial progress towards implementing a common policy until after this The proposals in the Action Programme for the Common Transport Policy presented to the Council of Ministers in May 1963 are themselves the result of compromises between conflicting objectives of the policy and also between existing practices in the member States, they are so framed that they stand some chance of being acceptable to all the countries in spite of the differences between their present transport systems The objectives of the policy are much more ambitious and extensive than the minimum necessary to secure the free working of the Common Market, and the instruments chosen, notably the system of forked tariffs, have far-reaching implications Nevertheless, some of the major transport problems are left untouched Own-account transport of goods is left free to develop in a way which could well frustrate the objectives of the policy to secure an optimal use of transport facilities of the Community as a whole There are no proposals about private cars, although the growth in their numbers and the pattern of their use affect road expenditure and traffic congestion, and thus the cost of operating goods vehicles, as well as the passenger revenue of the railways, the size of the railway system and its costs of provision

Any attempt to secure a real improvement in a community's use of transport resources should, the Broadsheet points out, rest on a full investigation of the social benefits and costs of transport operations, and the Commission is at present studying 'infra-structure costs' in order to reconstruct the basis of taxation on different forms of transport to redistribute the burden of Government expenditure as between different users It is suggested that the Commission should broaden these investigations to take into account costs and benefits dispersed among the community as a whole as a result of the expansion or contraction of different kinds of transport Policy could then be based on a much better knowledge of what different developments are likely to cost the community in terms of lost amenities, increased congestion, reduced scope and frequency of public services, etc

This kind of consideration is lacking in the report on the proposals for a Channel tunnel and Channel bridge Unfortunately, the report is also markedly lacking in either imagination or enthusiasm, and it appears to be preoccupied with financial and economic considerations in the narrower sense to the exclusion of social and political and even technical aspects whether short or long term Charged with considering the project for a tunnel proposed in March 1960 by the Channel Tunnel Study Group and the project for a bridge proposed in October 1961 by the Channel Bridge Study Group, its findings are in favour of the rail tunnel project It finds this means satisfactory from the technical point of view and constituting from the economic point of view a preferable solution to the continued use and development of established means of transport, while at the same time being free from the practical disadvantages of the bridge—which is likely to have a capital cost almost double The working group foresees no increase in traffic or other advantage to offset this higher cost, and even the financing of the rail tunnel it regards as an uncertain risk and recommends that the two Governments should contemplate direct guarantee of loans, but also that the bounds of the privately owned undertaking should be guaranteed This would be a new departure in United Kingdom practices and one that in France is only used on an extremely limited scale

It is recognized that the bridge, which offers a continuous road and rail Channel link in the open, is a more attractive proposal to road users than the tunnel project, in which vehicles are driven on and off special car ferry trains operating at intervals of 5–30 min according to demand, carrying either 150 or 300 cars, and completing the journey in an average of 65 min, of which 45 min would be occupied by the journey proper The working party regards the bridge as offering a new and serious hazard and source of delay to shipping, also its construction would involve international agreement as to safety regulations for sea traffic as well as to the bridge in principle It does not consider that these objections would be removed by foreseeable developments in navigational aids within the next few years However, it did not seriously consider a third proposal, for an immersed road-rail tunnel, which was submitted in March 1963 and for which the capital cost is estimated to be £236 million, compared with £329 million for the bridge and £160 million for the rail tunnel

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[*] Ministry of Transport *Proposals for a Fixed Channel Link* Pp iv+60. (Cmnd 2147) (London H M S O, 1963) 6*s* 6*d* net

[†] *Planning*, Vol 29, No 473 (8 July, 1963) *Transport in the Common Market* Pp 223–286 By Sylvia Trench (London Political and Economic Planning 1963) 6*s*

The proposals for a bridge and for a road tunnel both rely largely on tax reliefs to improve the financial prospects of their respective projects, but the risks do not appear to be excessively high, and while the means of Government assistance here proposed may be open to objection, the decision as to whether the project should be supported or not is one for Government level The decision, moreover, is urgent and ought not to be postponed If the determining considerations are those that fall outside the scope of the working party itself, and even if the proposals represent little that could not have been advanced half a century or more earlier, to delay is only to increase the ultimate cost of a favourable decision—a cost that has multiplied many times since the proposal was first advanced in the last century (see, for example, *Nature*, 1, 160, 303, 631, 1869)

EMPLOYMENT OF WOMEN IN INDUSTRY

A RECENT investigation of the industrial employment of women throughout their middle working lives*, to which the Director of the Nuffield Foundation contributes a foreword, is essentially in continuation of the investigations which the Foundation has for some time been promoting on the employment prospects of older men Insisting that the basic unit of society is the indissoluble complex of relationships that controls both men and women, Mr Le Gros Clark takes account of this subtle but changing element in dealing with the influx of women into employment. Considering first the life rhythms of modern women he concludes that there will be far fewer single women in middle life and a diminishing number of widows, though this large impact of widowhood will not be felt until most married women are passing beyond their employable age Of necessity, if employers still need female labour, they will have to depend on married women, and mostly on those of mature years Moreover, Mr Le Gros Clark insists also on the importance of the contribution which married women and mothers make outside the labour market, he regards as misleading the classification of many women by economists as merely 'dependants' Women as a whole are not free to go out to work until they feel that they have among them accounted for their multifarious domestic and family commitments

His analysis of available and relevant figures of part-time employment indicates that age makes remarkably little difference in the opportunities most women have of limiting the hours they work, and that the older women still have their domestic commitments as well as some impairment of health in mind when they stipulate for a part-time job Most of them would also seem to take it for granted that at their age they may have to gravitate to a catering. domestic or cleaning job Now that working hours per week are contracting in most industries, the stipulations made by many married women seem likely to appear more realistic It is not easy to say at what ages and for what reasons women finally move out of the labour market, but, in the present state of medical knowledge, women seem more susceptible than men to the kinds of ailment which one might expect to hasten their withdrawal from work outside their homes

Their rates of withdrawal from the manufacturing industries are most noticeable, and while the possibility of moving the older married women to lighter or modified work requires examination, it can at least be said that, although most middle-aged women are not on such exacting work, the differential withdrawal rate among those who are could easily affect the average rate at which women leave employment At present they do so in substantial numbers in their early fifties, and probably nearly one in three has departed by the time they approach sixty Some leave by choice and some subject to retirement regulations, but a proportion which is not negligible complain of industrial strain or incipient ill-health, so that women still at work in their late sixties represent only about a quarter of those who are at work ten years younger, and most are working on their own or managing the work of others, or in some 'domestic' form of employment

Mr Le Gros Clark suggests some of the female–male adjustments that are likely to be needed particularly if middle-aged married women are required in employment In large measure he predicts they will be able to make their own terms, particularly about their hours of work This is likely to be felt particularly in health, welfare and educational services, but he suggests too that the amenities, the codes of behaviour and the conventions habitual through industry are likely to be affected ultimately He points to the desirability, in the general education of girls, of making an effort to break down the barrier between the sciences and the arts and of stressing the æsthetic qualities of the former subjects This is largely a matter of finding imaginative teachers, but it has a great bearing on equipping girls for life, especially as, ultimately, married women may have to show themselves more prepared to take on posts of skill and responsibility

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* *Woman, Work and Age To Study the Employment of Working Women Throughout Their Middle Lives* By F Le Gros Clark Pp 111 (London The Nuffield Foundation, 1963) 7*s* 6*d* net

SCIENTIFIC RESEARCH IN BELGIUM

THE annual report for 1962 of the National Council for Scientific Policy, Brussels*, is of special interest in view of the present discussions on scientific policy in Western Europe generally Moreover, it exemplifies the type of report which the Office of the Ministry for Science might be expected to provide regularly for the United Kingdom and which, on a lesser scale, the annual report of the Advisory Council on Scientific Policy has sometimes supplied in part Following an introduction, the report is in two parts the first outlines the functions of the Council, including the general structure of scientific work in Belgium, the second records the activities of the Council during December 1, 1961–December 1, 1962, including those of its committees and institutions and Government measures affecting the work of the Council Among appendixes which list the membership of various committees, etc, is a note on the meeting of the Ministerial Committee for Scientific Policy and the Bureau of the Council last July at which the Budget for 1963 was fixed at 4,904 million francs, a 20 per cent increase on the previous year, and including an 18 per cent increase for the universities

The first part of the report is of the most general interest The budget for 1962 was for 4,080 million francs, an increase of 14 4 per cent over 1961 Of this, 2,316 million francs were for education, 1,156 million francs for economic interests, 179 million francs for agriculture, 153 million francs for defence (a decrease of 9 per cent) and 77 million francs for public health By institutions the distribution of the budget is as follows (figures in brackets give the percentage increase on 1961) universities and other higher educational institutions, 2,434 million francs (11 2), State scientific establishments, 748 million francs (11 7), public service institutions supported by the State, 627 million francs (27), industrial enterprises and co-operative centres, 170 million francs (16 4), and international organizations, 101 million francs (– 6 5) By sciences, etc, the distribution is as follows fundamental research, 533 million francs (16), of which 221 million francs was for the exact sciences, 114 million francs for biology and other natural sciences, and 80 million francs for the medical sciences, 601 million francs for applied research (18), of which 276 million francs was for agriculture and veterinary medicine and 325 million francs for industrial research, 826 million francs for nuclear science (19 7) and 120 million francs for economic and social science (33 3)

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* Conseil National de la Politique Scientifique Rapport Annuel, 1962 Pp 171 (Bruxelles Conseil National de la Politique Scientifique, 1963)

The second chapter of this first part of the report analyses further the research effort as supported by the Foundation for Scientific Research, and in special fields such as nuclear energy and space research, for which 14 8 million francs was allocated among four groups Fifteen groups working on molecular biology received 26 million francs, six on medical enzymology, 3 million francs, and seven on human genetics, 4 5 million francs Information regarding the universities, similar to, but less detailed than, that supplied in the annual returns from the University Grants Committee, is contained in the following chapter, which also includes figures for the support and staff of national scientific institutions Student numbers at the four universities of Ghent, Liège, Brussels and Louvain have risen by 23 7 per cent since 1958 to 28,346 in 1962, and other institutions of higher learning bring this total to 32,726 University grants have increased in the same period from 646 to 1,336 5 million francs

STRYCHNINE-RESISTANT CENTRAL INHIBITION

Strychnine-resistant Inhibition in the Brain

In the spinal cord it has been shown that strychnine very effectively depresses the activity of the postsynaptic inhibitory synapses which are formed by a variety of pathways converging on motoneurones[1–4] Strychnine depresses the inhibitory action of the olivo-cochlear bundle on sensory fibre terminals and on the hair cells of the organ of Corti[5] Since this alkaloid has this specific depressant action on so many types of inhibitory synapses it has been suggested that it acts competitively against the same inhibitory transmitter substance[6] Large and prolonged inhibitory postsynaptic potentials (IPSPs) have been recorded from a variety of neurones in the higher centres of the brain pyramidal cells of the motor cortex[7–10], of the sensory cortex[11–13] and of the hippocampus[14,15], and also mitral cells of the olfactory bulb[16], cerebellar Purkinje cells[17,18] and thalamic neurones[19–21] If there is the same inhibitory transmitter at all these synapses, it would be expected that strychnine would similarly be a very effective depressant

However, Green, Mancia and Baumgarten[22] and Baumgarten, Green and Mancia[23] found that strychnine failed to block the inhibition of mitral cells and cells of the plexiform layer of the olfactory bulb in response to stimulation of the lateral olfactory tract and of the anterior commissure In the investigation reported here it is shown that strychnine is without any detectable effect on three examples of postsynaptic inhibition in the brain even when given in doses that virtually eliminate postsynaptic inhibition in the spinal cord

Adult cats were anæsthetized by pentobarbital sodium and intracellular recordings made by glass microelectrodes filled with solutions of 3 M potassium chloride (5–40 MΩ) or 2 M potassium citrate (7–30 MΩ) Microelectrodes filled with 4 M sodium chloride (1–4 MΩ) were usually used for extracellular recording

In Fig 1*A* are the intracellular and just extracellular records from a hippocampal pyramidal cell in response to stimulation of the fimbria hippocampi Subtraction of these two records reveals that the pyramidal cell had responded by a typical large and prolonged inhibitory postsynaptic potential In Fig 1*B* it is seen that 10 min after the intravenous injection of 0 3 mg strychnine hydrochloride per kg body-weight a similar inhibitory potential was evoked in another pyramidal cell close to the first Postsynaptic inhibitory potentials of comparable amplitude and duration were observed in 174 out of 178 impaled hippocampal cells in strychnine-free preparations and

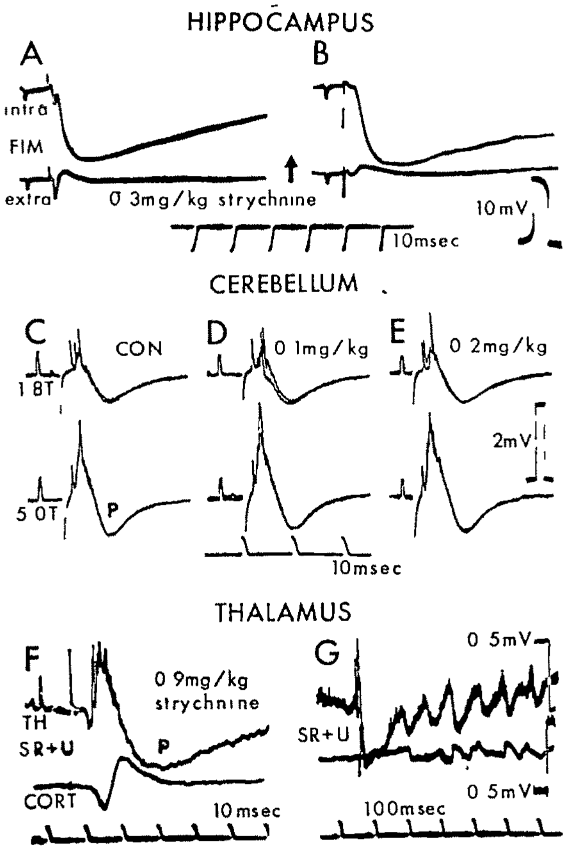

Fig 1 *A*, inhibitory postsynaptic potential from hippocampal pyramidal cell, produced by fimbria stimulation (*FIM*) *B*, similar inhibitory potential after intravenous injection of 0 3 mg strychnine hydrochloride per kg *intra*, intracellular, *extra*, extracellular recording In *A* and *B* positivity is upwards, in *C–G* positivity is downwards *C*, responses evoked by stimulation of parallel fibres at 1 8 and five times threshold (upper and lower traces) and recorded from the layer of Purkinje cell bodies in the cerebellum A negative wave with spike discharges is followed by a positive wave (*P*) which is associated with an inhibitory hyperpolarization of the cell-body membrane (ref 18) *CON*, control response before strychnine *D* and *E* are similar responses after intravenous injection of 0 1 and 0 2 mg strychnine hydrochloride per kg, respectively *F*, responses recorded from the ventrobasal complex of the thalamus (*TH*) and the post-cruciate gyrus (*CORT*) in response to a combined volley in the contralateral superficial radial (*SR*) and ulnar (*U*) nerves after intravenous injection of 0 9 mg/kg strychnine hydrochloride Both the thalamic positive wave (*P*) in *F* and the repetitive *P*-waves in *G* are associated with large inhibitory postsynaptic potentials (refs 20, 21) and are resistant to this large dose of strychnine

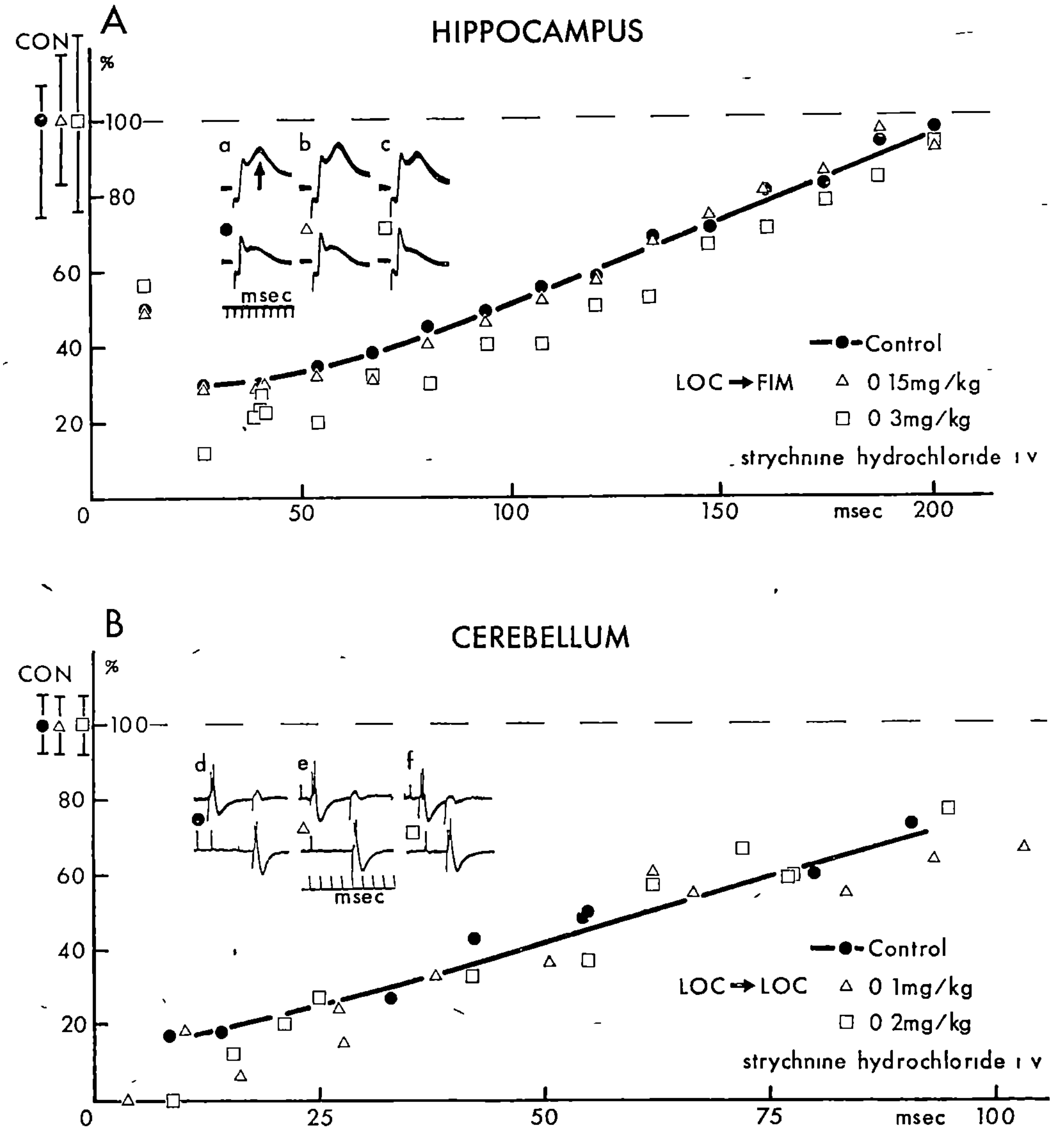

Fig. 2. *A*, upper traces in inset records are surface hippocampal responses to stimulation of fimbria. The first deflexion is due to antidromic invasion of pyramidal cells, the second (arrow) to synaptic excitation of these cells. The lower traces are the responses to the same test stimulus preceded 42 msec earlier by a conditioning local volley (*LOC→FIM*)—*a* is the control response, *b* and *c* are taken after intravenous strychnine injections. Symbols and doses indicated on the right in the figure. In the graph the size of the synaptically evoked pyramidal cell response to test volley is plotted against the conditioning-test interval. The inhibition lasts for about 200 msec and is unaltered by strychnine in doses up to 0·3 mg/kg. *B*, upper traces in insets show inhibitory effect of parallel fibre stimulation on the response of cerebellar Purkinje cells to a second and similar stimulus (*LOC→LOC*). Lower traces are test responses only. *d* is the control response, *e* and *f* the responses after strychnine administration. Symbols and doses indicated to the right. In the graph, the size of the negative wave is plotted against conditioning-test interval. Inhibition lasts more than 100 msec and is unaffected by 0·2 mg/kg strychnine hydrochloride. *CON*, control value, vertical bars indicating the scatter.

in every one of the 25 cells impaled after intravenous strychnine administration in doses of 0·3 mg/kg or higher.

In the cerebellum strychnine is likewise without action on the large extracellular positive potentials (Fig. 1*C*, *D*, *E*) which are produced by postsynaptic inhibitory action on Purkinje cells and recorded just outside the cell bodies[18]. Similarly, in the thalamus the large inhibitory postsynaptic potentials in the relay cells of the ventrobasal complex have as their counterparts large positive waves (*P*-waves) extracellularly[20,21]. Fig. 1*F* shows that the initial part of the *P*-wave set up by a large cutaneous volley was present and of normal size after a total dose of 0·9 mg/kg of strychnine, and in Fig. 1*G* there was still the normal series of rhythmic *P*-waves with associated burst responses. The intracellularly recorded counterpart of this response pattern is a series of large IPSPs.

In the hippocampus and the cerebellum strychnine also does not depress inhibitory actions such as those displayed in Fig. 2. In Fig. 2*A* the upper row of the insets show hippocampal surface records of responses to fimbrial stimulation. The first deflexion is a sign of the antidromic invasion into pyramidal cells, whereas the second wave denotes the synaptic activation of the same neurones. The lower row illustrates the effect of a local shock preceding the fimbrial stimulation by 42 msec, *a* gives the results before, and *b* and *c* after, the intravenous injection of 0·15 and 0·3 mg/kg strychnine hydrochloride, respectively. The graph in Fig. 2*A* is a plotting of the sizes of the synaptically evoked deflexion to fimbria stimulation against the time after a local stimulus, measured in per cent of the control value. The filled circles show the results before the strychnine administration, the open triangles after 0·15 mg/kg, and the open squares give the results after 0·3 mg/kg strychnine hydrochloride. Fig. 2*B* is a similar plotting for the cerebellar inhibition. The response here is the second of two potentials evoked by local stimulation of the parallel fibres about 2 mm from the recording electrode The latter was situated at the Purkinje cell body layer and picked up the *P*-wave associated with the inhibitory postsynaptic potential (cf Fig 1*C*, *D*, *E*) Filled circles give the control responses, open triangles the responses after 0 1 mg/kg and open squares the responses after 0 2 mg/kg strychnine hydrochloride The insets *d*, *e* and *f* show the inhibition of the second response after the same doses respectively The upper traces show the inhibition of the test response, the lower traces are the test responses alone Neither in the hippocampus nor in the cerebellum inhibitory curves is there any trace of a depressant action by strychnine Crawford, Curtis, Voorhoeve and Wilson (following communication) have also found that strychnine has no depressant action on inhibition of spontaneous or drug-induced repetitive firing of Purkinje cells, nor on the inhibition of sensimotor pyramidal cells following antidromic stimulation of the pyramidal tract

Our conclusion is that the powerful inhibitory action on hippocampal pyramidal cells, cerebellar Purkinje cells and ventrobasal thalamic relay cells is resistant even to large doses of strychnine.

It does not seem possible that this failure of strychnine action can be due to blockage by the blood-brain barrier There is ample evidence that the strychnine administration is having a convulsant action at the higher levels of the brain For example, in our experiments there was an increased rate of spontaneous discharge of cells, an augmented background noise and a large increase in the potential waves recorded from the surface of the cerebellum and hippocampus

Thus, there seem to be just two possible explanations for the failure of strychnine action on the inhibitions investigated One is that the transmitter or transmitters responsible for the inhibition examined in these higher centres are different from that which produces strychnine-sensitive postsynaptic inhibition in the spinal cord and the cochlea The other possibility is that the inhibitory transmitter may be the same in all areas mentioned, but receptor sites of the subsynaptic membrane may differ between the various parts of the nervous system Consequently, strychnine may differ in its effect on account of the effectiveness of its competitive attachment to the receptor of the inhibitory synapses on various nerve cells

The strychnine-resistant postsynaptic inhibitions are remarkable for the long duration of the unitary IPSPs and also for the potency of the IPSP Possibly these features are related to the strychnine-resistant property For example, they may arise either because there is a different inhibitory transmitter that is much more efficient or because the transmitter receptor sites react much more effectively Furthermore, the large size of the hyperpolarization suggests that conductance for K^+ ions plays a much larger part than with inhibitory actions on motoneurones[24] However, with all these IPSPs there is also an increased Cl^- ion permeability It will be appreciated that many of the strychnine-resistant inhibitions reported in the literature are due to pre-synaptic inhibition[25]

P Andersen *
J C Eccles
Y Løyning
P E Voorhoeve †

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* Rockefeller Foundation Fellow

† Visiting on a grant from the Netherlands Organization for the Advancement of Pure Research (Z W O)

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Strychnine and Cortical Inhibition

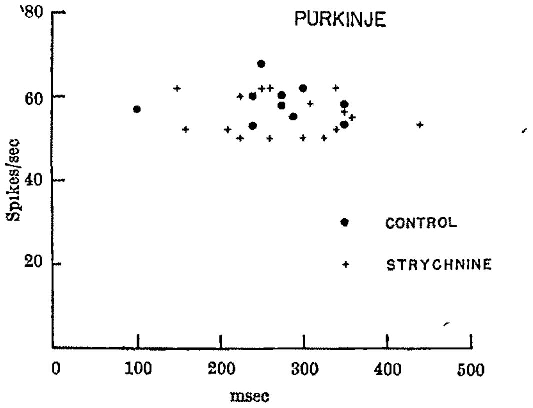
In view of the potent depressant action of strychnine on the postsynaptic hyperpolarizing inhibitory potentials of spinal motoneurones[1], it has been assumed that the enhancement of evoked potentials of the cerebral cortex which is produced by topically administered strychnine is associated with the depression of similar inhibitory potentials of cortical neurones[2] However, strychnine, which also augments the evoked potentials of the cerebellar cortex[3], has been shown not to block the prolonged hyperpolarizing potentials of Purkinje cells which result from local stimulation of this cortex[4] An investigation has therefore been carried out to determine the effect of this alkaloid on the inhibitions of Betz cells in the pericruciate cortex and of Purkinje cells in the vermis of the feline cerebellum

All experiments were performed on artificially respired cats which were anæsthetized with either 'Dial' or pentobarbital sodium, and paralysed with gallamine triethiodide Extracellular spike potentials of single neurones were recorded by means of the central barrel of five-barrelled micropipettes[5] and were photographed from an oscilloscope The cells were excited by the electrophoretic ejection of DL-homocysteic acid from one barrel of the multi-barrelled micropipette[5], and the duration of inhibition which resulted from an appropriate stimulus was measured as the period of suppression of this amino-acid-induced, or the spontaneous, firing The duration of this suppression of firing of both Purkinje and Betz cells was usually inversely related to the pre-stimulus rate of firing, which was varied by suitable adjustment of the electrophoretic current used to eject DL-homocysteic acid

Purkinje cells were identified by the position beneath the surface of the vermis the response to stimulation near the ipsilateral fastigial nucleus and the depression of spontaneous or induced firing which followed electrical stimulation of the surface of the cerebellar cortex within 1–2 mm of the recording site[4] This depression has been shown to be associated with an intracellularly recorded hyperpolarizing inhibitory postsynaptic potential[4] Betz cells were identified by the depth beneath the cortical surface (pre- and post-cruciate) and by the responses evoked by single and tetanic stimulation of the exposed pyramids[6] The spontaneous and amino-acid-induced firing of these cells was suppressed by repetitive stimulation of the pyramids (6 impulses, frequency 100/sec) which produces a hyperpolarizing inhibitory postsynaptic potential of Betz cells[6] ('recurrent' inhibition), and by stimulation of the cortical surface 3–4 mm from the site of recording[7].

Fig. 3 (upper) illustrates results from a Purkinje cell which was discharging spontaneously at a rate of approximately 55 spikes/sec. The control points (●) demonstrate that there was a considerable variation in the period of inhibition produced by local cortical stimulation at an interval of 2 sec. 3 min after 0·33 mg/kg of strychnine hydrochloride had been injected intravenously there was no change in the spontaneous firing rate and no significant alteration in the period of surface-evoked inhibition (+). Strychnine increased the spontaneous firing rate of other cells, possibly as a result of actions elsewhere in the nervous system[3], but nevertheless an examination of the period of inhibition with respect to this new firing rate, and to even higher rates produced by DL-homocysteic acid, failed to demonstrate any significant alteration in the relationship between firing frequency and the duration of inhibition. Similar experiments have shown that picrotoxin (0·9–1·1 mg/kg) and pentamethylene-tetrazol (80–90 mg/kg) are also without action on the afore-mentioned type of cerebellar inhibition.

Fig. 3 (lower) plots a curve for the depression of firing of a Betz cell by stimulation of the ipsilateral pyramid before (●) and 15 min after (+) the intravenous administration of 0·6 mg/kg of strychnine hydrochloride. This figure shows clearly that the relationship between firing frequency and the period of depression was unaltered by strychnine. Similar results were observed with the depression of firing produced by local cortical stimulation, both this and 'recurrent' inhibition of Betz cells were unaffected by doses of strychnine as high as 1·0 mg/kg.



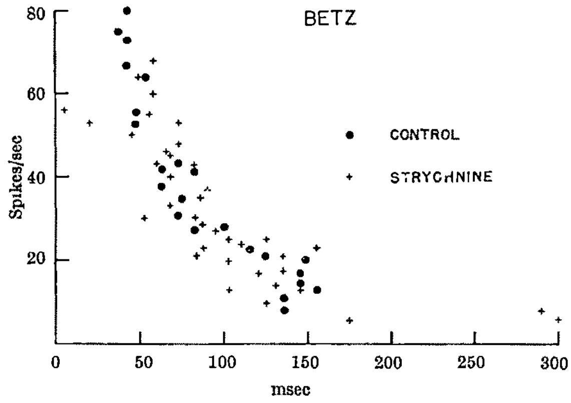


Fig. 3. Upper, duration of surface-evoked inhibition of a Purkinje cell plotted in relation to the spontaneous firing frequency (spikes/sec) before (●) and after (+) the intravenous administration of 0·33 mg/kg of strychnine hydrochloride. Lower, duration of the 'recurrent' inhibition of a Betz cell, evoked by six stimuli (100/sec) applied to the ipsilateral pyramid, plotted in relation to the frequency of firing (spikes/sec) elicited by the electrophoretic ejection of DL-homocysteic acid. Before (●) and after (+) 0·6 mg/kg of strychnine was administered intravenously

These results confirm that the inhibition of cerebellar Purkinje cells resulting from stimulation of the parallel fibres, and which is presumably mediated by the basket cells[4], is resistant to doses of strychnine which are more than adequate to suppress the postsynaptic inhibitions of spinal motoneurones. In addition, the 'recurrent' inhibition of Betz cells, and the inhibition of these cells produced by local cortical stimulation, are also apparently resistant to strychnine. A recent report[8] suggests that in unanæsthetized cats strychnine does block the 'recurrent' inhibition of Betz cells, but the method by which this inhibition was tested, using the invasion of a Betz cell by an antidromic volley, was probably not rigorous enough to permit a distinction to be made between the blockage of inhibition by strychnine and an increase in the background excitatory synaptic drive on the neurone.

Since it is possible that strychnine blocks spinal postsynaptic inhibition by preventing the access of the responsible transmitter substance to the appropriate sub-synaptic receptor sites[1], it may be proposed that this transmitter, presumably common to all strychnine-sensitive inhibitions, differs from the transmitters responsible for the strychnine-resistant postsynaptic inhibitions of cerebral, cerebellar, olfactory[9], thalamic[4] and hippocampal[4] neurones. Alternatively, one substance could be a common transmitter for all these various postsynaptic inhibitions, but, because of differences in nature of sub-synaptic receptors, this substance is blocked by strychnine at some synapses and not at others. It may be pertinent in this respect that in contrast to most forms of spinal postsynaptic inhibition, the hyperpolarizing inhibitory potentials of olfactory, thalamic, hippocampal, cerebral and cerebellar neurones are of prolonged duration. Furthermore, tetanus toxin, which presumably blocks the release of spinal inhibitory transmitters[1], reduces the 'recurrent' inhibition of evoked cortical activity[10], an observation which suggests that there are similarities between spinal and supra-spinal inhibitory transmitters. Preliminary experiments in which electrophoretically administered strychnine excited Purkinje cells provide an explanation for the excitatory action of this substance when applied to the cerebellar cortex[3]. A similar mode of action could account for the action of strychnine on neurones within the cerebral cortex. Alternatively there may be other forms of inhibition operating on cerebral and cerebellar neurones, apart from those studied here, which are sensitive to strychnine.

J. M. CRAWFORD
D. R. CURTIS
P. E. VOORHOEVE
V. J. WILSON*

Department of Physiology,
Australian National University,
Canberra.

* Present address: The Rockefeller Institute, New York. Recipient of grant *NB*—02619—04 from the Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

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CHANGES IN THE ESTERASES OF RAT BRAIN AND BLOOD SERUM FOLLOWING ACUTE CRANIAL EXPOSURE TO X-RAYS

By Dr. E. B. MASUROVSKY and Dr. C. R. NOBACK

College of Physicians and Surgeons, Columbia University, New York

THERE have been increasing reports, in recent years, of significant changes in the activity of certain esterases of the nervous system, and other important regulatory systems throughout the body, which result from acute cranial and/or whole-body exposure to X-rays and other ionizing radiations.

Zeaman *et al.*[1] have presented histochemical evidence of increased esterase activity in the brain tissue of mice 2–10 days after cranial irradiation with a 1-mm beam of 20-MeV deuterons at doses ⩾ 16,000 rads. They attributed this esterolytic activity to Type-*C* cathepsins activated in, and/or released from, the lysosomes within radiation-damaged cells. Cholinesterases (especially cholinesterase II), on the other hand, have been found to exhibit decreased activity in the tissues and blood serum of a variety of mammalian species following irradiation with X-rays[2,3]. The assay procedures utilized in these investigations, however, were not suitable for detecting changes which might have occurred in the individual enzyme forms which constitute a given esterase system. Such changes may be demonstrated by the application of high-resolution zone electrophoresis in combination with selective multi-substrate and inhibitor characterization of the esterase components of the resulting zymogram patterns. This analytical approach was utilized in the experiments reported here, concerning changes in the esterases of the central nervous system and associated hæmato-circulation of the rat, following localized X-irradiation of the brain.

Brain homogenates and blood sera were prepared from mature ♂ Long-Evans rats, exposed to 20,000 r. of collimated 184-kVp. X-rays (generated at 30 m.amp, filtered with 0·28 mm copper plus 0·50 mm aluminium, half-value layer = 0·67 mm copper) delivered to a 30-mm² area of the right visual cortex 48 h prior to sampling. The dose rate was approximately 300 r./min in air as measured with a calibrated Victoreen r.-meter, model 570.

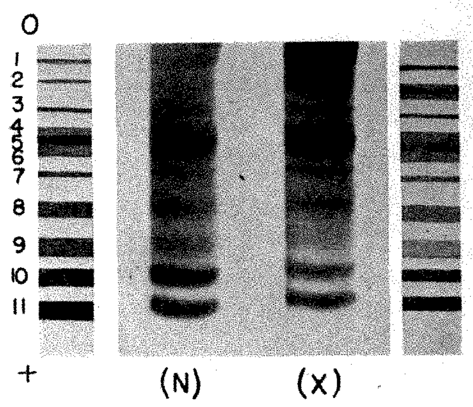
The animals (irradiated and normal controls) were killed by bleeding after relaxation under light ether. The blood thus obtained was allowed to clot at 20° C for 3 h, and the serum pipetted into centrifuge tubes for clarification. The brain of these animals was immediately perfused clear of blood under syringe pressure with > 500 ml. of a 0·9 per cent sodium chloride–2·4 per cent gum acacia solution at body temperature. It was then quickly excised from the cranial cavity and frozen solid with dry ice.

A cylindrical core, corresponding to the 30-mm² circular area exposed to the collimated X-ray beam (2·5 g), was removed from the right irradiated half and left control half of the brain, and individually minced in a glass—'Teflon' pestle homogenizer using normal saline at a 1 : 1 proportion with brain tissue. Alternate mincing and rapid freezing–thawing were performed six times before pipetting the homogenate into centrifuge tubes for clarification.

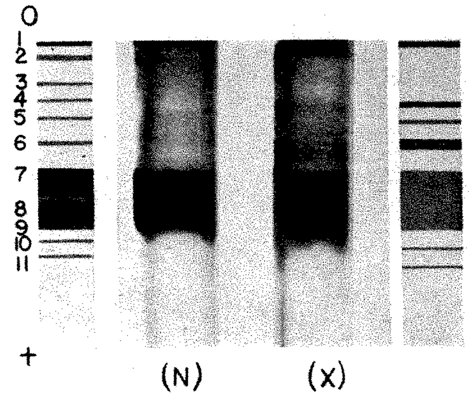
Brain homogenates and blood sera were centrifuged in a Servall model *RC*-2 refrigerated ultracentrifuge at 20,000*g* at 2° C for 60 min, following the method of Barron *et al.*[4]. The clear supernatant fractions of the brain homogenates were concentrated to about 40 per cent of their original volume at 3° C with 'Sephadex *G*-25' (Pharmacia) by the procedure of Flodin *et al.*[5], pipetted into clean tubes, and stored at 3° C prior to electrophoresis.

Electrophoretic separation of the samples was performed in polymerized acrylamide matrices consisting of a 12·5 per cent acrylamide lower gel and 3·3 per cent acrylamide spacer and sample gels, cast in soft glass tubes 64 mm long by 5 mm inside diameter, according to the technique described by Ornstein and Davis[6]. Eight replicate gel columns of both concentrated brain homogenate supernatant (100 µl.) and blood serum supernatant (10 µl.) were separated concurrently at a constant current setting (Beckman Duostat model *RD*-2) of 3 m.amp per tube (~ 120–155 V) for 80 min at 15° C using 0·05 M *tris*-glycine buffer, *p*H 8·3. Immediately following electrophoresis the gel-containing tubes were placed in ice water, and the gel plugs removed within 15 min for transfer to the appropriate substrate solution, selective inhibitor, or acetic acid/amido-Schwartz reagent.

Esterase substrates included α-naphthyl acetate, α-naphthyl butyrate, and 5-bromoindoxyl acetate (Sigma) in 0·2 M *tris*-maleate buffers, *p*H 6·0–7·5, prepared according to the methods of Allen and Hunter[7] and Barron *et al.*[4]. Fast blue *RR* (Sigma) was the diazo-coupling agent utilized. Esterase (including cathepsin) inhibition was performed with diisopropyl fluorophosphate (DFP) 10^{-6} M,



BRAIN TISSUE



BLOOD SERUM

Fig. 1. Protein patterns of rat brain tissue and blood serum from normal (*N*) and X-irradiated (*X*) animals. Major differences in brain tissue proteins appear at anodic bands *A* 2–4 and 6–10. Blood serum protein differences occur at anodic bands *A* 2, 3, 4, 6 and 10–11

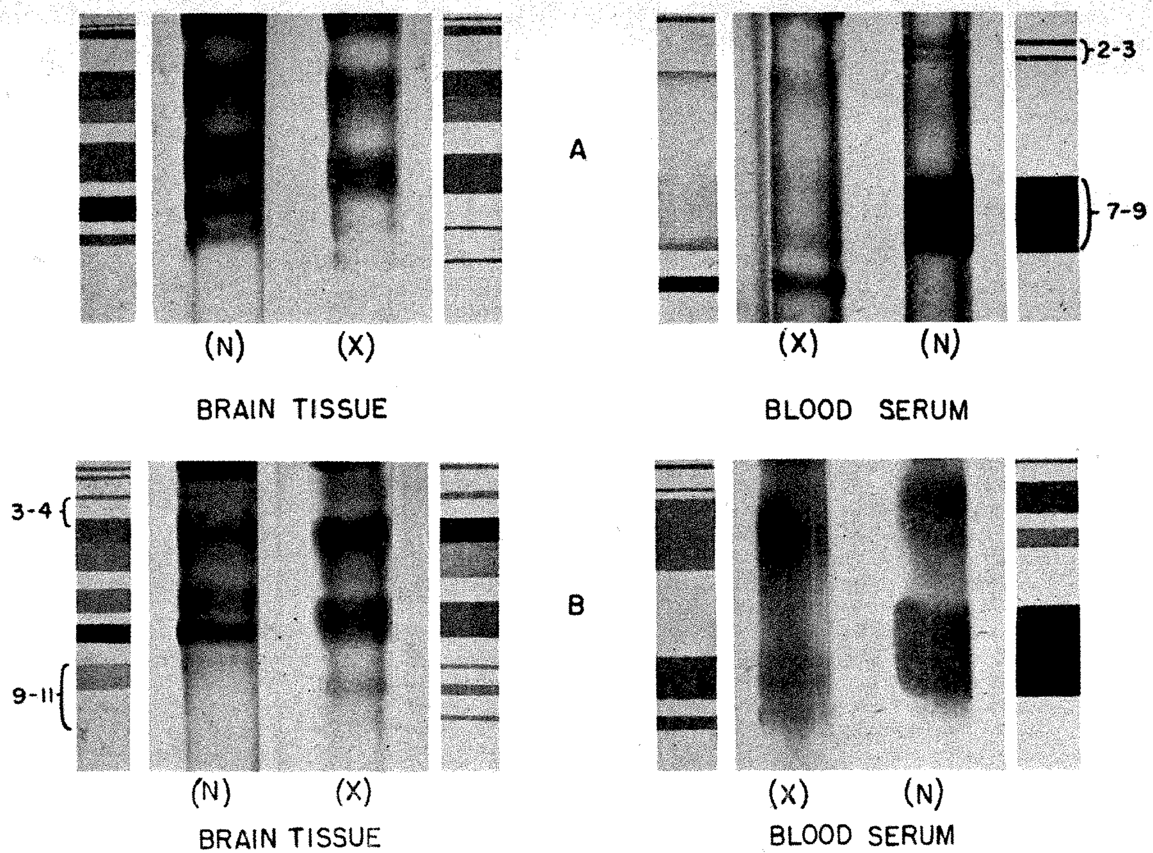


Fig. 2. Esterase patterns of rat brain tissue and blood serum from normal (*N*) and X-irradiated (*X*) animals. α-Naphthyl acetate (*A*) and α-naphthyl butyrate (*B*) substrates in 0·2 M *tris*-maleate buffers, *p*H 6·8. Major differences in brain tissue esterases at anodic band regions *A* 3–4 and 6–10. Blood serum differences in anodic band regions *A* 2–3 and 7–11

0·2 M *tris*-maleate buffers, *p*H 5·0–7·5, at 25° C for 2 h prior to transfer to the appropriate esterase substrate solutions. Incubation in the substrate solutions ranged from 2 to 16 h at 25° C. Protein staining in amido-Schwartz reagent was carried out for 16 h at 25° C, and electrophoretically de-stained in 10 per cent acetic acid at 8 m.amp for 90 min.

Figs. 1 and 2 show clear-cut differences in the protein and esterase patterns of the irradiated and control halves of the rat brains. The major differences in the protein patterns (Fig. 1), anodic bands *A* 2–4 and 6–10, correspond fairly closely to the different position and intensity of the esterases demonstrable with α-naphthyl acetate (*A*) which occur in those regions. This is brought out more clearly for the *A* 8–10 band region with α-naphthyl butyrate (*B*) as the substrate. 5-Bromoindoxyl acetate, the substrate utilized by Zeaman *et al.*[1] in conjunction with DFP to demonstrate Type-*C* cathepsins, did not reveal any noticeable differences in activity between irradiated and normal brain tissue, as only band regions *A* 3–5 (and possibly 11) showed any distinguishable reaction with this substrate.

DFP at 10^{-6} M markedly inhibited bands *A* 1, 4 and 6–7, but only partially blocked the activity of the other esterase bands. Thus, the differences in esterase activity produced by irradiation appear to be not only attributable to the Type-*C* cathepsins proposed by Zeaman *et al.*[1], but to other esterases as well. The proteolytic activity of these bands was tested with *N*-benzoyl-DL-arginine-β-naphthylamide (BANA) according to the method of Ravin[8], as modified by Barron *et al.*[4]. The results indicated a similar distribution as that demonstrated by the naphthol and indoxyl ester substrates with DFP, thereby confirming the proteolytic nature of some of these DFP-resistant esterases.

Blood serum protein and esterase patterns (Figs. 1 and 2) differ markedly between normal and irradiated animals. The major differences appear in anodic bands *A* 2, 3, 4, 6 and 7–11, with the albumin and pre-albumin regions (~*A* 7–11) exhibiting striking alterations from irradiation. Note that the esterase activity of the pre-albumin region is faint-to-absent in the blood from the normal animal, and quite distinct in the X-irradiated rat serum. However, the albumin region adjacent to it (bands *A* 7–9) shows just the converse esterase activity in these animals. This notable difference is brought out vividly by assay with both α-naphthyl acetate (*A*) and α-naphthyl butyrate (*B*). It is interesting that the amido-Schwartz protein stain appears to show a similar protein density in the albumin region of both sera, yet the esterase components of the irradiated serum were markedly inactivated. When α-naphthyl acetate was used as the esterase substrate bands *A* 2–3 were clearly distinguishable in the normal serum, but were almost totally absent in the irradiated serum electrophoretic pattern. 5-Bromoindoxyl acetate did not react with these bands (*A* 2–3), but did with bands *A* 7–9. Thus, although esterase bands *A* 2–3 coincide in some measure with esterase bands *A* 3–4 of the brain homogenates, they are most probably of a different constitution, at least so far as the nature of their esterolytic activities is concerned.

DFP greatly reduced the activities of all the esterase components of normal and irradiated sera except *A* 8, a possible cholinesterase I. BANA revealed significant proteolytic activity only in bands *A* 6–7.

The changes brought about in the esterases of brain cells and blood serum by cranial X-irradiation would therefore seem, on the basis of the differences in electrophoretic migration and substrate–inhibitor reactivity, to be largely indigenous to the respective systems. The most profound changes occurred in the blood, which is known to be more sensitive to the effects of X-rays than the central nervous system[3]. Several of the esterase components of the blood serum zymogram patterns were decidedly inactivated by irradiation, while the brain tissue zymogram patterns exhibited an apparent increase and/or decrease in the activity of certain esterolytic proteins These divergencies in the zymogram patterns may not only represent additions and/or deletions of enzymatically active molecules from the various cellular elements comprising the brain tissue, but also certain ancillary changes in their structural configurations and charge distributions as well Work is being carried out to characterize further those esterases which are effected by X-irradiation, and determine their role in the progression of functional changes brought about by irradiation of the central nervous system and its vital circulatory network

We thank Dr Roberts Rugh, Department of Radiological Research, for advice and assistance in the X-irradiation and other important phases of this work

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PARTIAL AGONISTS IN THE CENTRAL NERVOUS SYSTEM

By GLENDA M. LAYCOCK and A. SHULMAN
Department of Physiology, University of Melbourne

THE concept of a drug possessing dual action, both agonist and antagonist, has been derived from studies in a variety of *in vitro* systems Such drugs have been called partial agonists, and hypotheses have been proposed to explain their action in terms of 'occupation' theory[1,2] and 'rate' theory[3] of drug action Although drugs showing both agonist and antagonist action in the central nervous system have been reported previously[4-8], no explanation has been offered for these observations

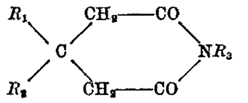
We wish to report the existence of substances with the properties of partial agonists among a series of β-substituted glutarimides and related drugs, the actions of which in mice range from convulsive and analeptic (agonist) to anticonvulsive and hypnotic (antagonist)[9-11] The following suggestions are based on quantitative titrations over a wide dose range with a series of structurally related and structurally unrelated hypnotics and analeptics in mice and on structure-action studies with β-substituted glutarimides and related drugs which will be reported fully elsewhere It is proposed that hypnotics and analeptics produce their opposed actions at common sites in the central nervous system[12-14] and that, in terms of 'rate' theory, convulsant and analeptic drugs are agonists with high k_2 (dissociation rate constant of drug-receptor complex), hypnotic and anticonvulsive drugs which antagonize convulsions induced by pentylenetetrazol are competitive antagonists with low k_2, and partial agonists have intermediate values of k_2 which may be closer to those of agonists or to those of competitive antagonists, 'occupation' theory can also be applied to explain the action of such drugs[15] It is considered that convulsive and analeptic drugs and likewise hypnotic and the foregoing anticonvulsive drugs differ primarily in their affinity for the common receptor surfaces

The glutarimide and related partial agonists (Tables 1 and 2) may for convenience be divided into two classes depending on whether their actions resemble more closely those of agonists or antagonists The first class of partial agonists, the k_2 values of which appear to lie closer to those of agonists, have lethal convulsive action and are analeptic to pentobarbitone, in higher doses they antagonize the maximum tonic extensor component of pentylenetetrazol or bemegride convulsion, provided that the mice are protected from the initial lethal convulsive action of the partial agonist Although they have no definite hypnotic action when given alone, in still higher doses they significantly increase the number and generally the sleeping time of mice given the HD_{50} dosage of pentobarbitone Mice which lose righting reflex may show associated signs of stimulation and on awakening a state of hyperexcitability and limb spasticity which may be followed later by mild convulsions These partial agonists are also analeptic to trimethadione and in higher doses synergize with its hypnotic action except in cases where death by respiratory depression intervenes

The second class of partial agonists, with values of k_2 presumably closer to those characteristic of antagonists, produce convulsions which, if not lethal, decrease in intensity to be followed by sedation and sometimes loss of righting reflex which is generally associated with stimulant action While the majority of such partial agonists are analeptic to trimethadione but not to pentobarbitone,

Table 1 CENTRAL NERVOUS SYSTEM ACTION IN MICE OF SUBSTITUTED GLUTARIMIDES

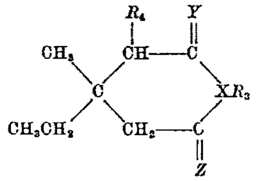
General formula



Drug type	Agonist			Partial agonist			Antagonist		
Drug action	Convulsive, analeptic*			Convulsive, analeptic*, Anticonvulsive†, hypnotic			Anticonvulsive†, hypnotic		
Substituent groups	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
	CH_3	CH_3	H						
				CH_3	C_2H_5	C_2H_5			
	CH_3	C_2H_5	H	CH_3	C_2H_5	C_3H_5			
				C_2H_5	C_2H_5	H	C_2H_5	$n\ C_4H_9$	H
	CH_3	$i\ C_3H_7$	H	CH_3	$n\ C_3H_7$	H			
				CH_3	$i\ C_4H_9$	H			
				CH_3	$n\ C_4H_9$	C_2H_5	CH_3	$n\ C_4H_9$	H
				CH_3	$n\ C_4H_9$	C_3H_5			
							CH_3	$n\ C_5H_{11}$	H
							CH_3	$n\ C_9H_{19}$	H

* Analeptic action tested against pentobarbitone sodium and trimethadione
† Anticonvulsive action tested against pentylenetetrazol and bemegride

Table 2 PHARMACOLOGICAL ACTION OF BEMEGRIDE DERIVATIVES



Substituent groups					Agonist	Partial agonist	Antagonist
R_3	R_4	X	Y	Z	Convulsant, analeptic*	Convulsant, analeptic*, anti-convulsant†, hypnotic	Anti-convulsant†, hypnotic
H	H	N	O	O	+		
CH_3	H	N	O	O	+		
C_2H_5	H	N	O	O		+	
C_3H_5	H	N	O	O		+	
C_6H_5	H	N	O	O		+	
$CH_2CH_2C_6H_5$	H	N	O	O		+	
H	CH_3	N	O	O		+	
H	H	N	S	S		+	
H	H	N	H_2	H_2		+	
H	H	N	O	H_2‡		+	
H	H	O	O	H_2		+	

* Analeptic action tested against pentobarbitone sodium and trimethadione

† Anticonvulsive action tested against pentylenetetrazol and bemegride

‡ New compound synthesized by Handley, G F, and Somers, T C (personal communication).

they are generally more powerful antagonists to the lethal convulsive action of pentylenetetrazol or bemegride and synergists of hypnosis induced by pentobarbitone or trimethadione

It may be seen (Tables 1 and 2) that the following series of very small changes in the components of the agonist bemegride (β-methyl-β-ethylglutarimide) gives rise to a number of partial agonists of distinct chemical types

(1) The introduction of a methylene group into the β-alkyl side-chain, for example, β-methyl-β-*n*-propylglutarimide and β,β-diethylglutarimide, (2) the introduction of a methyl group on to the α-carbon atom (α,β-dimethyl-β-ethylglutarimide), (3) the replacement of both oxygen atoms of the carbonyl groups by sulphur atoms (β-methyl-β-ethyldithioglutarimide), (4) the replacement of one or both carbonyl oxygen atoms by hydrogen atoms (4,4-methylethylpiperidone-2 and 4,4-methylethylpiperidine hydrochloride), (5) the replacement of the —CO—NH— grouping by the —CH_2—O— grouping (β-methyl-β-ethylvalerolactone), (6) the introduction of an ethyl, allyl, phenyl or phenethyl group on to the glutarimide ring nitrogen atom

These structural alterations presumably all increase the strength of the bond formed between the drug and receptor surface, since k_2 of a partial agonist is lower than that of an agonist, whereas certain small structural modifications of the hypnotic β-methyl-β-*n*-butylglutarimide appear to decrease the strength of the drug-receptor bond to produce partial agonists, for example, *N*-ethyl-β-methyl-β-*n*-butylglutarimide or the *N*-allyl analogue (7) and β-methyl-β-*iso*butylglutarimide (8) (Table 1) Types (1), (2), (3), (5) and (8) are partial agonists of the first class described, while (4), (6) and (7) belong to the second class, full experimental details and the possible mechanisms involved will be described elsewhere[15,16] It is of considerable interest that sodium diphenylhydantoin ('Dilantin'), which is highly effective in antagonizing convulsion induced by electroshock but not by pentylenetetrazol[17], shows many of the characteristics of a partial agonist of the second class

The foregoing observations permit the following conclusions

(*a*) The existence of drugs which are structurally closely related to both agonists and antagonists and which show both agonist and antagonist action supports the postulate that convulsion and hypnosis are initiated by these drugs at common receptor surfaces The progressive change within the homologous series of β,β-dialkylglutarimides (Table 1) from agonist (β-methyl-β-ethylglutarimide) to partial agonist (β-methyl-β-*n*-propylglutarimide) to antagonist (β-methyl-β-*n*-butylglutarimide) is analogous to that found among a variety of closely related homologues in *in vitro* systems[1-3,18,19]

In this context it is of marked importance that *N*-ethylation or *N*-allylation of the agonist bemegride and the closely related antagonist β-methyl-β-*n*-butylglutarimide gives rise, in both cases, to drugs with partial agonist action (Table 1) If hypnotic and convulsive action were taking place at separate sites, it would be very difficult to explain how the introduction of the same *N*-alkyl substituent could convert a convulsive drug to one which is able to act at both sites and likewise a hypnotic drug into one which can act at the convulsant as well as the hypnotic site However, if hypnotic and convulsive action both occur at the same site it might be expected that the *N*-ethyl or *N*-allyl substituent would decrease the exactness of fit to the receptor surface of a hypnotic drug, which most likely fits closely since k_2 is low, while increasing the capacity for binding of the smaller convulsant drug, thus enabling it to form a stronger bond with the receptor surface Thus the *N*-ethyl or *N*-allyl substituent by increasing k_2 of the hypnotic and decreasing k_2 of the convulsant could convert both to partial agonists

(*b*) Since the structural modifications which produce alteration in pharmacological action from agonist or antagonist to partial agonist are small, the associated changes in the value of k_2 would also be small; this might be anticipated from observations in rats and mice with analgesics and their antagonists[20] and has been demonstrated[3] for a homologous series of alkyltrimethylammonium cations on isolated guinea-pig ileum

(*c*) Since a small structural alteration at each position of the bemegride molecule leads to a change in pharmacological action from agonist to partial agonist, it would seem that the bemegride molecule as a whole, and presumably those of its closely related homologues and derivatives, must contribute to the strength of the drug-receptor bond and that the degree of steric and polar complementariness of the drug and receptor surface are most important in determining the resultant pharmacological response It may be predicted that, as in the case of β-substituents, a progressive change in action from agonist to partial agonist to antagonist will occur as substitution or modification of the α-carbon or nitrogen atoms or modification of the carbonyl groups of bemegride progressively increases the strength of the drug-receptor bond

(*d*) β-Substituted glutarimides are poorly ionized at physiological *p*H (refs 21 and 22) and presumably the type of bonds by which the drugs are bound to the receptor surface are van der Waals's forces, dipoles and hydrogen bonds Their pharmacological action and interaction with structurally related and structurally unrelated agonists and antagonists must therefore be a physical rather than a chemical process, a suggestion consistent with that proposed in many theories on the mode of action of central nervous system stimulant and depressant drugs[6,23] The variety of chemical types found among the partial agonists related to bemegride supports such a concept and suggests that the receptor surface lacks precise specificity in its requirement of drug structures to which it will respond, specificity is strict, however, with respect to the type of pharmacological response produced The increase in basicity associated with the conversion of the agonist bemegride to the partial agonists, 4,4-methylethylpiperidone-2 and 4,4-methylethylpiperidine hydrochloride suggests that the receptor surface has some cationic character

A hypothesis suggesting that β-substituted glutarimides are but one of a series of central nervous system stimulant and depressant drugs[4-8,20] which act and interact at common receptors in the central nervous system of mice, and probably of other animal species, will be reported elsewhere[15]

We thank the Directors of Nicholas Pty Ltd , Victoria, Australia, for supplying the drugs used in this investigation and for giving us access to the results of screening tests carried out in the Nicholas Institute for Medical and Veterinary Research, Victoria, during 1955–60 This work was supported by the National Health and Medical Research Council of Australia

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RESISTANCE TO FINGER COOLING RELATED TO PHYSICAL FITNESS

By Dr CHARLES J EAGAN

Arctic Aeromedical Laboratory, Fort Wainwright, U S Air Force, Alaska

PREVIOUS investigations[1,2] in this laboratory showed that subjects maintained higher peripheral temperatures during standardized cold exposure after a 3-week regimen of physical training The authors concluded that higher levels of physical fitness were "alone responsible for the physiological differences manifested between the control and experimental periods"[1] These conclusions have been widely quoted although it has been inferred by Hammel[3] and others that a "metabolic acclimatization"[3] was the salient feature, higher extremity temperature might be an incidental consequence of a higher metabolic rate after the training regimen This explanation was compatible with the results of the first investigation[1] but not the second[2] In subsequent work at this laboratory it was casually noted that there seemed to be little or no correlation between level of physical fitness and resistance to cooling of the fingers The two experiments to be described substantiate this

(1) The investigation of which this experiment is part was made during May–July 1962 Some of the results are reported elsewhere[4]

Two groups of subjects were compared Physical results are given in Table 1 The five mountaineers had all attained high levels of physical fitness in the course of training for and making an ascent of Mount McKinley, Alaska They returned directly from the expedition to the Arctic Aeromedical Laboratory, where they lived for three days The controls were 19 U S Air Force personnel of the laboratory staff, all were of mainly sedentary habitus

Table 1 COMPARISON OF THERMAL RESPONSES OF PHYSICALLY FIT MOUNTAINEERS AND SEDENTARY CONTROLS

Group and No of subjects		Mountaineer 5	Control 19	Significance of differences (P)
Age (yr)	Mean	27 6	29 6	
	Range	24–32	19–42	
Height (cm)	Mean	180 1	178 4	
	Range	170–192	168–190	
Weight (kg)	Mean	73 2	76 4	
	Range	64–81	61–97	
Physical fitness (units)	Mean	81 4 ± 2 6*	32 7 ± 2 4	< 0 001
	Range	76–88	19–61	
BMR (kcal/m²/h)	Mean	36 5	37 5†	NS
	Range	(33 3–39 8)	(34 1–39 5)	
Responses to severe cooling in water and air				
Finger temperature (°C) in ice water				
At individual nadir		0 96 ± 0 50	0 95 ± 0 24	NS
Average over 10 min		3 36 ± 0 76	3 86 ± 0 33	NS
Tolerance factor (units)‡ (air at −22° C, 850 kcal/m²/h 'windchill')		253 ± 50	234 ± 60	NS

* Standard error

† Basal metabolic rate was measured on only five of the control subjects

‡ Tolerance factor (units) = duration of cooling (min) × average finger temperature (°C) during the exposure

The same treadmill test[5] for physical fitness was used as in the first investigations mentioned[1,2]. The mountaineers were each tested twice, but most of the controls only once The thermal and metabolic tests were done with the subjects in the basal state, having slept overnight at the laboratory Finger temperatures were measured using stranded No 30 copper-constantan thermocouples and a recording potentiometer The thermocouples were attached at standard sites on the distal digital volar pads Conditions of severe cooling were imposed on the fingers, first on the index fingers immersed 2 8 cm in stirred 0 0° C water for 10 min while the subject was prone in bed. Then he arose, dressed in his usual clothes plus a heavy parka, moved to an adjoining room and exposed the index and middle fingers of the left hand in a refrigerated box at a temperature of −22° C, 'windchill' was approximately 850 kcal/m²/h This test was for 30 min, but was stopped if either finger supercooled below −5° C or superficial freezing was observed

A 'tolerance factor' equal to the duration of exposure in minutes multiplied by the average finger temperature during exposure was computed for each subject.

Table 1 shows the results. The responses to severe cooling of the fingers were essentially the same in the highly fit mountaineers and the sedentary controls

(2) The investigation of which this experiment is part was made during April–May 1963 The main part of the work was a comparison of thermal responses to cooling of the extremities in the following groups 6 Alaskan Eskimos, 6 Alaskan Indians and 6 Navajo Indians, all of whom had attended the Chemawa Indian School in Oregon for the nine months before the investigation, 6 University of Oregon students who were 'controls' All groups were matched for age and body-size The results reported here are of comparisons between equal numbers of the subjects separated on the basis of level of physical fitness The 'highly fit' group consisted of the 3 Eskimos, 3 Alaskan Indians, 3 Navajos and 3 controls who had the highest physical fitness scores[5] The 'less-fit' group consisted of the other 12 subjects The treadmill test[5] was made twice on each subject Fat percentages were estimated from skinfold measurements[6]

Resistance to cooling of the extremities was assessed using mild and severe cooling of the fingers (in 0° C air

Table 2 PHYSICAL DATA AND THERMAL RESPONSES ON SUBJECTS OF DIFFERENT LEVELS OF PHYSICAL FITNESS

Group* and No. of subjects	Highly fit 12	Less fit 12	Significance of differences (*P*)
Age (yr) Mean	19 7	19 8	
Range	18–22	17–23	
Height (cm) Mean	168 3	171 6	
Range	159–178	166–184	
Weight (kg) Mean	63 9	65 4	
Range	54 2–72 2	58 0–74 2	
Body fat (per cent) Mean	13 0	12 7	NS
Range	9 8–16 3	6 6–15 8	
Physical fitness (units) Mean	85 8 ± 2 0‡	67 2 ± 3 9	< 0 001
Range	76–99	34–82	
BMR (kcal/m²/h) Mean	34 7 ± 1 6	33 0 ± 2 2	NS
Range	24 7–44 4	26 3–43 7	
Finger cooling in 0° air for 30 min			
Finger temperature (°C)			
At group nadir (seventh min)	11 6 ± 1 7	11 0 ± 1 9	NS
At 30 min	17 0 ± 1 8	15 2 ± 2 0	NS
Average over 30 min	16 1 ± 1 4	14 9 ± 1 1	NS
Finger cooling in 0° water for 10 min			
Finger temperature (°C)			
At individual nadir	0 63 ± 0 23	1 24 ± 0 72	NS
At tenth min	6 50 ± 0 85	7 15 ± 1 13	NS
Average over 10 min	3 41 ± 0 47	3 92 ± 0 73	NS
Hand calorimetry (4 5° C water for 20 min)			
Finger excess temperature (°C)‡			
At individual nadir	1 78 ± 0 17	1 43 ± 0 23	NS
Average over 20 min	3 69 ± 1 02	3 43 ± 0 28	NS
Heat loss (cal/100 c c of hand/min)	238 1 ± 11 3	238 6 ± 12 6	NS

* There were three Eskimos, three Arctic Indians, three Navajos and three white controls in each group

† Standard error

‡ Finger excess temperature = finger temperature minus calorimeter temperature

and 0° C water, respectively) and moderate cooling of the whole hand involving rise-in-temperature calorimetry (starting at 4 5° C) Duration of cooling for each test is listed in Table 2 Finger temperatures were measured as already described Test conditions were similar to those for the first experiment Subjects slept overnight at the laboratory Finger cooling (both in air and in water) was carried out with the subject supine and in the basal state. Hand calorimetry was performed in the afternoon after lunch Cooling was not begun in any of the tests until finger temperatures were at least 35° C

Table 2 shows the results There is a marked similarity in the means for all factors except for physical fitness Most of the criteria for resistance to cooling had been shown to be efficient measures from comparisons of the Alaskan versus Navajo and control groups where highly significant differences were found (unpublished results) But level of physical fitness seems unrelated to the thermal responses to cooling of the extremities

The results of these two experiments seem conclusive When the resistance to cooling of the extremities is investigated under standardized conditions, there are no differences that can be attributed to level of physical fitness How, then, may these results be related to the opposite conclusion from previous work[1,2] where differences were found in the responses of subjects after training ? It may be relevant that habituation to a test procedure can improve resistance to cooling of the fingers[4] Perhaps more salient is the work of Osiba[7], who showed that a high protein intake combined with high physical activity resulted in a sustained increase in basal metabolic rate. Subjects on a regimen of strenuous physical training[1,2] who were allowed food *ad libitum* could be expected to eat more protein Thus, Osiba's conditions for a sustained elevation in basal metabolic rate would be satisfied In fact, Adams and Heberling[1,2] did find a higher resting metabolism for their subjects after training in the first investigation[1] but not in the second[2]

It is concluded that the results of Adams and Heberling are probably being wrongly interpreted when improved resistance to extremity cooling is ascribed to improved physical fitness There is probably no causal relationship The validity of the important finding of these workers that many of the indices for cold acclimatization can be explained on the basis of increased physical activity is not being questioned But the importance of change in level of physical fitness *per se* is indeed questionable

I thank the test subjects for their co-operation and especially for their forbearance during tests which caused severe cold pain I also thank the Bureau of Indian Affairs, the Superintendent and staff of Chemawa Indian School, Oregon, and the staff of the Department of Health, Recreation and Physical Education of the University of Oregon, especially Prof P O Sigerseth, for co-operation; Staff Sergeant Paul Felts, U S Air Force, for assistance; Dr Eugene Evonuk and Dr F A Milan for their interest and assistance

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CHANGES IN THE RATE OF METABOLISM FOLLOWING ADMINISTRATION OF ETHANOL IN THE MOUSE

By PROF F W. KINARD

Department of Physiology, Medical College of South Carolina, Charleston

THE mouse, with its ease in handling, convenience in housing, and relatively low cost, has been increasingly used by us in investigations of ethanol metabolism Of some concern is the finding of Marshall and Owens[1] that although the rate of oxidation is relatively constant 1 h after administration of ethanol, the rate may be twice as fast during the initial 60 min On the contrary, Nelson *et al* [2] found no statistically significant difference between the rates determined at the end of either 1 or 3 h Marshall's hypothesis was confirmed in general by Forney *et al* [3], but the early accelerated rate was found to occur during the first 30 min following injection of the alcohol This problem in metabolism, viewed somewhat differently by three groups, is of sufficient practical and theoretical importance to deserve further investigation If the mouse does not metabolize ethanol at a constant rate, the maximal rate of metabolism cannot be obtained by rate determinations during randomly selected intervals, for example, between the second and third hours after administration

The recent development of improved methods of homogenizing and the availability of accurate hypodermic syringes led us to attempt to develop a standard technique of determining the rate of ethanol metabolism in mice The various steps in the technique were examined critically The intervals of study were selected to include the important ½- and 1-h periods plus a longer period of 4 h

Mice, Carworth Farms white females, weighing 19–25 g, were given free access to Purina checkers and water until the beginning of the experiment The experimental animal was weighed, injected intraperitoneally with ethanol, 4 g/kg/body-weight, using a stock 20 per cent w/v solution of ethanol in physiological saline A Hamilton syringe, calibrated in 0 01 ml, was used routinely At the end of the experimental period, either 0 5, 1 or 4 h, the mouse was killed by breaking its neck The entire carcass was divided four or five times with scissors and homogenized for 5 min in sufficient water to give a 1 5 homogenate The homogenation was carried out in a semi-micro blending assembly, surrounded by ice water, and adapted to the motor drive of the gallon-size Waring blender Following homogenation, an aliquot of the homogenate, approximately 5 ml, was pipetted into a tared stoppered distilling flask The flask was re-weighed to determine the weight of homogenate added and to permit calculation of the weight of tissue utilized The reagents, that is, sodium sulphate, sodium tungstate and sulphuric acid, were added and the ethanol content determined by the distillation method of Aull *et al* [4]

The adequacy of the methods used was determined by the following experiments

(1) *Chemical method* Repeated analyses of the stock solution of ethanol were made, using calibrated pipettes and burettes instead of the Hamilton syringe This experiment was expected to show the degree of reproducibility in the chemical method

(2) *Homogenation, water* Ethanol, 0 5 ml of stock solution, was introduced by syringe into water, 25 0 g and homogenized for 5 min The resulting solution was treated as though it were tissue of an experimental animal and the ethanol concentration determined This experiment was intended to reveal losses during homogenation and variations occurring when measurements were made with the syringe

(3) *Zero hour mouse* An animal was killed, weighed, injected intraperitoneally by syringe with stock solution of ethanol, 0 5 ml /25 g body-weight The procedure from this point onwards was identical with that described for the experimental animals This experiment had a two-fold purpose, namely (*a*) to indicate whether pooling of the dose of ethanol in the peritoneal cavity, which occurs in the dead mouse without circulation, would result in complete dispersion of ethanol during homogenation, and whether the tissues would be sufficiently comminuted to permit representative sampling, (*b*) to give the exact concentration of ethanol in the mouse, as determined by analysis following the various procedures necessary to prepare the tissue for analysis The mean value of these determinations should indicate the concentration of ethanol considered to be present in an experimental animal, immediately following injection, and before metabolic activity had begun

The results of these investigations are summarized in Table 1 In the method described, as noted in homogenation, water and stock solution, respectively, the combined basic mechanical operations and the chemical method introduce the smallest errors The larger error, in the zero hour series, stems from the difficulty in completely homogenizing the various components of the carcass, thereby preventing representative sampling The duration of homogenation can be prolonged to secure better comminution, but this is accompanied by increased heating of the homogenizer and increased opportunity for evaporation It is now suspected that the 5-min period selected is not optimum The results, in the remaining three groups where experimental animals are used, appear to be within the range expected in this type of experiment

When the results in Table 1 are used to calculate the rates of ethanol metabolism found in Table 2, the standard deviations of the mean, as well as the range, increase noticeably Obviously, any variation is compounded since the rate of metabolism is calculated by the following steps (½ h) 398 1 − 375 2 = 22 9 × 2 × 10 = 458, (1 h) 398 1 − 342 5 = 55 6 × 10 = 556, (4 h) 398 1 − 159 4 = $\frac{238\ 7 \times 10}{4} = 594$ These results are not identical with those in Table 2 since the tabulated results represent the mean of the individual experiments The mathematical processes, therefore, play a part in producing the wide range in the results, particularly in the ½-h series

Table 1 ETHANOL IN TISSUE OR SOLUTION
(mg ethanol/100 g tissue, or solution)

	No of expt	Mean, *S D*	Range
Stock solution	12	399 6 ± 1 6	396–401
Homog , water	12	399 8 ± 2 9	392–402
Zero h mice	12	398 1 ± 11 5	378–409
½-h mice	12	375 2 ± 8 7	361–386
1-h mice	11	342 5 ± 7 3	332–353
4-h mice	11	159 4 ± 27 4	118–194

Table 2 RATE OF ETHANOL METABOLISM
(mg ethanol/kg/h)

	No of mice	Mean, *S D*	Range
½-h	12	455 0 ± 173 3	240–740
1 h	11	555 5 ± 73 1	450–660
4 h	11	595 5 ± 68 3	510–700

The decision to utilize the mean concentration of ethanol determined in the zero hour groups as the actual concentration of ethanol present in each individual experimental mouse at the start of the experiment was made in order to obviate the various errors introduced by the mechanical and chemical operations Instead of this approach, one can consider the concentration of the stock solution, as determined by analysis, to represent the actual concentration of ethanol administered Using the latter method, the mean values for the rate of ethanol metabolism, expressed as mg ethanol/kg/h, as indicated as follows, are approximately 7 per cent higher than those determined through utilization of the zero hour series: ½-h series 488, 1-h series 571, 4-h series 600

The mean calculated rate of oxidation, by Marshall and Owens[1], was 536 mg/kg/h This is in approximate agreement with my presently reported rate of 555 and well below the previously reported[2] rate of 625 in the 1-h group The rates given by Marshall and Owens for the direct determinations, 1,322 for ½ h, 1,123 for 1 h and 691 for 3 h, differ markedly, in the first two instances, from my present results of 455 for ½ h and 555 5 for 1 h, and less drastically, in the last instance, from the 595 5 I found in the 4-h group If the mice examined by Marshall and Owens oxidized ethanol during the first ½ h at the rate of 1,322 mg/kg/h, this would mean that only 462 mg/kg were oxidized during the second ½ h (1,123 − 661 = 462) During the 3-h period, oxidization of 2,073 mg/kg (3 × 691), with 661 mg/kg being oxidized during the initial ½ h, indicates that 282 mg/kg of ethanol were oxidized during each ½ h following the initial ½ h ($2{,}073 - 661 = \frac{1{,}412}{5} = 282$) Therefore, Marshall and Owens found a declining rate of oxidation, for example, 661 during the initial ½ h, 462 during the second, and a mean rate of 282 per ½ h over the remaining four periods of ½ h. By similar calculations, the rates of oxidation in our investigations were found to be 227 mg/kg during the initial ½ h, 328 during the second ½ h, and a mean rate of 308 per ½ h over the remaining six periods of ½ h each

In view of the fact that the lowest rate in our investigations was obtained in the ½-h experiments and the highest in the 4-h series, an explanation has been sought A possible explanation is that during the early phase of each experiment, insufficient alcohol had been absorbed to bring the oxidative reaction to its maximum rate, hence an overall slower rate was exhibited in the shorter experimental periods In the longer experiments, although we did not investigate this specific phenomenon, one would expect more alcohol to be lost by excretory routes, mainly through lungs and kidneys, with the result that such losses would sum with the loss due to oxidation to give an erroneously high rate of metabolism The loss of ethanol by excretion is probably not great in these experiments of relatively short duration, for of the total amount of alcohol administered, according to Lester and Greenberg[5], 1–5 per cent may appear in the expired air Contrariwise, Lundquist *et al*[6] consider that the loss of ethanol through the lungs is very considerable in the rat The best explanation appears to be that the shorter duration experiments indicate a rate which is not representative of the mean rate in a 3- or 4-h experiment In the longer experiments, this period of sub-optimal rate of oxidation occupies a proportionately smaller portion of the total time, thus leading to higher rates in the longer experiments

Statistical analysis of our results indicates that there is no significant difference between the rates during ½, 1 or 4 h . The disturbingly large standard deviations, especially in the ½-h series, indicate such wide ranges of individual values that this conclusion must be expected If one wishes to determine the rate of ethanol metabolism during the peak rate, the duration of the experiment must be sufficiently long to minimize the interval required for absorption As the period of investigation is prolonged, the quantity of ethanol lost by excretory routes will be increased Obviously, other factors can be controlled, such as magnitude of the dose administered and consequently the level of concentration in the blood In these experiments, the dose of 4 g/kg was chosen in order to work at the same concentration levels which had been used earlier It appears that the duration of such experiments can be limited to 3 or 4 h, but shorter periods of investigation yield rates which have not yet reached maximum values

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AGGREGATION OF CULTURED MAMMALIAN CELLS

By PROF MERWIN MOSKOWITZ

Department of Biological Sciences, Purdue University, Lafayette, Indiana

THE histotypic aggregation of dissociated embryonic cells has been extensively studied in recent years[1] This aggregation is observed with cells from young embryos, and the ability of the cells to aggregate declines and eventually disappears as the age of the embryo increases or if the cells are cultured[2]. I have observed that under appropriate cultural conditions cells of established strains form aggregates that resemble those obtained with dissociated embryonic cells Following this observation, an investigation of the factors affecting the aggregations of cells of established strains was undertaken and the initial findings are presented here The investigations carried out so far indicate that these factors are not susceptible to simple definition Different strains of cells vary markedly in their ability to aggregate, and different cultural conditions seem to be required for the aggregation of strains that have been observed to aggregate

The term 'aggregate' is used here to denote a group of cells tightly packed together into an oblong or spherical structure usually having a continuous layer of peripheral cells forming a relatively smooth surface (Figs 1 and 2) A group of cells loosely held together with a rough edge, appearing as if individual cells randomly attached to each other, is termed a 'clump' (Fig 3) These two types of groupings of cells were observed and they are distinguished inasmuch as there appear to be qualitative differences in their behaviour All the cells of a clump appear to attach and spread out when it is allowed to settle to a glass surface, and the cells of a clump are readily dissociated by trypsin An aggregate of cells acts like a tissue fragment under these conditions, it retains its structure on a glass surface and cells grow out from it as with a tissue explant, and it takes a relatively long while for trypsin to dissociate the cells in the aggregate

The fibroblastic cells used were clone cultures derived from the *Mox*[3] and *L* strains, and strains *WISH*, Girardi's Heart, *Hep* 2, and Salk Monkey Heart comprised the epithelial cells The cells were grown in nine volumes of Eagle's medium[4], modified by adding four times the vitamin concentration and three times the amino-acid concentration, plus one volume of horse (unless otherwise specified) serum or the dialysate of a tissue extract The latter protein-free medium is similar to one previously described[5], except that dialysates of saline extracts of porcine pancreas tissue were utilized instead of serum dialysates.

The cell inoculum was prepared by scraping off the surface growth from the culture vessel with a rubber policeman and dispersing the cells with a capillary pipette Only single cells were present in suspensions of fibroblastic cells, and single cells plus occasional clumps of two or three cells were present in suspensions of the epithelial strains Cells were agitated in tubes containing 1 ml of medium on a roller drum apparatus at 60 r p m or in 25-ml Erlenmeyer flasks containing 3 ml of medium on a rotary shaker at 70 r p m The cultures were incubated at 37° for 7 days without a change of medium

All the strains of cells used grew as monolayers in stationary cultures in serum supplemented medium, in the protein-free medium the *Mox* and *L* strains appear the same as in serum, the *WISH* and *Hep* 2 strains have a tendency to grow in patches, and the Girardi's Heart and Monkey Heart strains did not become attached to the glass but formed aggregates

Initial investigations by rotating cells on the roller-drum apparatus were carried out with the *Mox* strain In serum-supplemented medium the cells remained discrete and some clumps formed, in protein-free medium aggregates formed on the third day which increased in size during the remaining four days of incubation A number of clone cultures of the *Mox* and *L* strains were then tested on the roller drum in serum-supplemented and protein-free medium One culture aggregated only in protein-free medium, one culture aggregated only in serum-containing medium, two cultures aggregated in both media, and two cultures did not aggregate The *WISH* and Girardi's Heart of the epithelial strains aggregated on the roller drum to a slight extent and only in serum-supplemented medium

Further experiments were carried out on the rotary shaker The behaviour of the fibroblastic strains was similar to that observed on the roller drum, and, although all the epithelial strains aggregated, there was a variation in aggregative behaviour with different media.

The effect of variation in media on aggregation was tested by determining the aggregation of the epithelial cells in media supplemented with different sera and in protein-free medium. The results of such an experiment are given in Table 1. In addition to the variation in aggregation in a given medium, even the immediate past history of the strains appears to be a factor in their aggregatability. For example, the aggregation properties of cells grown for four passages in medium supplemented with agamma horse serum or fœtal calf serum were different when tested in various media than cells grown in horse serum-supplemented medium.

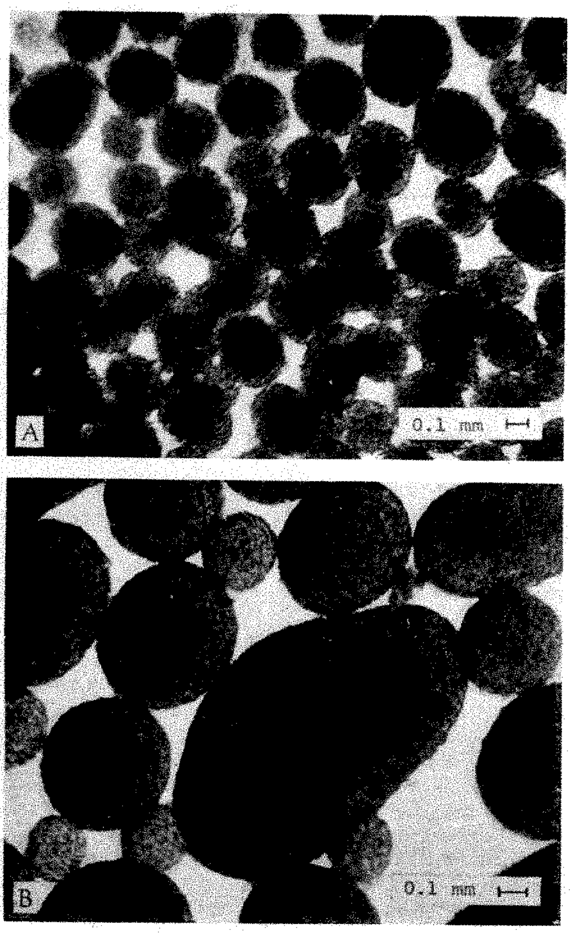

Fig. 1. Aggregates: *A*, strain *WISH*; *B*, *L* strain

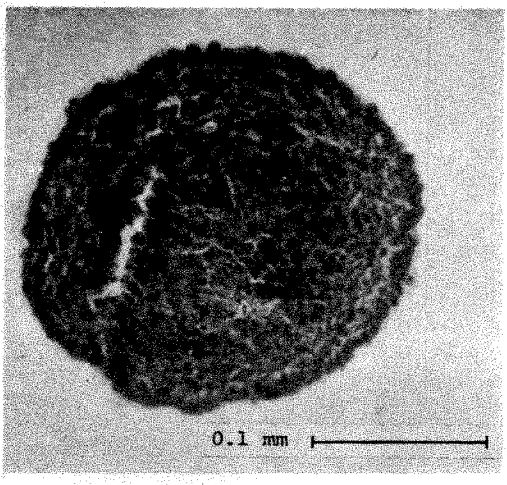

Fig. 2. Section through aggregate. *L* strain (safranin and fast green)

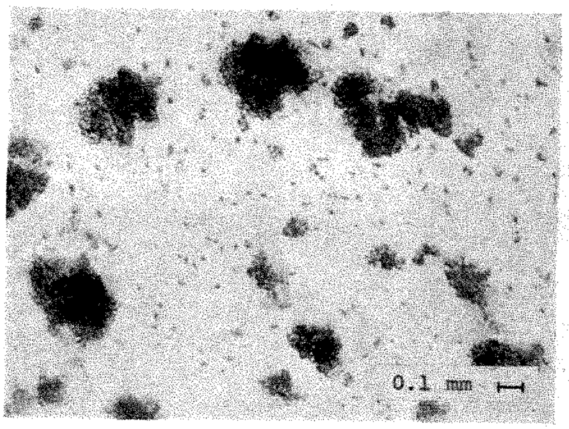

Fig. 3. Clumps. Strain *WISH*

The effect of the inoculum size was tested by determining the aggregation of the epithelial strains in media containing horse, agamma horse or fœtal calf serum with inocula of 5 × 10^5, 2 × 10^6, and 6 × 10^6 cells per flask. In flasks where aggregation occurred, it occurred earliest and there were larger aggregates with the smallest inoculum, the aggregates were slightly smaller with 2 × 10^6 inoculum, and extremely small aggregates formed late in the experiment with 6 × 10^6 inoculum. With Monkey Heart cells in fœtal calf serum, aggregates formed with the smallest inoculum and not with the larger inocula. An inoculum of 1 × 10^6 per flask was finally standardized in further experiments.

Inasmuch as the number of cells in suspension is a factor in the aggregation of the cells, the attachment of a portion of the cells to the glass surface with some of the media and conditions of rotation utilized affects the aggregation of the cells. In order to prevent the attachment of the cells to the glass, flasks were coated with silicone. Occasionally a thick layer of cells which had attached to the glass would break off and would be observed as a ribbon-like clump of cells. On the roller drum, aggregates would sometimes be seen among the cells attached to the glass. Sheets of cells were observed floating on the surface in a few fibroblastic cultures on the rotary shaker. Such sheets of cells were noted previously[6] and were quite distinct from the aggregates and clumps reported here.

In a few instances single cells were observed during the entire period of agitation. In the usual pattern of events, however, clumps formed within a few hours after agitation was initiated, and they usually increased in size during the course of the agitation period. They varied in size from a few cells to large clumps up to 0·5 mm in diameter. The density of clumps varied with different strains and media. In some instances aggregation occurred within 24 h, but aggregates usually did not form for two or three days. It appeared that cells comprising the clumps underwent some change which caused the clumps to acquire the property of an aggregate. The aggregates were often quite uniform in size (Fig. 1), but with some strains and media they varied in size from 0·2 to 1 mm.

Aggregation is not merely the association of cells which results when they are placed under conditions which do

Table 1. AGGREGATION OF CELLS IN DIFFERENT MEDIA

Cell strain	Medium supplement: Horse serum	Agamma horse serum	Fœtal calf serum	Tissue extract dialysate
Monkey heart	*A**	*C*	*C*	*C*
WISH	*A*	*C*	*A*	*C*
Girardi heart	*A*	*A*	*C*	*C*
Hep 2	*S*	*A*	*A*	*A*

* *A*, aggregate; *C*, clump; *S*, single cells.

not permit their attachment to the glass surface. Under such conditions cells usually remain single or form small clumps. The following observations suggest that aggregation occurred because of a change in the cells induced by the conditions under which they were cultured: the clumps, which formed in a matter of hours, did not invariably become aggregates; it took from 3 to 7 days for aggregates to form; and aggregates have properties qualitatively different from clumps. This change is undoubtedly related to a change in their surface properties, and it is apparent that subtle differences in the cultural conditions under which these cells are grown affect these surface properties. Specific substances, such as enzymes or other proteins, may be produced in association with the changes in the cells that cause them to aggregate. The possibility that such substances are produced will be investigated inasmuch as any new substance produced by the cells, or a marked increase in the production of one of their normal constituents, may provide information regarding the nature of the changes occurring in the cells which cause them to aggregate.

Although most established strains of cells have lost many of the properties of the cells of the tissue from which they were derived, the information to produce such properties is probably retained. The ability to join with other cells to form a tissue is one of these lost properties, and it may be speculated that the conditions which induce established strains to aggregate are those which permitted the expression of this bit of information. Further expression of the information within the cell may result in the formation of structures with a greater degree of organization. No specific histotypic structural features have thus far been observed, but we have on occasion noted what appeared to be organized regions in the aggregates. Investigations are in progress to determine if established strains of cells and mixtures of such different cell strains can form aggregates with structures resembling those of the tissue from which they were derived.

Leighton has observed the formation of relatively small aggregates when established cell lines were grown within a cellulose sponge[7]. He directs attention to the fact that most clinical carcinomas appear as aggregates or nests of cells, and suggests that factors intrinsic to the formation of aggregates are involved in the spread of the cancer. The specific conditions that cause the formation of aggregates in agitated cultures may thus be related to the development of neoplastic potentialities by the cells. This possibility will be explored.

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ON THE UNIVALENT FRAGMENTS OF HUMAN 7 S GAMMA-GLOBULIN

By D. STOLINSKY and PROF. H. FUDENBERG

Department of Medicine, University of California School of Medicine, San Francisco 22

HYDROLYSIS of 7 *S* rabbit antibody by papain yields three 3·5 *S* fractions separable by chromatography on carboxymethyl cellulose[1,2]. Two of these fractions, designated I and II by Porter, resemble each other in possessing univalent antibody activity[3–5] and in antigenic characteristics and molecular weight. Fraction III lacks antibody activity. Palmer, Mandy and Nisonoff[6] recently demonstrated that fractions I and II are derived from different parent molecules rather than being fragments of the same molecule (as originally thought), and showed that the relative amounts of fractions I and II vary reciprocally, depending on the chromatographic behaviour of the parent γ-globulin. Confirmatory evidence for this concept was recently furnished by Stelos *et al.*, using another method[7].

Various methods of fractionating the papain digest of human 7 *S* γ-globulin have been developed[8–12]. Only the method of Franklin, however, is comparable with that of Porter in producing three fractions. These fractions, designated *A*, *C* and *B*, correspond to fractions I, II and III, respectively, of rabbit γ-globulin[9]. (A small fourth fraction, designated *D* and probably representing intact 7 *S* material, is sometimes observed.) According to Franklin's results, the relative amounts of *A* and *C* in digests of pooled human Cohn fraction II varied, depending on the chromatographic and electrophoretic behaviour of the parent γ-globulin, whereas the proportion of *B* was relatively constant[9]. The basic fraction was rich in the positively charged fragment *C* while the more rapidly migrating fraction contained primarily the more negatively charged fragment *A*. However, no conclusions were made on the basis of this observation on whether or not *A* and *C* are derived from the same parent molecule. The apparent heterogeneity of Cohn fraction II in this respect might merely reflect its origin from γ-globulins of different individuals. The work recorded here attempted to clarify this point by sub-fractionating γ-globulin derived from single individuals on the basis of charge.

Sera from normal subjects, four patients with multiple myeloma, and three with an 'incomplete' anti-Rh antibody were the source of γ-globulin. In brief, the 7 *S* γ-globulins were isolated by diethylaminoethyl cellulose (DEAE) chromatography and separated into 'acidic' and 'basic' components by carboxymethyl cellulose chromatography or starch-block electrophoresis. These 7 *S* sub-fractions were then digested with papain and the resultant 3·5 *S* products were separated by sequential chromatography on carboxymethyl cellulose and DEAE columns. Experiments were also performed with fragments *A* and *C* of the 'incomplete' anti-Rh antibodies to evaluate their serological attributes.

Normal γ-globulin was prepared from 50-ml. samples of serum from two normal donors (P. S., Gm(a^{+}), Inv(a^{+}), and J. D., Gm(a^{-}), Inv(a^{-})) by elution from a 4 × 40 cm DEAE column[13] with 0·01 M phosphate buffer, *p*H 8·0. This yielded pure 7 *S* γ-globulin as shown by immunoelectrophoresis[14]. A 250-ml. sample of each protein was further separated into four sub-fractions on a 2 cm × 30 cm carboxymethyl cellulose column, using stepwise elution as follows: buffer 1, 0·005 M phosphate, *p*H 6·6; buffer 2, 0·01 M phosphate, *p*H 6·6; buffer 3, 0·025 M phosphate, *p*H 6·9; buffer 4, 0·05 M phosphate, *p*H 7·1, plus 0·04 M sodium chloride. In a further experiment, normal γ-globulin was applied to a carboxymethyl cellulose column with 0·025 M phosphate, *p*H 6·9, plus 0·025 M sodium chloride to elute the 'acidic' and 'intermediate' portions; 0·05 M phosphate, *p*H 7·6, plus 0·5 M sodium chloride (buffer 5) was then used to elute the most 'basic' portion. The 7 *S* γ-paraproteins of two patients with multiple myeloma were prepared similarly by elution from a DEAE column. The γ-cryoglobulins of two other myeloma patients were isolated and purified by repeated cold precipitation and washing. All the globulin preparations were concentrated to 15 ml. by dialysis against polyethylene glycol and then against phosphate-buffered saline, *p*H 7.0. The solutions were made 0.01 M in cysteine and 0.002 M in disodium ethylenediamine tetraacetate, mercuripapain (Worthington Biochemicals) was then added in the amount of 1 per cent of the protein by weight. Digestion was allowed to proceed at 37° C for 16 h and was then stopped by dialysis against 100 ml. of 0.01 M phosphate buffer, *p*H 7.6, in the cold. The amount of dialysable material formed was determined by a modified Folin technique[15]. The digests were fractionated in the cold by the two-step chromatographic method of Franklin[9] (carboxymethyl cellulose columns at *p*H 7.6, followed by DEAE columns at *p*H 8.0). The same batch of ion exchanger and the same conditions of flow rate and buffer were used for each run. The amount of protein in each fraction was determined by optical density at 280 mμ. γ-Globulin possessing incomplete anti-D (anti-Rh_0) activity was isolated in the same manner as the γ-globulin devoid of demonstrable antibody activity for this specific antigen. Anti-Rh activity was assayed by standard methods[16]. In all negative serological tests, serial dilutions were used to ensure that a prozone did not exist.

Table 1 shows the relative amounts of protein in the fractions (expressed as percentage of the parent protein) obtained from digests of normal γ-globulin (J. D.), its chromatographic sub-fractions, and the myeloma proteins. As shown, the most 'acidic' sub-fraction of normal γ-globulin (that is, the sub-fraction most easily eluted from the carboxymethyl cellulose column, and hence presumably possessing the lowest isoelectric point) yielded *A* but no *C*. The most 'basic' sub-fraction (that is, the sub-fraction eluted last) yielded mainly *C* and little *A*. The intermediate sub-fractions yielded intermediate ratios of *A* to *C*. Similar results were obtained with chromatographic sub-fractions of another normal γ-globulin (P. S.). The myeloma proteins resembled the artificially produced sub-fractions of normal γ-globulin in yielding markedly unequal amounts of *A* and *C*. (The property of cold precipitation was lost by the cryoglobulins after papain digestion, and was not present in any of the fractions or various mixtures thereof.) In confirmatory experiments, 'fast' and 'slow' sub-fractions of the two normal γ-globulins (P. S. and J. D.) were isolated by starch-block zone electrophoresis[17]. The relative amounts of *A* and *C* fragments obtained from papain digests of 'fast' and 'slow' sub-fractions isolated by starch-block electrophoresis were similar to those obtained from the 'acidic' and 'basic' chromatographic sub-fractions, respectively. Agar diffusion examination of the isolated *A*, *B* and *C* fragments of the 'acidic' portion obtained by chromatographic fractionation gave bands of identity with the comparable fragments obtained by isolation of the 'fast' γ-globulin from starch-block electrophoresis. The same was true of the papain fragments of the 'basic' (chromatographic) and 'slow' (starch-block) fractions of the 7 *S* γ-globulin.

Table 2 shows the representative results obtained with one (Ri) of three incomplete anti-Rh antibodies. The parent γ-globulin possessed incomplete anti-Rh activity as measured by antiglobulin test and by its ability to agglutinate *D*-positive erythrocytes treated with ficin or suspended in 30 per cent albumin. No agglutination was observed in saline. The papain digest of the Ri γ-globulin and its *A* and *C* fractions retained anti-Rh activity as measured by the antiglobulin test with rabbit anti-serum to the *A* and *C* fragments, but showed no detectable activity against red cells pre-treated with proteolytic enzymes (ficin, trypsin and papain) or suspended in albumin. The *B* fraction was inactive by all three methods. These results are in accord with those obtained by Fudenberg, Mandy and Nisonoff[18] with rabbit 7 *S* hæmagglutinins. (The localization of anti-globulin activity of incomplete anti-Rh antibody to the *A* and *C* fragments was previously observed by Deutsch *et al.*[19].) In a further experiment, the intact anti-Rh γ-globulin (Ri) was sub-fractionated chromatographically into 'acidic' and 'basic' portions. The papain digest of the 'acidic' portion yielded mainly fraction *A* and little *C*, whereas the 'basic' portion yielded the reverse. As before, anti-Rh activity was confined to *A* and *C*. As a control on the chromatographic method, a part of the 'acidic' anti-Rh γ-globulin (*A*/*C* ratio 59:1) was mixed with a ten-fold excess of the 7 *S* γ-globulin (not sub-fractionated) of 'normal' sera lacking anti-Rh activity. The mixture of 'acidic' anti-Rh and of normal γ-globulin was then digested with papain. Although comparable amounts of *A* and *C* were obtained, the anti-Rh activity was limited to fraction *A*. When the 'basic' fraction of anti-Rh γ-globulin was mixed with normal γ-globulin, the anti-Rh activity was confined almost exclusively to papain fraction *C* of the resultant mixture.

These observations indicate that the differences in the net charge of γ-globulin molecules in the serum of a single individual are reflected in the varying proportions of *A* to *C*. The relative amounts of *A* and *C* depend on the charge of the parent γ-globulin. The fact that only these two fractions, *A* and *C*, are obtained from a parent γ-globulin consisting of molecules having a spectrum of charges is probably attributable to the chromatographic method[6,20]. Since molecular weight data[8,9,11] indicate that each 7 *S* γ-globulin molecule consists of two sub-units of the *A*–*C* type and one of the *B* type, it seems likely that some γ-globulin molecules contain either two *A* or two *C* sub-units, that is, two identical univalent fragments. Present results, however, are not sufficient to support the hypothesis that this holds true for all γ-globulin molecules. The fact that the electrophoretically homogeneous myeloma proteins (Table 1) yielded either almost two parts of *A* and almost no *C*, or two parts of *C* and almost no *A* (relative to one part of *B*), is in keeping with this hypothesis. (The trace amounts of *A* in myelomas 1 and 4 and of *C* in myelomas 2 and 3 presumably are due to small amounts of normal (non-myeloma) γ-globulin in the myeloma sera.)

In parallel experiments, the 3.5 *S* fragments of two 7 *S* γ-globulins containing incomplete anti-Rh activity were prepared by sequential treatment with pepsin and a reducing agent[21], their behaviour when tested by anti-

Table 1. RELATIVE AMOUNTS OF FRACTIONS OBTAINED FROM PAPAIN DIGESTS OF VARIOUS GAMMA-GLOBULINS*

	Fraction *A*	*C*	*A*+*C*	*B*	*D*	Peptides	Ratio *A*/*C*
Normal γ-globulin							
Total	34.7	27.9	62.6	28.6	0.8	8.0	1.24
Sub-fraction 1 ('acidic')	48.7	0	48.7	34.3	0	17.0	0
Sub-fraction 2	63.2	1.2	64.4	21.5	0	14.1	52.7
Sub-fraction 3	37.3	19.0	56.3	34.7	0.7	8.3	1.96
Sub-fraction 4	37.8	35.1	72.9	17.6	0	9.5	1.08
Sub-fraction 5 ('basic')	6.7	38.1	44.8	31.3	10.4	13.5	0.18
Paraproteins							
Myeloma 1	16.8	48.9	65.7	22.7	4.1	7.5	0.34
Myeloma 2	53.3	12.1	65.4	26.7	3.3	4.6	4.40
Myeloma 3 (cryoglobulin)	79.4	0.3	79.7	17.2	0	3.1	265.0
Myeloma 4 (cryoglobulin)	8.8	48.1	56.9	24.9	7.3	10.9	0.18

* All values expressed as percentages of the parent protein, except the ratio *A*/*C*.

Table 2. TITRES* OF ANTI-RH ANTIBODY (RI) AND ITS PAPAIN DIGEST FRACTIONS BY VARIOUS TECHNIQUES

γ-Globulin preparation	Method Antiglobulin	Ficin†	Albumin	Saline
Intact (7 *S*)	1:10,000	1:10,000	1:200	—
Papain digest	1:1,250	—	—	—
Fraction *A*	1:1,000	—	—	—
Fraction *B*	—	—	—	—
Fraction *C*	1:1,000	—	—	—

* End-point of final dilution causing detectable reaction. Initial concentration of protein solutions was 10 mg/100 ml.

† Similar results were obtained when cells were treated with trypsin or papain.

globulin, ficin and albumin methods was identical with that of similar fragments prepared with papain The 3 5 *S* fragments of a human 7 *S* anti-*A* isoagglutinin also failed to agglutinate the appropriate erythrocytes, whether or not the latter were treated with enzymes or suspended in a high protein medium However, combination of these fragments with the homologous antigens did occur, as shown by the occurrence of agglutination when an antiserum specific for the 3 5 *S* fragments was added (In a few experiments, the digest of the antibody appeared to retain a slight ability to agglutinate erythrocytes treated with enzymes or suspended in a high protein medium, but in each case this was found to be caused by the persistence of small amounts (1–3 per cent) of intact 7 *S* material, removable by gel-filtration on 'Sephadex *G*-200') Similar results have been obtained previously with rabbit bivalent 7 *S* agglutinating antibodies[18]

The results obtained with the papain digest of the incomplete anti-Rh antibodies merit further comment, in that they provide some basis for speculation on the relationship of the structure of such antibodies to their 'incomplete' character The serological behaviour of the papain digest and its *A* and *C* fractions is what would be expected of truly univalent antibodies, that is, combination with antigen (as detected by the antiglobulin technique), but inability to cause agglutination of appropriate erythrocytes, even when the latter are pre-treated with ficin or suspended in a high protein medium The parent 'incomplete' antibody, which reacts by all three methods, presumably is bivalent[18] The means by which enzyme treatment of erythrocytes or the presence of a high protein medium enables 'incomplete' blood group antibodies to produce agglutination remain to be clarified The mechanisms responsible for the serological behaviour of 'incomplete' antibodies are at present being investigated in studies with univalent fragments of chromatographically sub-fractionated human γ-globulin

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REPRODUCTION IN A FREE-LIVING POPULATION OF DOMESTIC RABBITS, *ORYCTOLAGUS CUNICULUS* (L.), ON A SUB-ANTARCTIC ISLAND

By E. SHIPP
School of Biological Sciences, University of New South Wales, Sydney
AND
K. KEITH, R L. HUGHES and K. MYERS
CSIRO Division of Wildlife Research, Canberra

SINCE 1948 the Australian National Antarctic Research Expedition (ANARE) has maintained a station at Macquarie Island (54° 30′ S , 158° 57′ E) Situated in latitude mid-way between New Zealand and Antarctica, the Island represents the southern limit of rabbit distribution in the Australasian region

The climate is bleak, temperatures varying little from the mean of 40 0° F The mean difference between the hottest and coldest months is 6 0° F and the mean diurnal range is only 5 7° F

Precipitation is evenly distributed throughout the entire year (Table 1) and the average relative humidity is extremely high (92 per cent) Wind gusts exceeding 60 m p h are frequent in every month The average annual sunshine is 1 8 h/day and is concentrated during the period of maximum plant growth (October–March) when a mean monthly maximum of 3 h/day may be reached[1] Even under these bleak conditions the domestic rabbit has continued to thrive since its original introduction by sealers about 1880 The relatively narrow variations in so many climatic factors provide the ecologist with a unique opportunity to investigate those factors which determine the duration of breeding

Observations were made in two separate years by two of us (1950–51 (E S) and 1956 (K K)) while members of ANARE parties at Macquarie Island The number of animals examined each month appears in brackets in Table 1 The large animals were selected in shooting, and the obviously immature ones were ignored in the analysis of the results Body-weight, number of embryos, stage of pregnancy (determined from gross inspection of the uterine horns) and number of corpora lutea were recorded soon after animals were shot Testes were fixed in 10 per cent formalin and were later cleaned and weighed Frozen sections of testes were cut at 20μ and stained with hæmatoxylin and eosin

The combined reproductive data for the two separate years 1950–51 and 1956 are shown in Fig 1

It can be seen that the proportion of females either pregnant or lactating increased from a few per cent in July to 100 per cent in December and thereafter decreased until the cessation of breeding in May. Both testis weight and spermatogenetic activity (determined by the percentage of testes containing either sperm or spermatids) showed a similar trend, although maximum reproductive activity in the males was reached earlier and persisted longer Two other trends were apparent in females Pre-natal reproductive wastage, based on corpora lutea counts of pregnant females, was highest in the earlier months of breeding, being 50, 72 and 80 per cent in September, November and December compared with 15 per cent in February and 24 per cent in March and

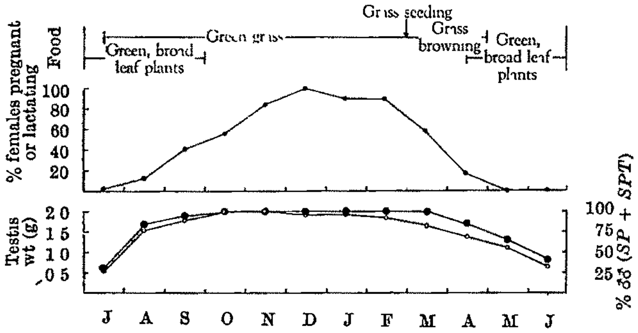

Fig 1 The breeding season of rabbits on Macquarie Island Upper graph ●, percentage females pregnant or lactating, lower graph ○, testis weight in grams, ●, percentage males in which the testis was producing either sperm or spermatids (or both), *SP*, sperm, *SPT*, spermatids

April The number of eggs shed at each ovulation showed a gradual increase in all but the last month of breeding, being 6, 7, 7 4, 7 8, 6 8 in September, December, February, March and April respectively

A prominent feature of reproduction is the relatively long breeding season (July–May) The short annual anœstrus (May–July) corresponds with the season of no plant growth when the preferred pasture species (grasses) are not available (Fig 1). Anœstrus is accompanied by variable loss of weight (Table 1), which is probably caused by fluctuations in both the availability and nutritional value of food

The importance of nutrition is underlined by comparisons between reproductive data (August 1950–April 1951) from two adjacent but dissimilar areas, one with luxuriant plant growth, the other over-grazed and relatively bare, to the extent that rabbits were digging for roots A very small creek, which rabbits were not seen to cross, separated these areas sharply

It can be seen from Fig 2 that females occupying the well-vegetated habitat apparently have a longer and more effectual breeding season than those living in the denuded area In the vegetated area the height of breeding, as judged by the percentage of females pregnant, was reached about one month earlier and continued at a level some 6–20 per cent above that found in females taken from the over-grazed area. The results also suggest that females on the better pasture maintain maximal breeding for about one month longer

The picture in the males is essentially similar Males from the vegetated area almost always have a higher mean

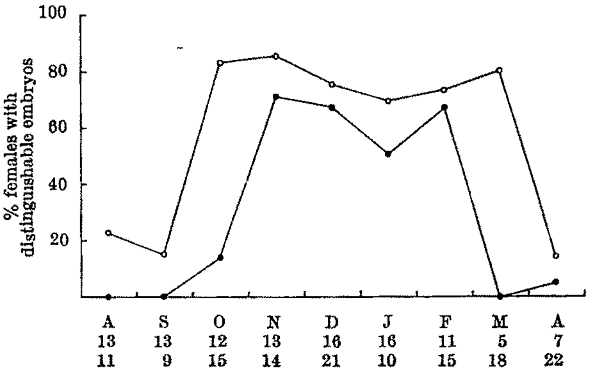

Fig 2 Effect of habitat difference on breeding, 1950–51 season, Macquarie Island
Upper curve ○, well-vegetated area, lower curve ●, denuded area Pregnancy was ascertained from gross inspection of the uterine horns The figures which appear below the letters denoting the month refer to the sample size The upper and lower figures correspond to the upper and lower curves, respectively

testis weight (Table 2) The annual enlargement of testis and accompanying weight increase occurs two months earlier and probably persists at least a month longer than that found in males living in the bare region The testis tubules of all but one male from the better pasture contained either sperm or spermatids during the period August–April (Table 2) In contrast, males from the denuded area during the same period exhibited reduced spermatogenic activity during August, September and particularly April In addition, low levels are shown in Fig 1 for June and July when males were taken only from the bare area

These differences in reproductive performance, and in weight (Table 2), between the rabbits of the two areas may be primarily related to nutritional values of the pastures, though some effect may be ascribed to increased cover and variations in microclimate

The two factors which appear to be correlated with the initiation and maintenance of breeding are day length and season of plant growth Day length *per se* appears to be of little importance, as wild rabbit populations throughout their entire range in the Australian region have overlapping breeding seasons (pregnancy and lactation) which cover every month of the year[2-4] However, a large portion of the Australian range is of a Mediterranean-type climate where summer anœstrus is the rule, and this is in marked contrast with the Macquarie Island breeding pattern A common factor pertinent to breeding in

Table 1 MACQUARIE ISLAND RABBIT AND CLIMATIC DATA

1950–51	June	July	August	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May
Mean body-weight males	1,239	1,369	1,524	1,490	1,475	1,435	1,519	1,548	1,605	1,650	1,654	1,371
Sample size	(27)	(20)	(32)	(39)	(25)	(25)	(29)	(37)	(26)	(23)	(48)	(4)
Mean body-weight females	1,178	1,362	1,532	1,419	1,433	1,562	1,532	1,518	1,589	1,575	1,618	1,390
Sample size	(11)	(30)	(24)	(22)	(28)	(27)	(37)	(30)	(26)	(23)	(29)	(5)
Temperature (mean hourly) (°F)	36 2	38 5	35 9	39 1	40 0	40 1	41 4	43 2	43 7	43 8	40 1	39 5
Precipitation (in)	3 13	2 14	2 83	3 56	3 08	4 27	4 02	4 30	2 92	4 83	3 42	4 28
1956												
Mean body-weight males	—	1,500	—	1,464	—	1,439	—	1,630	1,529	1,560	1,617	1,617
Sample size		(8)		(28)		(9)		(8)	(6)	(18)	(30)	(13)
Mean body-weight females	—	1,475	—	1,469	—	1,700	—	1,659	1,512	1,505	1,570	1,550
Sample size		(8)		(16)		(3)		(10)	(8)	(9)	(26)	(6)
Temperature (mean hourly) (°F)	40 5	37 5	38 5	38 5	39 7	40 8	44 7	44 9	43 7	43 0	42 8	41 5
Precipitation (in)	3 65	2 98	2 93	2 47	3 22	1 51	2 83	6 05	2 32	2 87	2 88	2 90

Table 2 EFFECT OF HABITAT ON BREEDING CONDITION AND BODY-WEIGHT, 1950–51 SEASON, MACQUARIE ISLAND

	Group No	August	Sept	Oct	Nov	Dec	Jan.	Feb	March	April
Mean testis weight (g)	*a*	2 1	2 3	2 1	2 2	2 0	1 9	1 9	—	1 6
	b	0 8	1 3	1 9	1 9	1 8	1 9	1 9	1 7	1 3
Proportion of males with sperm or spermatids	*a*	$\frac{15}{15}$	$\frac{16}{16}$	$\frac{10}{10}$	$\frac{11}{11}$	$\frac{14}{14}$	$\frac{13}{13}$	$\frac{17}{17}$	—	$\frac{7}{8}$
	b	$\frac{12}{16}$	$\frac{18}{20}$	$\frac{13}{13}$	$\frac{13}{13}$	$\frac{10}{10}$	$\frac{17}{17}$	$\frac{9}{9}$	$\frac{2}{2}$	$\frac{25}{37}$
Mean body-weight males and females (g)	*a*	2,153	2,096	2,068	1,954	2,124	2,040	2,090	2,068	2,068
	b	1,816	1,812	1,700	1,812	1,898	1,898	2,011	1,954	1,954

Group *a*, animals from well-vegetated areas, group *b*, animals from denuded area almost all regions, including Macquarie Island, is nutrition In all areas where adequate studies have been made, breeding is initiated by the commencement of the growing season—the shooting of green grass or equivalent preferred plant species being a potent stimulator (Fig 1) Reproduction falls off and eventually ceases in the latter portion of the growing season when pastures deteriorate The importance of nutrition in domestic rabbit reproduction was recognized by Hammond and Marshall[5]

Preliminary observations have been made on natural populations of wild rabbits in sub-tropical Queensland, Australia Although the season of plant growth is here coincident with spring and summer rains, the proportion of pregnant and lactating adult females is reduced during the hottest months suggesting that temperature and not nutrition may be limiting[4]

We thank the many members of the ANARE who helped with the field work on the Island

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DETERRENT EFFECT OF INSECTICIDES ON MALARIA VECTORS

THE effect of residual insecticides on malaria vectors is commonly assessed in the laboratory by using treated panels or containers lined with paper impregnated with the insecticide under examination, with which anophelines from laboratory cultures of known age and feeding conditions are brought into contact The effect of the toxicant investigated is usually judged by knock-down counts and by immediate and 24-h delayed mortality figures For the assessment in the field, the investigator relies largely on the numbers of vector species caught in treated houses resting or biting, and, since the pioneer work of Muirhead-Thomson[1] in Africa, on the results obtained in experimental huts fitted with exit traps, in which it is possible to count the number of mosquitoes dead on the floor and the number found in the exit trap dead or alive and, by holding the latter for a 24-h period, to estimate the delayed mortality Feeding conditions and ovary development of the females caught are usually noted, and to this has been added in recent years the determination of their physiological age by one of the methods described by Detinova[2]

These comparatively simple techniques have shown that the action of the residual insecticides against a winged insect like an anopheline mosquito, free to enter human dwellings in search of food or shelter, is more complex than would perhaps have been suspected Kennedy[3] was probably the first to observe in the laboratory that DDT had an irritant effect on mosquitoes, an observation that was confirmed in the field by Muirhead-Thomson[1], Davidson[4] and many others, but the implications of the irritant effect of this insecticide on the transmission of malaria are not yet completely understood A repellent effect of DDT on house-haunting anophelines was noticed by Gebert and was confirmed later by Muirhead-Thomson[5], Downs and Bordas[6] and others DDT, it appeared, prevented anophelines from entering treated houses, and that it was not due to the irritation produced by this insecticide was shown when other chlorinated hydrocarbons with non-irritant properties, like BHC and dieldrin, also prevented anophelines from entering treated houses The importance of this action of residual insecticides has perhaps not been fully appreciated in the past, but recent observations in Africa indicate that it must have great operational significance

In the course of a successful malaria eradication pilot project in the district of Kigezi in Uganda, experimental huts with mud walls and thatched roofs were used, fitted with exit traps of the type described by Muirhead-Thomson[1] and Davidson[4] Each hut was baited with two men, and it was found that the one treated with 2 g DDT (75 per cent w d p) per m^2—the dosage applied in the area—caught, during the nine months it was in operation, only 30 5 per cent of the number of *Anopheles gambiae*—the local vector—that were captured in an identical unsprayed control hut[7] A clear-cut finding like this, it was considered, deserved further investigation, and, since the success of the campaign in Kigezi had reduced the population of *A gambiae* to practically nil after one year of spraying, it was decided to build a new series of huts in another part of Uganda where DDT or other residual insecticides had not been used in public health campaigns This was done in the district of Masaka, and there it was found that DDT in the form of 75 per cent w d p and at the dosage of 2 g per m^2, and dieldrin in a 50 per cent w d p formulation and at the dosage of 0 8 g/m^2, greatly reduced the number of *A funestus* and *A gambiae*—the two vectors in the area—entering sprayed huts Since in Kigezi our experimental huts, and indeed all the local huts in the area, had been sprayed not only indoors, as is the common practice, but also on the outside 'ingress surfaces' with which a mosquito will probably have been in contact when gaining access to a hut, it was decided to have some of the new experimental huts sprayed inside only and in others to have the insecticide applied to the inside surfaces as well as to the 'ingress surfaces' on the outside These were the door and door frame of the hut (they had no windows other than that to which was fitted the exit trap) and the portion of the eaves and upper part of the walls close to the approximately 0 5 m -1 m opening between the roof and walls through which mosquitoes entered

The reduction produced by DDT and dieldrin on *A funestus* in a seven-month period of observation is summarized in Table 1.

Table 1

	A funestus caught in window trap and floor captures		Per cent reductions in relation to the number caught in control hut	
Hut No	Females	Males	Females	Males
3 (control)	2,692	1,780	—	—
1 (DDT inside only)	534	334	80 2	81 2
4 (DDT inside and 'ingress surfaces')	299	203	88 9	88 6
2 (dieldrin inside only)	528	379	80 4	78 7
5 (dieldrin inside and 'ingress surfaces')	573	380	78 7	78 7

The greatest reduction observed was in hut No 4, where the irritant effect of the DDT applied to the outside surfaces may have contributed to preventing mosquitoes gaining access to the hut Altogether, DDT and dieldrin produced a reduction of 82 1 per cent in females and 81 8 per cent in males of *A funestus* There was also a marked reduction in the numbers of *A gambiae* caught in this experiment, but the numbers in this case were too small to warrant analysis Of great interest was the finding that mosquitoes of the genus *Mansonia*—practically all *Mansonia* (*Mansonioides*) *uniformis*—were not reduced in numbers by either DDT or dieldrin whether applied to indoor as well as outdoor 'ingress surfaces' or to indoor surfaces only There was in fact a slight increase of 9 per cent in the treated huts when compared with the control, unsprayed hut, and this, it should be noted, was observed in a particularly large sample 43,595 individuals, practically all females

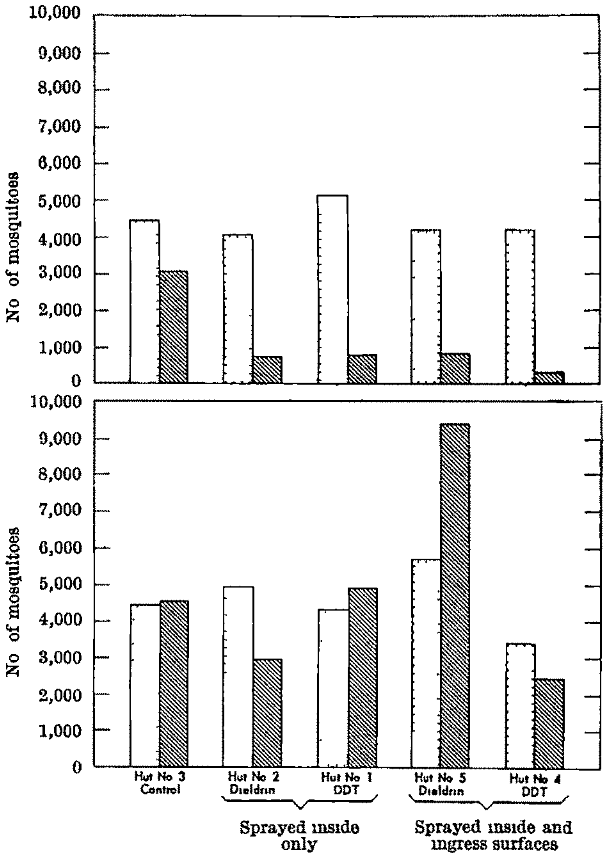

Fig. 1. Effect of DDT and dieldrin on female anophelines (above) and culicines (below) entering experimental huts. 'Before-spraying' (stippled) adjusted to equal time as 'after spraying' (hatched)

The very different effect of DDT and dieldrin on the anophelines and culicines in this experiment can also be appreciated by comparing in each individual hut the results before spraying with the results obtained after application of the insecticides. This has been done graphically in Fig. 1, where the period 'before spraying' has been adjusted to equal time as 'after spraying'.

Another series of observations made in Masaka, comparing the effect of DDT and dieldrin in metal-roofed and thatch-roofed huts, confirmed the phenomenon of reduced entry in anophelines. There was an overall reduction of 89·9 per cent in females and 96·4 per cent in males of *A. funestus* in the treated huts. A sufficient number of *A. gambiae* females were caught this time to show a reduction of 71·4 per cent. As in the previous experiment, *Mansonia* was not reduced in numbers in the treated huts, but showed, on the contrary, a slight increase of 12·5 per cent.

A full account of these observations will be published elsewhere, but it may be worth emphasizing here the importance of the phenomenon of reduced entry when assessing the effect of residual insecticides in anopheline mosquitoes. That the reduction was not an artefact in our results is clear from the finding of *Mansonia* in the same or slightly larger numbers in the treated huts, and also by the work carried out by other investigators like Wharton, Wilkinson[8] and Kuhlow[9], proving that a reduced catch in a treated hut was due in fact to a reduced entry and not to increased and unaccountable exit.

The reduced entry appears, therefore, as a very important factor when considering the overall action of residual insecticides, since a reduced entry means in fact a reduced contact between man and vector. Mortality figures, feeding conditions, physiological age of the mosquitoes captured, will not give a complete picture without a comparison of the numbers caught in treated and untreated huts, or those caught before and after treatment in the same hut.

If the importance of the reduced entry seems clear, the cause or causes of the phenomenon still remain uncertain. That irritation is not the main cause is proved by the fact that a reduced entry has been observed with non-irritant insecticides, such as dieldrin and BHC. That the presence of the insecticides is felt before contact with the deposits inside treated houses also seems clear. The effect then must be felt at a distance, and this must be due either to vapour released from the insecticide deposits or to particles drifting in the air in the vicinity of treated houses. Wilkinson[8] suggested that a reduced catch might be due to the masking of the attractive human odour by the smell of the insecticide, but this would leave unexplained the fact that engorged females are prevented from entering treated dwellings in search of a resting-place. We believe, therefore, that a cause or causes of a more general nature must be at work and that the use of the term 'repellency' either in the sense of contact repellency or of repellency at a distance, would not be adequate. We propose instead the use of the term 'deterrency' or 'deterrent effect' to embrace all possible causes that may prevent mosquitoes from gaining access to houses treated with residual insecticides.

J. DE ZULUETA
J. R. CULLEN

World Health Organization,
Palais des Nations,
Geneva.

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THIS Institute assesses the toxicities of new insecticidal compounds to mosquitoes entering experimental huts. Because toxicity of the insecticide is greatly influenced by the time that mosquitoes spend resting in houses, our work has developed into a very specialized subject requiring a detailed knowledge of feeding and resting habits of the species of mosquito under assessment[1-3]. Our techniques have become more elaborate to assess concurrently the toxic effects of an insecticide and its effects on the behaviour of hut-entering mosquitoes. The chief remaining source of error in our routine assessments is that we have no measure of the proportion of hut-entering mosquitoes that leave through the eaves, or of the survival of these mosquitoes after egress. We have therefore investigated eave-egress in two ways by means of specially constructed huts.

(1) *By surrounding an experimental hut with a cage of mosquito netting.* A simple experimental hut, with a window-trap, was surrounded by a cage of mosquito netting, the sides of which were furled up to allow mosquitoes to enter the hut and unfurled to ground-level to trap mosquitoes leaving the eaves. The species examined were *A. gambiae* and *M. uniformis* which were collected by (1) hand-catches indoors; (2) a window trap in the east wall, (3) hand-catches outdoors within the cage. It was possible to examine the behaviour of unfed and fed mosquitoes, but not of gravid mosquitoes, because collections were made daily. Overall egress throughout the night was determined by combining the results of three periods, examined separately: dusk (5 p.m.–8 p.m.), darkness (8 p.m.–5.30 a.m.) and dawn (5.30 a.m.–7.30 a.m.).

The results in Table 1 are for forty collections. They show that nearly all unfed *A. gambiae* and *M. uniformis* left the experimental hut by dawn, with one-third of the total egress of *A. gambiae*, compared with 97 per cent of *M. uniformis*, leaving by the eaves.

Table 1 SHOWING THE EGRESS OF *A gambiae* AND *M uniformis* INTO A WINDOW-TRAP AND THROUGH THE EAVES OF AN UNTREATED EXPERIMENTAL HUT, IN THE MAGUGU AREA OF TANGANYIKA

	Unfed			Fed		
	Resting indoors	Egress by window	Egress by eaves	Resting indoors	Egress by window	Egress by eaves
A gambiae						
No	4	148	78	2,772	422	361
Per cent	2	64	34	78	12	10
M uniformis						
No	20	25	1,498	211	126	840
Per cent	1	2	97	18	10	72

Table 2 SHOWING THE NUMBERS OF *A gambiae* INDOORS (RESTING + DEAD ON FLOOR), IN WINDOW-TRAPS AND IN EAVE-TRAPS OF EXPERIMENTAL HUTS IN THE TAVETA AREA OF KENYA

Month	Untreated hut			Hut with dichlorvos in dibutyl phthalate			Hut with dichlorvos only		
	Indoors	Egress by window	Egress by eaves	Indoors	Egress by window	Egress by eaves	Indoors	Egress by window	Egress by eaves
1	807	999	142	1,263	338	292	961	302	182
2	704	811	64	438	139	163	747	590	116
3	631	265	42	601	299	165	919	648	124
4	470	259	62	831	547	149	654	630	108
5	290	158	57	690	373	106	357	272	54

The proportion of recently fed *A gambiae* in the window-trap was similar to that found in similar types of untreated experimental huts in Kenya[4], with about equal numbers of the recently fed *A gambiae* leaving by eaves and window-trap

The behaviour of *M uniformis* was quite different from that of *A gambiae* 72 per cent of recently fed *M uniformis* left by the eaves compared with 10 per cent of *A gambiae* This finding indicates that the present type of experimental hut, used for insecticide-assessment[5], would be unsuitable for assessing insecticides against *M uniformis* and other species with similar habits

(2) *By experimental huts fitted with eave-traps* The investigations were made in three wooden portable huts 8 ft. × 8 ft. and with walls $5\frac{1}{2}$ ft high Each hut was fitted with eight egress traps situated between the top of the walls and the roof, which was thatched but completely lined with ivory-faced fibreboard Each egress-trap extended 2 ft along the top of a wall and was V-shaped, the mouth being fitted with mosquito-netting to form a broad inset funnel with a mouth half an inch wide Light entered each trap from outside through a broad slit at the bottom, screened with wire mosquito-gauze Mosquito entry was through eight apertures, each 2 ft long, situated between shutters on the top of the walls and the roof, and alternating with the egress-traps The shutters were sited in relation to the roof to form V-shaped ingress-traps The ingress apertures were 1 in wide in these studies and were screened from mosquitoes inside the hut by narrow strips of cloth placed 3 in. in front of the shutters Provision was made for fitting window-traps in each wall. and the huts were made ant-proof by a method successfully used at Magugu[5] The work recorded here involved all eave-traps and one window-trap placed in the east wall

Investigations were made in an untreated hut and another containing a dichlorvos dispenser (Ciba VIII) that contained the insecticide in solution with dibutyl phthalate The third hut also had a dichlorvos dispenser (Ciba IX) which contained only technical dichlorvos. Mosquitoes resting in the huts were counted *in situ* in addition to observations required to determine overall mortalities[3], so that egress could be related to toxicity of the insecticide

Table 2 shows details of the egress of *A gambiae* into window-traps and eave-traps over a period of five months, and Table 3 shows that the proportion of the total egress that occurred through the eaves increased with overall mortality, as had previously been suspected[3] This latter finding indicates that the present type of experimental hut, used for routine insecticide assessment, is not entirely suitable for assessing irritant or repellent insecticides against *A gambiae* due to loss in efficiency of the window-trap when the insecticide is repellent

Table 3 SHOWING THAT THE PROPORTION OF THE TOTAL EGRESS OF *A gambiae* THAT LEFT BY THE EAVES VARIED DIRECTLY WITH OVERALL MORTALITIES TO DICHLORVOS

Month	Untreated hut		Hut with dichlorvos in dibutyl phthalate		Hut with dichlorvos only	
	Overall mortality (%)	Total egress by eaves (%)	Overall mortality (%)	Total egress by eaves (%)	Overall mortality (%)	Total egress by eaves (%)
1	2	12	85	46	80	38
2	10	7	86	54	32	16
3	11	14	34	36	19	16
4	16	19	24	21	27	15
5	8	27	11	22	11	17

Experimental huts fitted with veranda traps as well as window-traps might be better

The results in Table 3 confirm as yet unpublished findings from experimental huts at Magugu that it is the dichlorvos itself that affects the behaviour of *A gambiae* and not the plasticizer as was first suspected[3]

Finally, the details of the eave-egress of *A gambiae* and *M uniformis*, described here, show that the observations of Zulueta and Cullen—although striking—do not constitute proof that the malaria vectors were greatly deterred from entering treated huts The reduced numbers of *A funestus* and *A gambiae* that they observed may equally be explained by the insecticides irritating many of them to leave by the eaves An increase in numbers of *M uniformis* is in fact what would be expected in an insecticide-treated experimental hut with only a window-trap as normally most of them leave the hut by the eaves the same night as they enter it, toxic effects of the insecticide could reduce the numbers leaving the hut but irritant or repellent properties could not appreciably increase the number leaving

I thank Mr R E Rapley for designing and building the portable trap-huts at Taveta

A SMITH

Tropical Pesticides Research Institute,
Arusha,
Tanganyika

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GAS YIELDS FROM ELECTRON-IRRADIATED COTTON CELLULOSE

By DR F. L DALTON, M. R HOULTON and J A SYKES

Radiation Branch, Isotope Research Division, Wantage Research Laboratory, U K Atomic Energy Authority

MEASUREMENTS by several workers have shown that the principal gaseous products produced when cellulose is irradiated in vacuum or in an inert atmosphere are hydrogen, carbon monoxide and carbon dioxide[1-3] In all cases irradiations have been carried out in sealed tubes at a limited number of high radiation doses in the range 10^7–10^8 rads Such experiments allow the build-up of considerable pressures of reactant gases above the cellulose, and hence introduce the possibility of back reaction In some cases, the outgassing procedure has not been sufficiently rigorous to remove all oxygen from the cellulose, so that some oxidative breakdown may have

Table 1. TOTAL GAS YIELDS (≏HYDROGEN YIELD)

Dose (Mrads)	Gas yield (μmoles/g)	$G_{\text{gas production}} = G_{\text{Hydrogen}}$
0·0955	0·61	6·2
0·0955	0·58	5·8
0·382	2·17	5·5
0·764	3·90	4·9
1·05	4·89	4·5
1·43	6·26	4·2
1·43	6·39	4·3
1·91	7·85	4·0
2·87	10·6	3·6
5·73	19·7	3·3
9·56	29·0	2·9
19·1	49·4	2·5
28·7	68·5	2·3
38·2	88·0	2·2
47·8	109	2·2
57·3	128	2·2
76·4	168	2·1
95·6	210	2·1
119	250	2·0
239	500	2·0
382	815	2·1

Table 2. MASS SPECTROMETER ANALYSES AND DEDUCED G VALUES

Dose (Mrads)	Mole % H_2	Mole % CO	Mole % 'mass 34'	Mole % CO_2	Yield CO (μmoles/g)	Yield 'mass 34' (μmoles/g)	Yield CO_2 (μmoles/g)	G_{CO}	$G_{\text{Mass}_{34}}$	G_{CO_2}
4·78	100	—	—	—	—	—	—	—	—	—
12·4	98·9	0·74	—	0·30	0·26	—	0·11	0·020	—	0·0080
23·9	98·7	0·81	—	0·42	0·46	—	0·24	0·019	—	0·0099
28·7	98·9	0·66	—	0·34	0·45	—	0·23	0·015	—	0·0078
38·2	98·6	0·98	0·06	0·36	0·86	0·05	0·32	0·022	0·001	0·0080
57·3	98·5	0·94	0·06	0·47	1·09	0·08	0·60	0·018	0·001	0·010
76·4	98·1	1·07	0·10	0·73	1·80	0·2	1·22	0·023	0·002	0·015
95·6	98·0	1·14	0·06	0·71	2·39	0·1	1·49	0·024	0·001	0·015

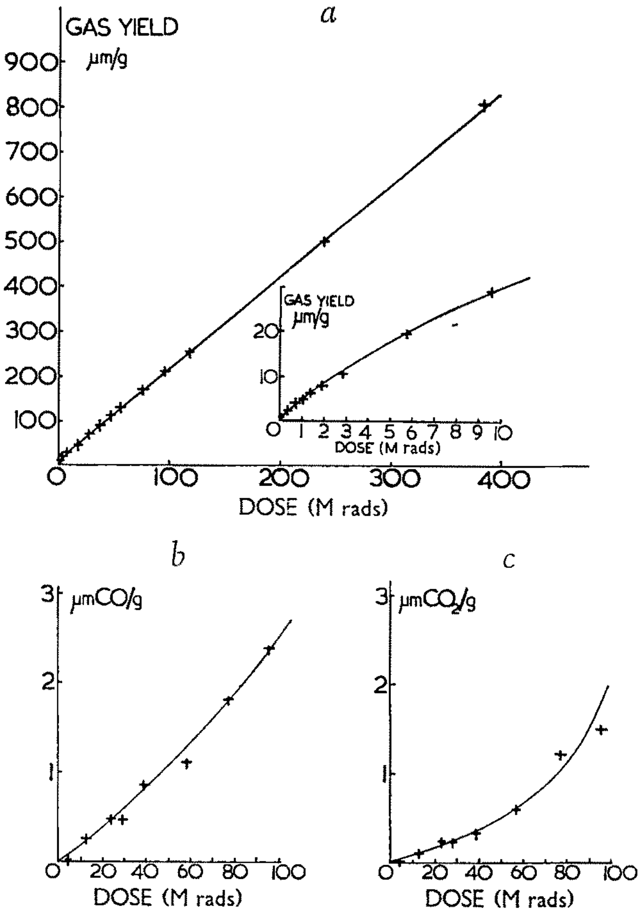

Fig. 1. (a) Total gas yield as a function of radiation dose (inset expands the scale at low doses); (b) carbon monoxide yield as a function of radiation dose; (c) carbon dioxide yield as a function of radiation dose. In all cases the dose rate was 1·30±0·02 Mrads/min

occurred. No systematic study of the G value of products as a function of dose has been reported.

The purpose of this article is to show that the G value of hydrogen, the principal gaseous product, is dependent on total dose and that the reaction products depend critically on the efficiency of the outgassing of the cellulose.

The cellulose used was a 'middling' American cotton which was received as a gift from the Shirley Institute at Manchester, where it had been purified by boiling in 1 per cent caustic soda solution containing 0·15 per cent castor oil soap for 3 h at 35 lb./in.^{2} excess pressure, washing with hot water, treating with cold 0·1 normal hydrochloric acid, washing with cold water and then re-boiling at 35 lb./in.^{2} in 1 per cent caustic soda without soap. After this it was washed as before and dried.

Samples of 0·1–2 g in weight were outgassed on a conventional high vacuum apparatus at 60° C overnight, the pressure being below 10^{-5} mm mercury. It was shown that further pumping under these conditions for a period of one week did not affect the results obtained. The cellulose, contained in a small tube attached to a bulb of sufficient size to prevent the pressure of product gases rising above 1 mm mercury, was then sealed off and irradiated at ambient temperature by means of a 4-MeV Metropolitan Vickers linear accelerator, the dose rate being 1·30±0·02 Mrads/min. Radiation heating was kept below 20° C at the centre of the cellulose by playing compressed air over the reaction vessel. After irradiation, the amount of gas produced was measured by means of a Saunders–Taylor apparatus[4] and its composition afterwards determined by mass spectrometry.

In view of the possibility of small quantities of atmospheric nitrogen contributing to the carbon monoxide peak, carbon monoxide was also determined by gas chromatography. These latter results confirm the mass spectrometry data and will not be discussed further. To remove gas from the cellulose completely, the irradiated material was held at 40° C overnight before admission to the Saunders–Taylor apparatus. This produced no change in the gas yield at doses above 1½ Mrads, but eliminated the retention of small but significant quantities of hydrogen in the cellulose at very low doses.

The results obtained are shown in Tables 1 and 2, while Fig. 1 shows yield curves for hydrogen, carbon monoxide and carbon dioxide. It is clear that the gas is almost entirely hydrogen, a result which is in conflict with the conclusion of other workers. Fig. 2 shows a plot of G value versus dose for total gas (≏ hydrogen), and it can be seen that the G value rises from near 2 at high doses to around 6 at 0·1 Mrads. Both carbon monoxide and carbon dioxide are produced at an increasing rate as the total dose increases. This behaviour is typical of secondary products. The initial G values are 0·02 and 0·008 respectively. From Table 2 it can be seen that a material of mass 34, which may well be hydrogen peroxide, is also produced but in very low yield with a G of about 0·001.

In view of the much lower rate of production of carbon monoxide and carbon dioxide obtained in this work than

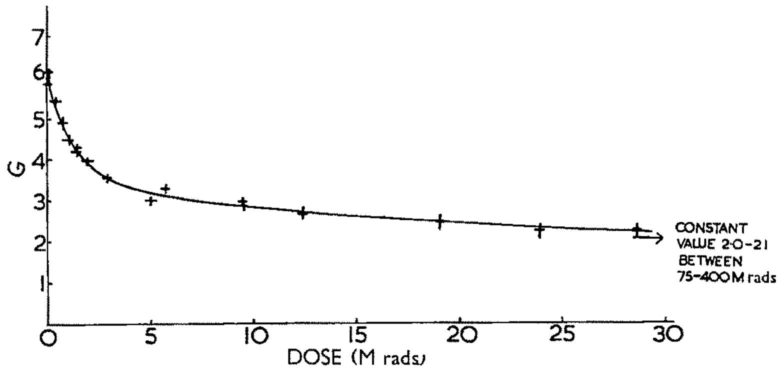

Fig. 2. $G_{\text{total gas}}$ ($=G_{H_2}$ to within experimental limits) as a function of radiation dose

in earlier studies, a check was made on the effect of outgassing. It was found that if 5 g of cellulose were packed into a tube of about 15-ml capacity, evacuated to a pressure of 0·002 mm mercury at room temperature and sealed off, irradiation to a dose of 47·8 Mrads gave a gas which was 82 per cent hydrogen, 5 per cent carbon monoxide and 13 per cent carbon dioxide. This compares with an interpolated analysis of 98·6 per cent hydrogen, 0·96 per cent carbon monoxide and 0·41 per cent carbon dioxide by our usual technique. From this experiment it would appear that unless the outgassing of the cellulose is carried out rigorously a considerable amount of carbon monoxide and carbon dioxide may be formed by oxidative processes, and this may well explain the discrepancy between our results and those of earlier workers. There is also some indication that if the pressure of product gas is allowed to build up above the sample, carbon monoxide and carbon dioxide yields are affected somewhat, but this has not yet been investigated thoroughly.

The change in *G* value with dose is of considerable interest and could arise from several causes, three of the most likely of which are outlined here.

(1) The existence of a small number of hydrolytically weak links in cellulose has been debated for many years, such links, if also weak in radiolysis, would lead to the observed behaviour.

(2) Waite[5] has shown that reactions in condensed phases may be partly or wholly diffusion controlled, and that in such a case, though the rate may be expressed by a conventional 'mass action' equation, $R = k(A)(B)$, the rate constant k is no longer a true constant but varies with time. In view of the complex nature of the degradation of cellulose we may envisage several bi-molecular processes occurring and in so rigid a medium diffusion control is to be expected. If a repair mechanism behaved in this way, the *G* value versus dose curves could well have their observed form.

(3) We have found by electron spin resonance investigations that the concentration of unpaired spins in cellulose irradiated under conditions identical to those used for the gas yield work rises to a maximum within 10 Mrads and then remains sensibly constant. (This result is in agreement with a more detailed examination of electron spin resonance of irradiated cellulose recently published[6].) This would suggest that radical–radical reactions are occurring at an increasing rate during the early stages of irradiation, and if at least a proportion of such reactions remove hydrogen atoms, then the observed behaviour would result.

It is not proposed to present a detailed theory of cellulose radiolysis, since an examination of the solid residues, which is at present being carried out, should enable this to be done more reliably. In this connexion it is of interest to note that the *G* values for both chain breaks and acid production behave in a similar manner to the hydrogen yield and fall to a limiting value as the dose is increased.

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MECHANISM OF FORMATION OF EXCITED CN IN ATOMIC REACTIONS

By DR. D. W. SETSER* and DR. B. A. THRUSH

Department of Physical Chemistry, University of Cambridge

MANY reactions involving free atoms yield excited species. In general, electronically excited species are commonly formed in recombination reactions, whereas the excitation of molecules formed in transfer reactions is normally vibrational rather than electronic. The following afterglow reactions are examples of recombinations which yield electronically excited species, active nitrogen from N+N (ref. 1), NO emission from N+O (ref. 2), air afterglow from O+NO (refs. 3 and 4), sulphur dioxide afterglow from O+SO (refs. 5 and 6) and HNO emission from H+NO (refs. 7 and 8). For some of these reactions, the radiating state does not correlate with ground-state atoms. In these cases, emission occurs from levels with energies below that corresponding to dissociation into normal atoms, and there is evidence that, during recombination, molecules are formed in excited electronic states which correlate with normal atoms and from which they can cross into the radiating state (this crossing is normally a radiationless process induced by collisions). It is to be expected that all the stable molecular states which can be formed from ground-state atoms will be populated when such atoms recombine as, apart from states which correlate via an avoided crossing, the only energy barrier to combination is that due to rotation which is normally negligible.

For diatomic molecules, the potential curves of excited states do not normally cross that of the ground state as the internuclear distance is increased. If potential surfaces for the atomic transfer reaction:

$$A + BC = AB + C$$

are constructed by one of the normal methods[9,10], it is found that the potential surface leading to an electronically excited state of AB lies above that leading to the ground state except where $r(A - B)$ approaches infinity. This is represented diagrammatically in Fig. 1*a*, which shows that the reaction yielding electronically excited AB would be expected to have a higher activation energy than that yielding AB in its electronic ground state.

Such arguments should also apply to atom transfer reactions involving polyatomic species, and it has recently been shown[11] that the transfer reaction

$$NO + O_3 = NO_2 + O_2$$

has an activation energy of 2·5 kcal/mole to yield ground-state NO_2 molecules and 4·2 kcal/mole for the formation of electronically excited NO_2 molecules which give the red emission characteristic of this reaction. In fact, very few transfer reactions which yield electronically excited species are known, and most exothermic transfer reactions which are known to produce excited products give instead molecules in their electronic ground states with excess vibrational energy in the newly formed bond, for example, $H + O_3$ (ref. 12), $O + ClO_2$, NO_2 (ref. 13), $O + O_3$ (ref. 14), $H + Cl_2$, Br_2 (ref. 15). The preferential formation of ground-state molecules is to be expected on the model given, but the presence of vibrational excitation is clearly a function of the shape of the potential surface near the saddle point and will not be discussed here.

A particularly interesting case is the mechanism of CN chemiluminescence which is observed in many reactions between active nitrogen and substances containing carbon. The reaction of active nitrogen with halogenated methanes

* Present address: Department of Chemistry, Kansas State University.

has been investigated in detail by Broida *et al.*[16,17], who have shown that, in these systems, excited CN radicals are formed almost exclusively in the $A^2\Pi$ state (which emits the CN red system) in vibrational levels up to $v' = 10$ They also showed that the $v' = 0$ level of the $B^2\Sigma^+$ state (which emits the CN violet system) is populated by collision-induced transitions from the adjacent levels of the $A^2\Pi$ state[18]. Bayes[19], who also investigated CN red emission from reactions involving active nitrogen, used the notation P_2 for this distribution, which has its maximum population at or near level $v' = 7$ of the $A^2\Pi$ state In the reaction of active nitrogen with HCN, C_2N_2 or ClCN he observed a different distribution which he classed as P_1 and showed it was not formed by the relaxation of the P_2 distribution.

We have recently made kinetic and spectroscopic investigations of these reactions and of the reaction of oxygen atoms with cyanogen, observing both CN red and violet systems in emission Our results confirm Bayes's conclusion that the CN red emission from these reactions can be classified as two groups, P_1 ($v' \leqslant 3$) and P_2($v' \geqslant 3$) We further find that each of these groups is associated with a characteristic distribution in the CN violet emission, the P_2 distribution corresponding to level $v' = 0$ of $B^2\Sigma^+$ being predominantly populated as observed by Broida[16-18] The P_1 distribution in the red system is accompanied by CN violet emission mainly from the higher levels of the $B^2\Sigma^+$ state, transitions from levels $v' = 5$ and 6 and the headless bands just above them being the most intense, the 'tail' bands are also observed decreasing in intensity from $v' = 11$ to $v' = 15$, which is the highest level seen In our experiments this emission and the associated P_1 red distribution predominated in those reactions where the parent molecule contained a CN group (that is, active nitrogen with HCN or C_2N_2 and O + C_2N_2), while the P_2 emission was dominant in the reaction of active nitrogen with halo-methanes where the CN bond was formed in the reaction The active nitrogen plus cyanogen chloride system showed both types of emission from both red and violet systems; this point is discussed later

The excitation energies involved in the CN violet emission which accompany the P_1 distribution are very high (133 kcal/mole for the fairly prominent $v' = 11$ level), and it is almost impossible to find reactions which are sufficiently exothermic to produce CN radicals with these amounts of energy from the CN-containing compounds used We therefore consider that these levels of the $B^2\Sigma^+$ state and the P_1 levels of the $A^2\Pi$ state which accompany them are populated in collisions between previously formed CN radicals and some excited species. This view is substantiated by the observation of NO emission from levels $v' = 0$ and 1 of the $A^2\Sigma^+$ state and $v' = 0$ of the $B^2\Pi$ state when NO is introduced into the O + C_2N_2 reaction showing that NO molecules are acquiring up to 133 kcal/mole of energy in collisions with some species A similar experiment is not feasible for reactions involving active nitrogen owing to the rapidity of the reaction[20]

$$\mathrm{N + NO = N_2 + O}$$

The large excitation energies involved and the fact that both CN and NO have an unpaired electron, which could assist spin reversal in another molecule during a collision, suggest that the species by which they are excited is the metastable triplet state of $N_2(^3\Sigma^+_u)$ which has an energy of 142 kcal/mole This species is known to be present in active nitrogen[21], and there is good reason to believe that it or CO molecules in the corresponding triplet state ($^3\Pi$), which has an energy of 139 kcal/mole, are formed in the O + C_2N_2 reaction. The ratio of the CN violet emission intensity to the red P_1 emission intensity showed some variation between different reactions suggesting that other species capable of exciting only the CN red system might be present.

The reaction between active nitrogen and halomethanes where CN bonds are formed during the reaction shows P_2 emission as a well-defined flame. For this and other reasons, they are believed to be chain reactions and the observed products are explained by a generalized chain mechanism of the type.

$$\begin{array}{llll}
\mathrm{N_2}^* & + \mathrm{C}X_4 & = \mathrm{C}X_3 & + X \\
X & + \mathrm{C}X_4 & = \mathrm{C}X_3 & + X_2 \\
\mathrm{CN} & + \mathrm{C}X_4 & = \mathrm{C}X_3 & + X\mathrm{CN} \\
\mathrm{N} & + \mathrm{C}X_3 & = X\mathrm{CN} & + 2X \\
X & + \mathrm{C}X_3 & = \mathrm{C}X_2 & + X_2 \\
X & + \mathrm{C}X_2 & = \mathrm{C}X & + X_2 \\
\mathrm{N} & + \mathrm{C}X_2 & = \mathrm{CN} & + X_2 \\
 & & = X\mathrm{CN} & + X \\
\mathrm{N} & + \mathrm{C}X & = \mathrm{CN} & + X
\end{array}$$

where X is H, Cl or Br Although the heats of formation of some of the species involved are not known, it is probable that all the essential reactions are exothermic if the parent molecule is a partly or fully halogenated hydrocarbon.

In this scheme, the last reaction is very exothermic. Accepting the lower of the two recently published values of D(C–N), that is, 174 kcal/mole (refs 22 and 23), rather than 184 kcal/mole (ref. 24), this reaction is 94 kcal/mole exothermic for X = H, at least 80 kcal/mole for X = Br (ref 25), and probably 80–90 kcal/mole exothermic for X = Cl, D(C—Cl) being unknown From previous observations it would be expected that this reaction would yield vibrationally rather than electronically excited CN radicals, however, CN is a somewhat unusual species, in that the $A^2\Pi$ state crosses the ground state ($X^2\Sigma^+$) at an internuclear distance of 1 50A and lies below it for greater nuclear separations[26]. If potential surfaces are constructed for this reaction

$$\mathrm{N} + \mathrm{C}X \rightarrow \mathrm{N - C} - X \rightarrow \mathrm{NC} + X$$

the surface leading to the $A^2\Pi$ state which emits the CN red system will lie below that leading to the ground state where $r(\mathrm{C-N}) > 1{\cdot}50A$, as illustrated in Fig. 1*b*. The intersection of the $A^2\Pi$ and $X^2\Sigma^+$ states occurs at an energy of 60 kcal/mole corresponding to level $v' = 7$

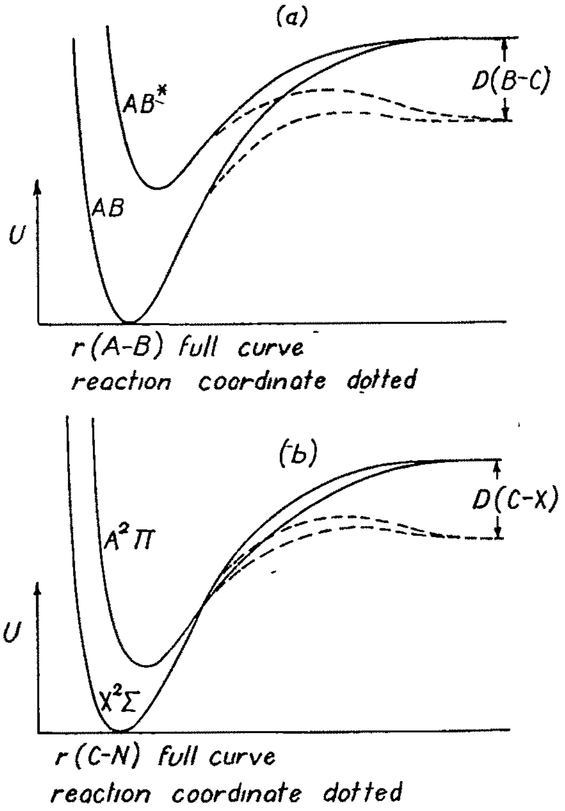

Fig 1

of the $A^2\Pi$ state, it is therefore to be expected that CN radicals formed with energies close to or greater than this will be produced preferentially in the $A^2\Pi$ state which gives the CN red emission. This reaction provides a convincing explanation of the P_2 distribution of the CN emission which has its maximum intensity at level v′ = 7 of the $A^2\Pi$ state and extends up to level v′ = 10 corresponding to an excitation energy of 74 kcal/mole It also explains why the $B^2\Sigma^+$ state, which has its outer limits at smaller internuclear distances than the $A^2\Pi$ state[26], is populated from the latter state rather than directly

In our experiments, the only other reaction which gave significant P_2 emission was that between active nitrogen and cyanogen chloride. While this is a complex reaction giving surface deposits, the P_2 emission is explained by the reactions

$$N + NCCl = N_2 + CCl$$
$$N + CCl = NC^* + Cl$$

The first reaction is exothermic, whereas the corresponding step for HCN would be thermoneutral and probably has a large activation energy.

Thus, the emission from high vibrational levels of CN violet systems including 'tail' bands and the associated P_1 red emission are believed to be due to collisional excitation of previously formed CN radicals By contrast, the P_2 emission is due to formation of electronically excited CN in $A^2\Pi$ state in a highly exothermic transfer reaction The crossing of potential curves which makes the latter process possible is due to the configurations of the states involved, the ground state $X^2\Sigma^+$ is $KK(\sigma g2s)^2(\sigma u2s)^2(\pi u2p)^4(\sigma g2p)$, while the $A^2\Pi$ state is . $(\pi u2p)^3(\sigma g2p)^2$ The promotion of an electron from the $\pi u2p$ to the $\sigma g2p$ orbital increases the bonding at greater internuclear distances but decreases it at shorter distances[27] (the u and g notation can be used since the transition $B \rightarrow A$ which is $^2\Sigma^+_u \rightarrow {}^2\Pi_u$ is not observed) C_2 is another molecule in which excited electronic states cross the ground state at large internuclear distances, and a similar explanation may hold for the 'high pressure' bands from the v′ = 6 level of the C_2 Swan system ($^3\Pi_g \rightarrow {}^3\Pi_u$) which can appear unaccompanied by other bands of C_2 under conditions when C atom concentrations are thought to be high[28]. It has been shown[29] that the recently discovered $^3\Sigma^-_g$ state of C_2 (ref 30) perturbs the v′ = 6 level of the $^3\Pi_g$ state It is likely that if C_2 molecules are formed with the appropriate energy in a reaction involving C atoms, they would be formed preferentially in the $^3\Sigma^-_g$ state (configuration . $(\pi u2p)^2(\sigma g2p)^2$), which at these energies has its outer turning point at a greater internuclear distance than to the ground state . . . $(\pi u2p)^4$, $^1\Sigma^+_g$ or the first excited state . $(\pi u2p)^3(\sigma g2p)$, $^3\Pi_u$ of C_2 Thus the selective excitation of the 'high pressure' bands of C_2 would be associated with a high population of the $^3\Sigma^-_g$ state which has only been observed by the infra-red $^3\Sigma^-_g \rightarrow {}^3\Pi_u$ emission[30]

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ANOMALOUS BEHAVIOUR OF SOME AMINO-ACIDS AS ICE NUCLEATORS

By N. BARTHAKUR and DR. J MAYBANK

Saskatchewan Research Council, Saskatoon, Canada

FOR a long time, certain inorganic compounds of hexagonal structure with lattice parameters similar to those of ice have been known as good cloud-seeding materials Recent work has shown that a few crystalline organic substances will nucleate ice almost as effectively as silver iodide Power and Power[1] were first to determine the nucleating efficiency of some amino-acids which nucleate ice from supercooled vapours at threshold temperatures as high as −4 5° C However, it was Schaefer[2] who first found proteins to be good ice nucleators Head[3,4] has claimed some steroids and α-phenazine to have excellent ice-nucleating properties. Mason and Fukuta[5] have recently investigated more closely the epitaxial growth of ice on organic crystalline substrates and found several steroids to be the most effective substances, forming ice crystals at temperatures close to −1° C Fukuta[6] has recently found metaldehyde to have remarkable ice-nucleation properties, producing ice crystals at temperatures as high as −0 4° C

Several fundamental nucleation techniques were utilized in the present work to investigate more closely variations in the behaviour of some amino-acids as ice nucleators with the view of their practical utility as potential cloud-seeding agents Following the technique of Mason and Maybank[7], mixing cloud chamber tests were carried out using wire-supported supercooled soap films as detectors of ice crystals or active ice nuclei

The appearance of one crystal in 10^4 particles was arbitrarily defined as the threshold for activity for each substance Each compound was tested for ice-nucleating ability at −18° C, and then at successively higher temperatures to determine a threshold for their activity The relevant temperatures were measured within a centimetre of the film surface using a copper–constantan thermocouple No significant difference in results was obtained by using as detectors dishes of supercooled sugar solution enclosed in a copper cylinder Supercooled sugar solution has an advantage over the soap film technique due to the slower growth-rate of the ice crystals.

As recent research has indicated the occurrence of some amino-acids in trace amounts in the atmosphere[8,9], some of these simpler, naturally occurring compounds were first tested for their activity. Peptides, which are polymers that contain only a small number of amino-acid units, were then selected for similar experiments. Table 1 gives the threshold temperatures for ice nucleation of those amino-acids and peptides found to be active at or above −10° C. The bulk water freezing temperatures were determined for these compounds by observing the freezing behaviour of millimetre-size water drops containing suspended particles of each test material. The mean supercooling values so obtained are also listed in Table 1 in order of increasing activity.

Table 1

Compound	Solubility (g/100 g of water)	Mixing chamber threshold temperature (°C)	Bulk water freezing temperature (°C)
L-Valine	5·36 at 20° C	−7·0	−15·0
D-Valine	9·15 at 16·5° C	−6·0	−14·5
L-Tryptophan	1·14 at 25° C	−5·0	−13·5
DL-Tryptophan	Slightly soluble in cold water	−10·0	−12·5
Glycine	25·3 at 25° C	−10·0	−12·0
Glycyl-DL-serine	—	−7·5	−7·0
L-Leucylglycine	—	−6·5	−6·0
L-Isoleucine	4·12 at 25° C	−6·0	−6·0
D-Isoleucine	4·12 at 25° C	−6·5	−6·0
L-Leucine	2·43 at 25° C	−5·0	−5·0
D-Leucine	2·02 at 19° C	−5·5	−5·0
L-Methionine	—	−5·0	−5·0
D-Methionine	—	−5·0	−5·0

Out of 40 amino-acids chosen for investigation, it was found that the racemic variety is generally less effective than the optically active type. D-Leucine and L-leucine nucleated ice at −5·5° C and −5·0° C respectively, whereas DL-leucine gave a threshold temperature of −12·0° C. Similar results are found for valine, isoleucine and tryptophan. Differently prepared samples of DL-leucine were tested for their activity and found to yield varying threshold temperatures. This might be due to the dependence of nucleating site density and crystal form on the method of preparation.

Efficient cloud-seeding nuclei require stability in sunlight. Two-hour exposures to a 550-W mercury vapour lamp at 20 cm or to strong sunlight produced no significant change in activity of the powdered samples.

Table 1 shows, for the first five compounds, the discrepancy between the threshold temperature obtained from the freezing of supercooled water vapour and that of bulk water. This is particularly pronounced in L-valine, D-valine (each of which possessed a very well marked onset of activity in the mixing chamber) and L-tryptophan. Maybank[10] observed a similar effect in the case of three clay minerals, namely, gibbsite, kaolinite and vaterite, and concluded that the freezing ability of a particular nucleus is dependent on the manner in which it is introduced into the supercooled water droplets, particularly if solubility is a factor. The discrepancy with some of the amino-acids might be explained in a similar way.

Serine, tyrosine, cysteine and threonine are all found to be inactive, regardless of their stereoisomers. Several peptides such as DL-phenylalanylglycine and glycyl-L-leucine were observed to have moderate threshold temperatures of −11° and −12° C, respectively. Despite the fact that serine was ineffective, glycyl-DL-serine gave a fairly consistent threshold temperature of −7·5° C.

It is interesting to note that glycine, alanine and other amino-acids have been recently reported to be synthesized in the laboratory by irradiating simpler compounds[11]. It is suggested that amino-acids, the origin of which in the atmosphere, rain and snow is yet unknown, might be synthesized by radiation and by lightning discharge, thus influencing the precipitation mechanism by the formation of additional ice nuclei.

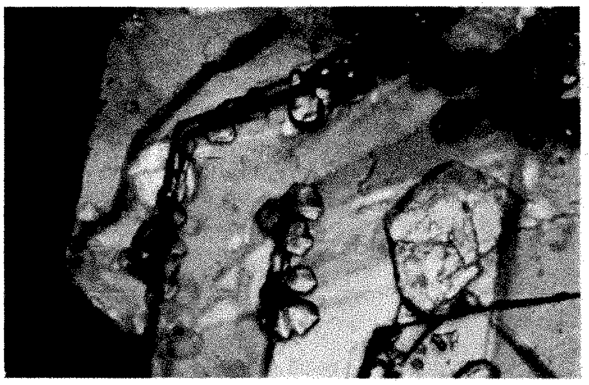

Fig. 1. Pseudo-hexagonal ice crystals growing preferentially on the steps of a D-leucine substrate at −15° C. (× c. 190)

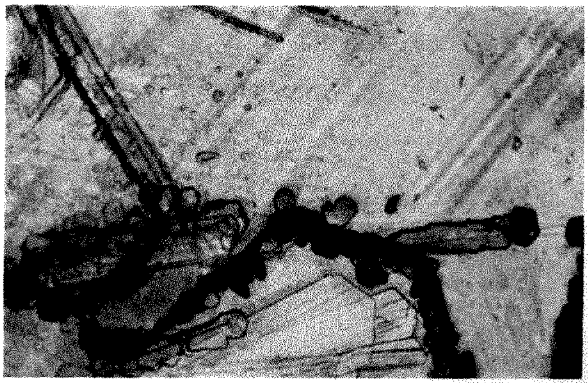

Fig. 2. Oriented ice crystals on D-valine at −15° C showing preferential nucleation at shrinkage cracks. (× c. 470)

Epitaxial growth of ice on amino-acid substrates was investigated in the microscope cold-stage and is illustrated by the photo-micrographs in Figs. 1 and 2. Ice crystals were found to grow preferentially on the steps and shrinkage cracks of the organic substrates. Certain crystals showing many active sites were taken for close observation. Ice crystals appeared and grew usually only on a few active sites, leaving the other sites free of ice. By varying supersaturation and temperature, no difference in behaviour was observed. By repeatedly growing and melting ice on the substrate, under apparently identical conditions, the sites previously active became less and less so, while other sites gradually became nucleating centres. Decreasing activation might result from the fact that many amino-acids are soluble in water, although in some cases only with difficulty in cold water; so during the process of repeated growth and evaporation of the ice crystals on the substrate the active sites were simply being destroyed by water solvation.

Experiments conducted to ascertain whether certain particles act as sublimation or freezing nuclei produced inconclusive results. In the temperature range of 0° C to −30° C, water vapour was always observed to condense on the substrate and then afterwards freeze. Below this temperature range, it was found very difficult to decide whether water vapour was first condensed or not. Unlike mica[12], no part of any of numerous amino-acid crystals investigated was found to permit ice crystals to survive in an unsaturated atmosphere. This might be an indication that amino-acid crystals will not act as sublimation nuclei.

Nucleation has been shown to occur with compounds possessing —OH, =O, —NH_2 groups. Amino-acids fall into this category. Ice-nucleating ability of these organic substances is not yet well understood. From the investigation reported here, it does not appear to be closely related to their lattice geometry. Disregistry between various amino-acid nucleants and ice crystals is found to be well beyond the effective region of 0·005–0·015, where ice crystals grow coherently on substrates according to Turnbull and Vonnegut[13]. Designating the interfacial energy between the substrate and the crystal as σ_{sc}, it may be written as the sum of two terms:

$$\sigma_{sc} = \gamma + \alpha\,(\delta - \varepsilon)$$

where γ is an interaction term due to different bond type, chemistry, etc., and $(\delta - \varepsilon)$ is a structural term, with δ the disregistry between the substrate and forming crystal on low-index planes of similar atomic arrangement. In this equation $\varepsilon = \dfrac{(a - a_0)}{a_0}$, where a_0 and a are the lattice parameters of the nucleus in the strain-free and strained condition, respectively. Vonnegut[13] determined the value of α to be 180 ergs/cm². For amino-acid substrates, it could be the first term γ which plays the most important part. As was pointed out by Mason and Fukuta[5], the important factors determining the nucleating ability of these compounds are the presence and arrangement of hydrogen bonding groups on their surfaces. The exposure of different bonding groups at the steps and cracks might explain why they are preferred sites for the ice-nucleation mechanism, despite the fact that crystal dissymmetry is not itself apparently important.

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RHEOLOGY OF DILUTE AQUEOUS SUSPENSIONS OF SOME REACTIVE SOLIDS

By A. K. CHATTERJI and G. W. KAPSE

Central Building Research Institute, Roorkee (U.P.) India

WE have made some rheological measurements of dilute aqueous suspensions of some solids which react with water (as lime, Portland cement and plaster of Paris). It is found that, when the reaction occurs for a short time, the plastic viscosity of these suspensions (which are non-Newtonian in character) can be altered to a large extent by accelerating or retarding the reaction rate of the material with water. Acceleration of the reaction increases the viscosity and its retardation has the opposite effect.

Results are explained on the assumption that reaction between these materials and water is a chemisorption process. In such a process the position of the bulk Fermi-level of the particles controls the coverage of chemisorbed molecules. Further, when the water molecules are adsorbed on the surface of the particles, they have their charged groups protruding outwards. These groups help to orient the water molecules in the region immediately surrounding the particles, and the layers of water there are stabilized. Acceleration of the reaction leads to stabilization of the structure of water in a larger volume than would have happened if the reaction were allowed to proceed normally. Increased extent of stabilization of water lattice may be responsible for enhanced viscosity when the reaction rate is augmented. When the reaction rate is lowered, the coverage being smaller than when the reaction rate was normal, the volume over which water structure gets stabilized is smaller than that which would have been normal, and lowering of viscosity of the suspension is observed.

Forslind's view[1] about the viscosity of hydrogels appears to be applicable in such dilute suspensions. The present view, in addition, stresses the importance of the position of Fermi-level in the reacting particles, and it is concluded that the rheological properties of dilute aqueous suspensions of solids, which react with water by the chemisorption process when the reaction products are not formed in sufficiently large quantities as to alter the water structure, are governed by the position of the bulk Fermi-level of the particle in suspension.

Materials used in these experiments are (*a*) lime (CaO); (*b*) normal Portland cement; (*c*) plaster of Paris; and these were prepared as follows: (*a*) Lime was obtained by heating AnalaR calcium carbonate at 900°–1,000° C for 4 h. The mass obtained was powdered and sieved in B.S.300 mesh and the portion passing was used in all subsequent operations. Portions of the powder thus obtained were heated separately with 0·1 per cent NiO and with 1 per cent ZnO at 800° C for 4 h. (*b*) Normal Portland cement was sieved in B.S.300 mesh and the fraction passing was used. (*c*) Plaster of Paris ($CaSO_4$,1/2H_2O) was prepared by heating $CaSO_4 2H_2O$ at 170°–180° C for 1 h. As a given material was drawn from the same lot and as no sintering could be detected on heating, the specific surface area of the materials used in a particular set of experiments was taken to have remained unaltered.

The rate of reaction of these materials with water was changed as follows. (1) With lime: (i) for acceleration, (*a*) activated lime (heated with NiO) was taken; (*b*) 0·104 per cent of sodium acetate (an accelerator) was mixed with water; (ii) for retardation, (*a*) deactivated lime (heated with ZnO) was taken; (*b*) 0·5 per cent sucrose (retarder) was mixed with water. (2) With cement: 3 per cent $CaCl_2$ was mixed with water for acceleration and 0·9 per cent sucrose for retardation. (3) With plaster of Paris: 0·118 per cent and 0·15 per cent boric acid were used as retarders and 0·148 per cent and 0·17 per cent of K_2SO_4 were used as accelerators. Percentages are by weight of the material.

Rheological measurements were carried out by the multiple point method using a rotational viscosimeter (MacMichael viscosimeter) in which only low shearing rates could be used. The speed of the rotating cup was varied from 5 to 40 revolutions per minute. The rotating cup has an internal diameter of 2·50 cm, and a bob 2·00 cm diameter was selected for use. The bob was immersed to a depth of 4·50 cm in the dispersion under test, in all the experiments. Torsion wire No. 26 was used for plaster of Paris dispersions and wire Nos. 28 and 34 were used for the lime and cement dispersions.

All measurements were carried out at 27° ± 0·5° C and 50 per cent relative humidity. Deionized distilled water was used throughout. The water-to-material ratio was taken as· lime, 4 : 1, cement, 3 3 1; and for plaster of Paris, 3 : 1 and 4 1.

The material was dispersed by stirring it in the required quantity of water or solution for 10 min, the temperature being maintained between 26·5° C and 27° C The dispersion was then quickly poured into the annular space between the cup and the bob of the viscosimeter, and measurement of deflexion was started at the lowest rate of rotation. The latter was raised to the next higher stage of choice and the deflexion noted This was continued until the highest rate of rotation was reached. The deflexion with decreasing speed of rotation was then noted. The time taken to change from one rate of rotation to another was kept constant The entire measurement comprising both increase and decrease in speed was completed in 2·5–2 75 min, and this time was kept similar in all the samples.

Results are given in Figs 1, 2, 3 and 4. As the rate of shear is proportional to the rate of rotation and the torque to the deflexion produced, the results are given as plots between rotation per min and the deflexion.

Fig 1 gives the results for lime. It will be seen that compared with the deflexions (at a given speed) for the suspension of normal lime (curve *a*), the deflexions for NiO-treated lime suspension (curve *b*) and the suspension with accelerator (curve *c*) are greater and those for the suspension of lime treated with ZnO (curve *d*) and for the suspension of lime with retarder (curve *e*) are less. Fig 2 shows the results of measurements on cement dispersions. Curve (*a*) gives the behaviour of normal Portland cement dispersed in water, curve (*b*) when an accelerator ($CaCl_2$) has been added It is seen that the deflexion in this case is less than that for normal cement When sucrose (a retarder) is added, at lower rates of rotation, the deflexion decreases, and at higher rates the deflexion initially tends to equal and then to exceed the deflexion (curve *c*) found in dispersion of normal Portland cement Figs 3 and 4 show the results with dispersions of plaster of Paris. Curves (*a*) in these figures are for suspensions of plaster of Paris as such Curves (*b*) are for those suspensions in which accelerators were added and the curves (*c*) are for those in which retarders were used A change in mixing times from 1 min (Fig. 3) to 10 min (Fig. 4) shows that with increased mixing time the dispersion behaves as a dilatant when accelerator is used.

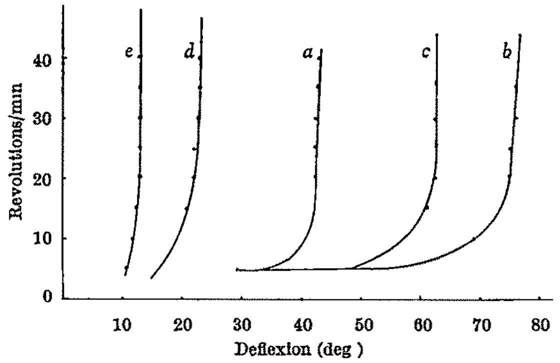

Fig 1 Suspension of lime in water
Curve (*a*) for normal lime, (*b*) for lime treated with NiO, (*c*) for lime with sodium acetate (accelerator), (*d*) for lime treated with ZnO, (*e*) for lime with sucrose (retarder)

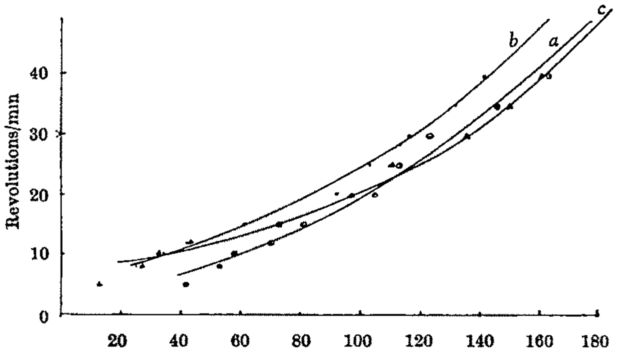

Fig 2 Suspension of cement in water
Curve (*a*) for normal Portland cement, (*b*) with $CaCl_2$ (an accelerator), (*c*) with sucrose (a retarder)

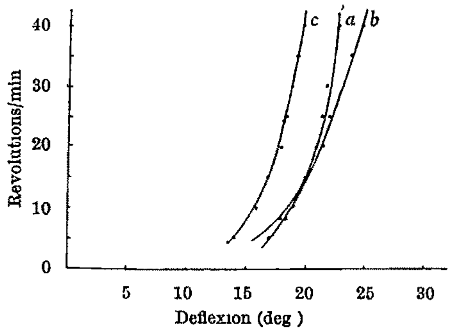

Fig 3 Suspension of plaster of Paris in water Mixing time, 1 min (*a*) Normal plaster of Paris, (*b*) with K_2SO_4 0 148 per cent (accelerator), (*c*) with boric acid 0 118 per cent (retarder)

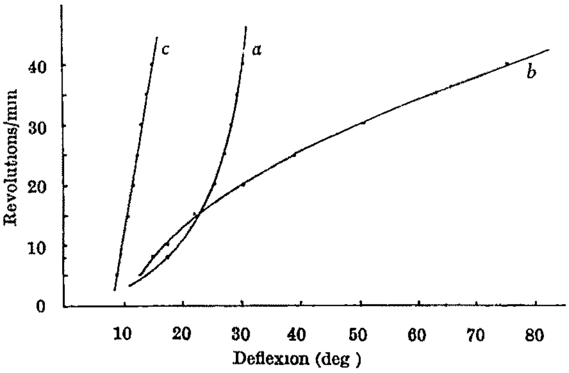

Fig 4 Suspension of plaster of Paris in water Mixing time 10 min (*a*) Normal plaster of Paris, (*b*) with K_2SO_4 0 17 per cent (accelerator), (*c*) with boric acid 0 15 per cent (retarder)

The change in the deflexion produced in the solutions of chemicals, used as accelerator or retarder, compared with the deflexion produced with deionized distilled water is about 1 per cent

When lime is heated with NiO and ZnO, the reactivity of lime so produced with water is enhanced or decreased respectively[2], such treatment also raises or lowers the Fermi-level of lime[2] When lime is treated with NiO, viscosity of the suspension increases, and when it is treated with ZnO the viscosity decreases Again, when an accelerator (sodium acetate) is added viscosity increases, and the reverse occurs with the addition of a retarder (sucrose) to the suspension Since a rise or fall in the Fermi-level of lime crystal enhances or lowers the reactivity of lime with water, the function of agents which accelerate or retard the reaction between lime and water is probably to raise or lower the Fermi-level of lime crystal except where retardation is due to purely physical obstruction

Coverage of adsorbed water molecules on the surface of lime (*n*-type semiconductor) is larger the higher its Fermi-level The reverse occurs when the Fermi-level is lowered This adsorbed layer of water influences the molecules of water in immediate surroundings of the crystal to form stable layers with the molecules in them having some degree of orientation With larger coverage, the volume of the medium having stabilized water layer also increases, and retardation of reaction, which occurs through a lowering of Fermi-level, results in a decrease of the volume having stabilized water layer Stabilization of larger volume water lattice increases the viscosity of the suspension and a decrease in the volume of stabilized water lowers it.

As the reaction is allowed to proceed only for a short while, the possibility of calcium ions disturbing the water lattice is low. Thus, the result of these measurements on the lime–water suspensions represents the influence of rate of interaction of the suspended lime with water on the viscosity of such suspensions

When an accelerator ($CaCl_2$) is used along with normal Portland cement the deflexion, at a given rate of revolution, is reduced This may occur because of loosening of the water structure by chlorine ions[1] and also by removal of calcium ions by the clinker crystals by adsorption The latter may occur through capture of 'holes' by the adsorbed ions from the clinker minerals on the same line as the adsorption of lime from aqueous solutions by Fe_2O_3 (*p*-type reaction)[3] Actual transfer may occur through (OH) groups associated with calcium ions As the clinker minerals lose 'holes'. the position of Fermi-level in these crystals is raised and their reactivity with water is enhanced[4] in the same manner as the acceleration of setting of Portland cement occurs when chrysotile asbestos is present[5] Thus it appears that the acceleration of setting of cement (and the retardation, too) through chemical action may find explanation from the instantaneous position of the Fermi-level in the clinker minerals when these minerals react with water Sucrose may retard the reaction between lime and water if the HO—C—H groups in sucrose get adsorbed on the lime surface through an 'acceptor' type reaction Fermi-level is lowered and retardation of the reaction between lime and water becomes possible Among the clinker minerals in Portland cement, as di- and tri-calcium silicate and tri-calcium aluminate, evidences are in favour of higher density of conduction electron in the latter two minerals[4] With di-calcium silicate, then, the possibility of an 'acceptor' type reaction is less than with the other two minerals, and hence the cause of retardation of setting of cement by sucrose may possibly be both physical and chemical Formation of a surface precipitate will mechanically hinder the reaction with water of di-calcium silicate, while the reactions with tri-calcium aluminate and silicate will be retarded by the chemisorption process on the same lines as in lime This clarifies the views expressed regarding the mechanism of the action of sucrose as a retarder of the setting process in Portland cement[6] Observed lowering of viscosity of cement suspension on addition of sucrose at low speeds can then be explained as due to both mechanical hindrance provided by the surface precipitates on di-calcium silicate and chemisorption on others At higher speeds, precipitates (of sucrose) are thrown out, which goes into the solution and enhances the viscosity due to its own account[7] and also due to enhanced reaction rate

For plaster of Paris, addition of an accelerator (K_2SO_4) increases the viscosity, while the effect of a retarder (boric acid) is to lower it These facts may also be explained on the same principles, and on this basis the initial reaction between plaster of Paris and water should be considered as a solid–liquid reaction This confirms the views of Hansen[8]

The results are thus explained on the assumption that the reaction between these materials and water is a chemisorption process A chemisorbed layer of water is responsible for the stabilization of water lattice The rheological property of the suspension will be governed by how far the absorbed layer of water is able to stabilize the water structure Incidentally, an explanation of the action of accelerators and retarders on setting of cement has been given on the basis of chemisorption of the ions added As these chemisorption processes are governed by the position of the Fermi-level in the suspended particle, the position of the Fermi-level in the solids is considered to be the factor controlling the rheological behaviour of dilute suspensions of materials which react with water by the chemisorption process, as well as controlling the acceleration and retardation of the setting process of cement.

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ENHANCEMENT OF LIGHT OUTPUT FROM A SUPERNOVA

By Dr S A. COLGATE
Lawrence Radiation Laboratory, University of California, Livermore, California
AND
Dr. A G. W. CAMERON
Goddard Institute for Space Studies, National Aeronautics and Space Administration, New York

INTEREST has been aroused by the discovery that certain radio sources are associated with superluminous objects in distant galaxies In particular, the source *3C* 273 is 8–9 astronomical magnitudes brighter than a supernova explosion at the maximum of the light curve[1], and much of the emitted light appears to be synchrotron radiation[2] Occasional flares occur in the light curve of this object, during which an excess energy of $\sim 2 \times 10^{46}$ ergs/sec is emitted within an interval of 10^5–10^6 sec[1]

Among suggestions which have recently been made to account for the large energy radiated by strong radio sources we may mention the following three

(1) A chain reaction in a compact cluster of older stars in which one Type I supernova explosion triggers another[3]

(2) Collective formation of a cluster of more massive stars with similar evolutionary time-scales, which results in numerous Type II supernova explosions in a limited period of time[4]

(3) Formation of a very massive star (10^6–10^8 $M_\odot$), which implodes, releasing a substantial fraction of its rest-mass energy[5]

We have investigated a possible mechanism within the framework of the second of the above theories which may be able to account for the intense light flashes observed in *3C* 273 (ref 1) A normal Type II supernova explosion would not be visible against the strong light output from the *3C* 273 object However, the energy in one of the light flashes is the same order of magnitude as the expected kinetic energy in the ejected envelope from a star of 10–100 $M_\odot$ which undergoes a Type II supernova explosion If occasionally circumstellar gas of density $\sim 10^{-16}$ g/cm^3 accumulates in a very compact cluster of massive stars, then the interaction of the shock-ejected stellar envelope with such a gas could convert much of the kinetic energy into visible light in the required period of time The postulated gas density is much higher than

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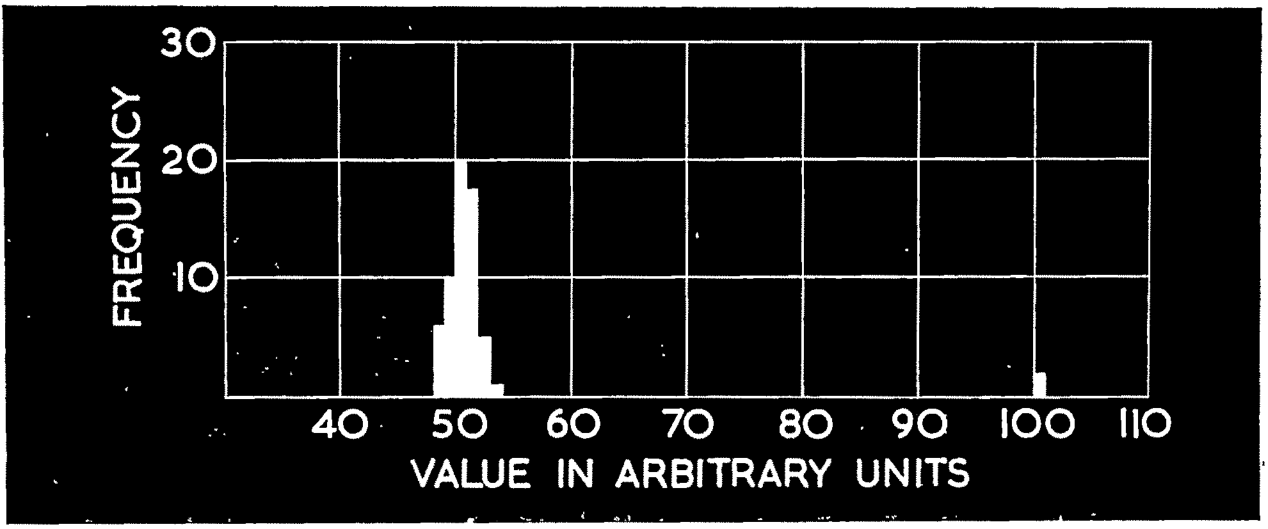

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normal interstellar gas densities, which assures us that this mechanism will have only a rare occurrence, but it would not be surprising to have such a density accumulate in a region such as that under discussion.

The short time-scale of the light flashes indicates that the energy must be released in a rather small volume of space Solar flares are believed to result from the annihilation of magnetic field energy in the solar corona, they last for $\sim 10^3$ seconds The annihilation time varies as the square of the dimensions of the annihilation region. The amount of energy released in a *3C* 273 light flash is so many orders of magnitude greater than that in a solar flare, and the duration is so little greater that it is extremely unlikely that there is any similarity in the processes It seems very unlikely that the light flash energy can be stored in space and released by any triggering mechanism, such storage would have to exceed 200 ergs/cm^3 in the volume that could be illuminated by a light pulse in 10^6 sec

Our philosophy in selecting the most likely origin of the light flash has been to minimize the necessary energy content of the space within which the light flash is to be produced The following crude analysis indicates that much of the kinetic energy of a shock-ejected supernova envelope (from a Type II explosion) can be converted into visible light if a significant fraction of the envelope is slowed by collision with circumstellar gases

Massive stars are believed to undergo implosions, that initiate Type II supernova explosions, when nuclear evolution has converted their cores to iron, and the temperature has risen sufficiently to start the endothermic conversion of the iron to helium[6] We have found that the implosion of the core continues until ordinary nuclear densities are somewhat exceeded[7,8] In a recent theory by one of us[9], the deposition in the envelope of the energy of the neutrino flux from inverse β-decay in the collapsing core permits a fraction (~ 10 per cent) of the rest mass of the core to be converted into kinetic energy of expansion The core is that fraction of the star that collapses adiabatically and in these calculations it has been ~ 20 per cent of the total mass Thus a total energy release of several solar rest masses ($\sim 3 \times 10^{54}$ ergs) is predicted The time of this release ($\sim 10^{-3}$–10^{-2} sec) is short enough so that a shock wave necessarily forms in the star external to the core

Initially the shock wave will not be strong enough either to reverse the implosion of the matter or to eject it from the gravitational potential However, as it proceeds outward it increases in strength both because of the density gradient and because of the additional neutrino energy deposition At some point the shock becomes strong enough not only to reverse the imploding velocity but also to overcome the gravitational potential The matter corresponding to this point asymptotically approaches final velocity after leaving the star, but the shock strength which accelerated it corresponded to the gravitational energy per gram at the time of shock passage If nearly all the mass of the star is ejected, the mean energy of the shock becomes $\sim c^2/50$ erg/g The corresponding mean ejection velocity for the bulk of the mass of the star would be $u_0 = 4 \times 10^9$ cm/sec Consequently, only a two-fold increase in the strength of the shock as it proceeds in the density gradient of the envelope results in matter ejected with this velocity

The increasing strength of such a shock as it proceeds in the envelope has been previously calculated in detail[10] The resulting velocity distribution in the non-relativistic region can be approximated by

$$u = u_0\left(\frac{10M}{M_0}\right)^{-1/5.5}, M \leqslant M_0/10 \qquad (1)$$

where M is the integral mass external to the matter the velocity of which is u, and u_0 is the mean velocity of ejection (4×10^9 cm/sec for 100 solar masses)

If circumstellar gas of low density ρ surrounds the supernova, then half the incident shock energy $Mu^2/2$ will be converted into heat in a distance R, such that $M = 4\pi R^3\rho/3$, in a time $\tau = R/3u$ sec The factor 3 in the denominator of the last expression makes allowance for the fact that most of the volume of the sphere is at a substantial fraction of R

The heat deposited behind the shock may alternately be retained by the fluid or in the opposite limit may be completely radiated away If the heat is retained, then in a spherically divergent flow the gas expands and the heat is converted into kinetic energy, leaving only a small fraction to be radiated away when the expansion causes the gas to become transparent In the present problem we seek the opposite limit where the energy is radiated at the rate of deposition. Two conditions ensure this rapid radiation limit (1) At thermal equilibrium, the radiation energy density Q is larger than the particle energy density (2) The radiation mean free path (λ) is larger than the particle thermal relaxation length (l) behind the shock

The last condition ensures that the thermal energy gradient at the shock front is at least as steep as Q/λ, and the first condition ensures that with this gradient the heat flow $Qc/4$ will be greater than the shock heating rate $Q(u_s - u_f)$, where u_s is the shock velocity and u_f is the fluid velocity behind the shock For u_s, $u_f \ll c$

$$\frac{u_s - u_f}{u_s} = \frac{\gamma - 1}{\gamma + 1} = (\text{compression})^{-1} \qquad (2)$$

so that $Qc/4$ is always greater than the shock heating rate

We shall assume that thermodynamic equilibrium prevails, and then examine this requirement to find the conditions that optimize the visible radiant energy flux

If we assume a uniform density distribution and a mass equal to the incident mass, the total radiation rate becomes

$$W_t = \frac{Mu^2/2}{\tau} = \frac{1.5Mu^3}{R} = 0.4m^{0.45}u_0^3M_0^{0.55} \text{ erg/sec} \qquad (3)$$

which becomes as large as possible at the maximum ejected mass, $M = M_0/10$, giving:

$$W_{t\,\max} = 0.15\,\frac{M_0u_0^3}{R} \text{ erg/sec} \qquad (4)$$

The maximum spectral fraction will be radiated in the visible when $3kT = h\nu = 5 \times 10^{-12}$ erg; or $T = 1$ eV

The initial density ρ_0 corresponding to this radiation rate is determined by

$$caT^4/4 = \rho_0u^3/2$$

or

$$\rho_0 = \frac{1.1 \times 10^{-4}T^4}{u_0^3} \qquad (5)$$

The radius for equal mass at this density is determined by

$$4\pi R^3\rho_0/3 = M_0/10 \qquad (6)$$

giving:

$$W_{t\,\max} = 0.033u_0^2M_0^{2/3}T^{4/3} \text{ erg/sec} \qquad (7)$$

Choosing $T = 1$ eV, $u_0 = 4 \times 10^9$ cm/sec, and $M_0 = 100\,M_\odot$ gives $W_t = 5 \times 10^{46}$ ergs/sec, mostly in the visible at a radius $R = 3 \times 10^{16}$ cm

The assumption of equilibrium must be examined for these conditions

In general, the ion slowing-down path-length due to dynamic friction with the shocked plasma electrons is small compared with the total gas thickness, provided the electron temperature is held below 5 keV by radiation Since the relative ion energy is 8 MeV, this condition requires that the electrons radiate at least $10^3\ kT_e$ However, either the electron temperature must be in equilibrium with the radiation at a few eV, or low-energy (a few eV) bound–bound transitions must be available for non-equilibrium radiation as in the solar corona For the latter case to be effective, the high atomic number species must not be completely stripped of bound electrons (three or more must remain bound) This latter condition for the carbon–nitrogen–oxygen group requires $T_e \leqslant 50$ eV, so that again radiation in the visible requires a relatively low electron temperature One is forced to the conclusion that should the electron temperature rise above 100 eV behind the shock front, the visible radiation would become a small fraction of the total, and indeed the remaining radiation process, bremsstrahlung with Compton scattering, is small enough so that the electron temperature would run away to such a high value, $\gg 10$ keV, that the ion slowing-down length would become larger than the gas thickness, and no further shock would occur.

Therefore, we must demonstrate a self-consistent radiation loss rate large enough to maintain a low electron temperature at the heating rate of the shock (equation (5))

The collisional shock thickness is determined by the dynamic friction between the electrons of the shocked plasma and the incident ions of the cold gas

According to Spitzer[11], the energy loss time of a test charge of mass M and velocity u large compared with the thermal electron velocity of an ionized plasma becomes

$$\tau_s = \frac{u^3 M^2}{(M/m) 8\pi e^4 n_e z^2 z_1^2 \ln \Lambda} \text{ sec} \tag{8}$$

For the low densities involved, $\ln \Lambda \simeq 20$, and taking $z = z_1 = 1$, the slowing-down length becomes:

$$L = \tau_s u = 5\ 7 \times 10^{-17} u^4 / n_e \text{ cm} \tag{9}$$

or:

$$L = 10^{-40} u^4 / \rho_0 \text{ cm} \tag{10}$$

For the conditions of (6) and (7) this gives

$$L/R = 4\ 5 \times 10^{-3} \tag{11}$$

which confirms that there is a small fractional shock thickness, provided the radiation cooling is adequate.

The principal radiation loss from such a low-density plasma will occur due to the excitation of bound–bound transitions of the small fraction of high atomic number elements $z \geqslant 3$. If we assume an element composition similar to that of solar matter, then the CNO fraction, $f = 10^{-3}$, becomes the dominant radiation source For transparent plasmas in the temperature range 5–50 eV, extensive studies of radiation loss from low-lying resonant transitions[12,13] show that the effects can be approximated by an effective allowed transition of 10 eV with a cross-section of 10^{-15} cm^2 for each atom If the calculated radiation rate is then greater than the shock heating rate[7], the electron temperature will fall to a low enough value so that the radiation rate is limited to partial blackbody radiation with a larger fraction in the visible

The energy radiation rate becomes

$$W = (\text{volume})\, n_e n_i\, \overline{\sigma v_e}\, (h\nu) \text{ erg/sec} \tag{12}$$

With the assumption that nearly all the internal energy is radiated behind the shock, the shock compression would approach infinity However, an upper limit is determined by the shock thickness, since a thinner layer would be subject to Taylor instability The compression then becomes R/L and the 'transparent' radiation rate becomes

$$W_R = 4\pi R^2 L f\, [6 \times 10^{23}\, (R/L)\, \rho_0]^2\, \overline{\sigma v}\, (h\nu) \text{ erg/sec} \tag{13}$$

Using condition (6), which maximizes the total available energy, and evaluating $\overline{\sigma v}$ for $5 \leqslant T_e \leqslant 50$ eV as 3×10^{-7} cm^3/sec results in

$$W_R = 2\ 7 \times 10^{65} \frac{f M_0^3}{u^4 R^5} \text{ erg/sec} \tag{14}$$

Comparing this with the energy generation rate of the shock (equation (4)) shows that for radii less than the maximum $R_{\max}$ the resonant radiation can be greater than the shock rate Therefore, the stability condition for a radiation-cooled shock can be met and the maximum radius becomes.

$$R_{\max} = 3\ 7 \times 10^{16} f^{1/4} M_0^{1/2} u^{-7/4} \text{ cm}$$

which for $f = 10^{-3}$, $M_0 = 100\, M_{\odot}$, and $u = 4 \times 10^9$, gives $R_{\max} = 3\ 7 \times 10^{16}$ cm and a radiation rate of 5×10^{46} ergs/sec

At least 90 per cent of this radiation at the maximum radius will be in the ultra-violet, but at smaller radii the electron temperature will be reduced and a larger fraction will be in the visible. Consequently, it appears feasible to radiate an amount of energy from a shell interacting with circumstellar gas comparable with the amount measured by Smith and Hoffleit[1]

In addition, it can be noted that the thickness of the required circumstellar gas is small enough ($\leqslant 10$ g/cm^2) so that the cosmic ray spectrum that may be produced by the shocked outer layers of the supernova[10] will be only slightly modified

For general consistency, it is desirable to show that the model of a supernova explosion used here leads to the usual luminosity of a Type II supernova at the maximum of the light curve, if there is no circumstellar gas present The absolute photographic luminosity of a Type II supernova at maximum is approximately -18 (ref 14) corresponding to an energy emission in the photographic wavelength range of slightly less than 10^{43} ergs/sec We may estimate the light emission from the expanding envelope in a very crude way by considering the decrease in temperature of the shock-heated envelope as it expands adiabatically, and by assuming that this temperature is also characteristic of the expanding envelope at optical depths near unity

We have seen that the outermost layers of the pre-supernova are accelerated to near-relativistic velocities. Hence, after shock ejection, such layers will rapidly become optically thin while the temperature is very high. The bulk of the material is ejected at smaller velocities, and, as a result of the contraction of the inner parts of the pre-supernova, it will lie inside a radius $\sim 3 \times 10^9$ cm This material will be heated by the shock wave to temperatures $\sim 4 \times 10^9$ °K (ref 8) The energy density of radiation will then considerably exceed the thermal kinetic energy of the matter, and so the adiabatic expansion will be approximately characterized by a ratio of specific heats $\gamma = 4/3$

Early in the expansion, $kT/3 \gg \langle h\nu \rangle_{pg}$, so that the luminosity $L \propto T r^2$, where r is the radius of the envelope near optical depth unity We have $T \propto (\text{density})^{1/3} \propto r^{-1}$ Hence $L \propto r$ Later in the expansion, when $kT/3 \leqslant \langle h\nu \rangle_{pg}$, L becomes a sensitive function of T; at the least $L \propto T^4 r^2 \propto r^{-2}$ Hence it is evident that the maximum light emission occurs when $T \approx 10^4$ °K This will occur approximately for a radius $r \approx 3 \times 10^9\ (4 \times 10^9/10^4) = 1\ 2 \times 10^{15}$ cm Hence $L_{\max} \approx 4\pi r^2 \sigma T^4 \approx 10^{43}$ ergs/sec There is great uncertainty in this figure, but it is in order-of-magnitude agreement with the observed luminosity

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LETTERS TO THE EDITOR

PHYSICS

Current–Voltage Relations for Mobility-controlled Sheaths; a Simple Method for Determining Ion Mobility

Recently, the constriction of fairly high pressure discharges by applied radial electric fields has been observed by Kenty[1]. The discharges, which are very sensitive to electrostatic effects, are of the low current diffuse type in which the ions disappear mainly by volume recombination[2]. For d.c. fields, annular rings are provided just inside the wall of the tube. The discharge can be greatly constricted by making these rings negative to the discharge, while at the same time the positive ion current per unit length can be measured.

These observations can be understood in terms of the idea that when the wall is made highly negative a thick positive ion wall sheath is formed in which the current is due entirely to mobility flow. Since the thickness of the sheath can be measured quite easily by direct visual observations, and since almost all the applied voltage appears across the sheath, it has been suggested[1] that the constriction effect might be used to measure ion mobilities in a very simple way. It is readily shown that the relationship between the wall current, J, and the voltage across the sheath, ΔV, is of the form:

$$\Delta V = (J/\mu)^{1/2} g \qquad (1)$$

where μ is the mobility and g depends on the sheath thickness and the size and geometry of the discharge. Hence, knowing the sheath thickness, essentially all one needs to determine the mobility is an ammeter and a voltmeter. In this communication we derive equation (1) and calculate g for sheaths of cylindrical and spherical symmetry. For comparison, we also include the plane-parallel case, which has been treated by Cobine[3].

We assume that in the sheath: (1) only positive ions are present; (2) no production of ions occurs; (3) diffusion can be neglected; (4) the energy gained from the field in a mean free path is negligible compared with the thermal energy of the ions; (5) at the inner edge of the sheath, which is somewhat diffuse in practice, the field is approximately zero. Condition (2) will be fulfilled if photoelectric and secondary emission at the negative (sheath causing) electrode are small, and if the voltage across the sheath is well below that necessary for breakdown. The equations which describe the sheath are then:

$$\nabla^2 V = -4\pi q n \qquad (2)$$

$$\operatorname{div} j = 0 \qquad (3)$$

$$j = -nq\mu \operatorname{grad} V \qquad (4)$$

where V is the potential, n, the ion density, j, the current density, q, the ionic charge, and μ, the mobility. These equations do not, of course, provide a good description of the sheath in the low field region near the inner edge, since assumptions (1) and (3) are obviously not valid there. Since the field rises very rapidly, however, this region is quite small, and for thick sheaths the results are rather insensitive to the precise position chosen for the inner boundary.

We first eliminate n by making use of the fact that from equation (3) the net current across a surface of symmetry is constant. Denoting the plane-parallel, cylindrical and spherical cases, respectively, by an index $k = 0,1,2$, we write:

$$J_k = 4\pi\alpha_k r^k |j| \qquad (5)$$

where, for each symmetry, r is the appropriate co-ordinate and J_k is proportional to the appropriate wall current. It is convenient to take:

$$\alpha_0 = (4\pi)^{-1},\ \alpha_1 = 1/2,\ \alpha_2 = 1 \qquad (6)$$

so that J_0 is the wall current per unit area of the plane, J_1 is the wall current per unit length of the cylinder, and J_2 is the total wall current to the sphere. From equations (2) and (4), on making use of the fact that for the three geometries of interest $\nabla^2 V = r^{-k} \mathrm{d}/\mathrm{d}r(r^k \mathrm{d}V/\mathrm{d}r)$, we then have simply:

$$\mathrm{d}/\mathrm{d}r(r^k \mathrm{d}V/\mathrm{d}r)^2 = (2J_k/\alpha_k\mu) r^k \qquad (7)$$

Neglecting the field dependence of the mobility, this gives, for the field:

$$-\mathrm{d}V/\mathrm{d}r = [2J_k r_s^{1-k}/(k+1)\alpha_k\mu]^{1/2}[(r/r_s)^{1-k} - (r_s/r)^{2k}]^{1/2} \qquad (8)$$

and for the potential distribution:

$$V_s - V(r) = [2J_k r_s^{3-k}/(k+1)\alpha_k\mu]^{1/2} f_k(r/r_s) \qquad (9)$$

where the subscript s refers to values at the inner sheath edge, and:

$$f_k(z) = \int_1^z (t^{k+1} - 1)^{1/2}\, \mathrm{d}t/t^k \qquad (10)$$

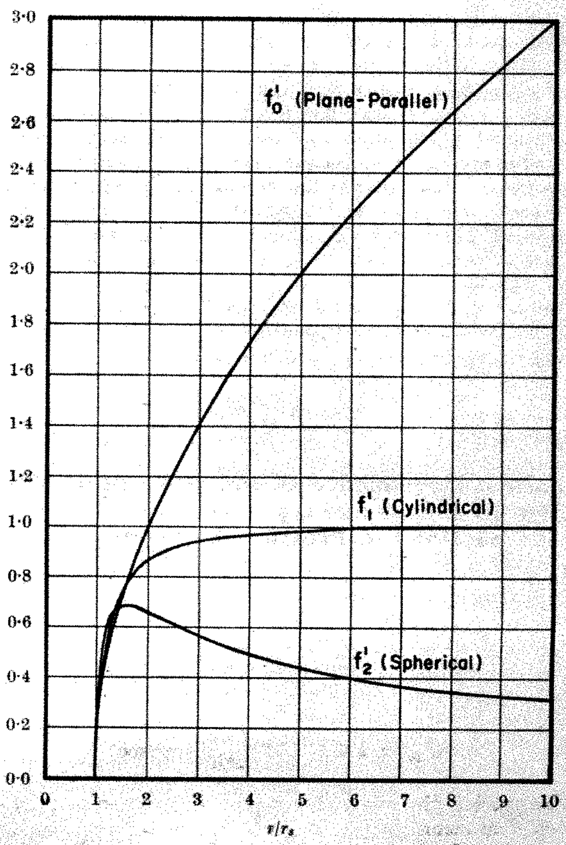

Fig. 1. Field variation in sheath

On setting $r = r_w$, the co-ordinate of the wall, equation (9) reduces to equation (1).

The integrals defining $f_k(z)$ are readily evaluated. We obtain:

Plane-parallel:

$$f_0(z) = (2/3)(z-1)^{3/2} \tag{11}$$

Cylindrical:

$$f_1(z) = (z^2-1)^{1/2} - \cos^{-1}(z^{-1}) \tag{12}$$

Spherical:

$$f_2(z) = z^{-1}(z - 1 + 3^{1/2})^{-1}(2z + 1 - 3^{1/2})(z^3 - 1)^{1/2} + (3^{3/4}/2)[(1 + 3^{1/2})F(\varphi, k) - 2\cdot3^{1/2}E(\varphi, k)] \tag{13}$$

where $F(\varphi, k)$ and $E(\varphi, k)$ are the incomplete elliptic integrals of the first and second kind, of modulus $k = \sin(\pi/12)$ and argument $\cos\varphi = (3^{1/2} - 1 + z)^{-1}(3^{1/2} + 1 - z)$. For small z equation (13) reduces to $f_2 \sim (2/3^{1/2})(z - 1)^{3/2}$, in agreement with the result for plane-parallel geometry. For large z it becomes:

$$f_2(z) \sim 2z^{1/2} - 3^{1/4}[(1 + 3^{1/2})K(k) - 2\cdot3^{1/2}E(k)] + 0(z^{-3/2}) \\ \cong 2z^{1/2} - 2\cdot24,\ z \geq 3 \tag{14}$$

This approximation is accurate to within about 5 per cent at $z = 3$ and improves with increasing z. For intermediate values of z, $f_2(z)$ is listed in Table 1.

Table 1. POTENTIAL DISTRIBUTION IN SPHERICAL SHEATH

z	$f_2(z)$	z	$f_2(z)$
1·1	0·0335	2·1	0·689
1·2	0·0877	2·2	0·754
1·3	0·150	2·3	0·818
1·4	0·216	2·4	0·880
1·5	0·284	2·5	0·942
1·6	0·353	2·6	1·003
1·7	0·422	2·7	1·063
1·8	0·490	2·8	1·121
1·9	0·557	2·9	1·179
2·0	0·624	3·0	1·236

The functions $f_k'(z)$ are plotted in Fig. 1, which indicates the small extent of the low field region, where the theory breaks down. The cylindrical case is of particular interest, because in this geometry the field is practically uniform across the entire sheath, so that the effect of neglecting the field dependence of the mobility is quite small.

The foregoing theory has been used, together with data on a cylindrical xenon discharge, to measure the mobility of xenon ions[1]. Agreement with published mobilities is satisfactory, indicating that the foregoing conditions 1–5 are sufficiently fulfilled.

M. A. WEINSTEIN
C. KENTY

General Electric Lamp Research Laboratory,
Nela Park, Cleveland, Ohio.

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Thermal Dependence of the Absorption Band Edge in Zincblende-type Cadmium Sulphide obtained in Pressure Experiments

THE discontinuity in the optical absorption edge shift of wurtzite-type cadmium sulphide (CdS–*W*) under a pressure of 27,000 kg/cm², reported in Edwards–Drickamer's experiments[1], was assumed as a phase transition, essentially complete at about 40,000 kg/cm², perhaps[2] due to the zincblende phase (CdS–*Z*). The high-pressure X-ray experiments recently carried out by Rooymans[3] in CdS–*W* under a pressure of 20,000 kg/cm² have shown indeed a rocksalt-type structure (CdS–*R*) with a lattice constant, measured under pressure, of a_0 = 5·30 Å and an X-ray density of 6·44 corresponding to $\Delta V/V = -25$ per cent during the transition CdS–*W*→CdS–*R*.

Spectroscopical observations carried out in our laboratory show that, after the removal of pressures, variable in range 25,000 kg/cm²–40,000 kg/cm², to which a set of CdS–*W* powder was subjected, the position of the absorption edge is always situated at a constant value of 5860 Å with respect to 5070 Å of the original CdS–*W*. According to a recent remark by Rooymans[4], the CdS–*W* after pressure treatment is mainly of the CdS–*Z* structure.

This communication aims at reporting the pressure-method used to prepare this unusual structural form of the CdS semiconductor and its fundamental optical properties, that is, the value of the optical activation energy and its thermal dependence estimated from measurements on optical absorption-edge. The CdS–*Z* structural form has been prepared as a definite and separate phase by pressing, under 40,000 kg/cm² at room-temperature, very fine powder of CdS–*W* into tablets, the press form being simultaneously evacuated. In pressing, uniform distribution of the material in press form received special attention. Samples, reddish in colour and glass-like after the first pressure treatment, became microcrystalline after a second treatment; the average crystal size increases after further pressure treatments of powdered material, and closely packed crystallites were visible under an ordinary microscope; the pyknometrical density of polycrystalline samples is found to be 4·10 g/cm³. The removal of the pressure, after the first treatment of CdS–*W* powder,

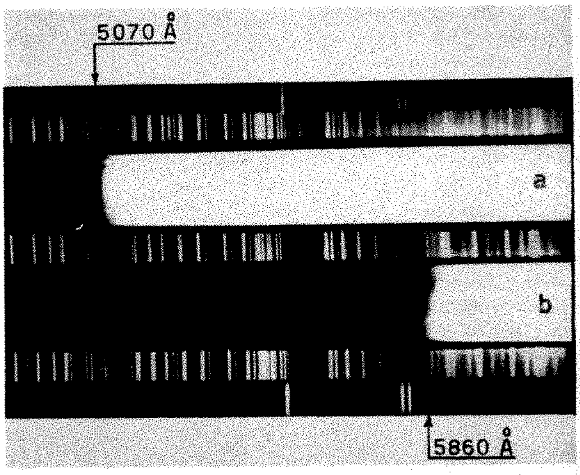

Fig. 1. Shift of the room-temperature absorption-band edge after CdS–*W* (*a*) to CdS–*Z* (*b*) pressure transition

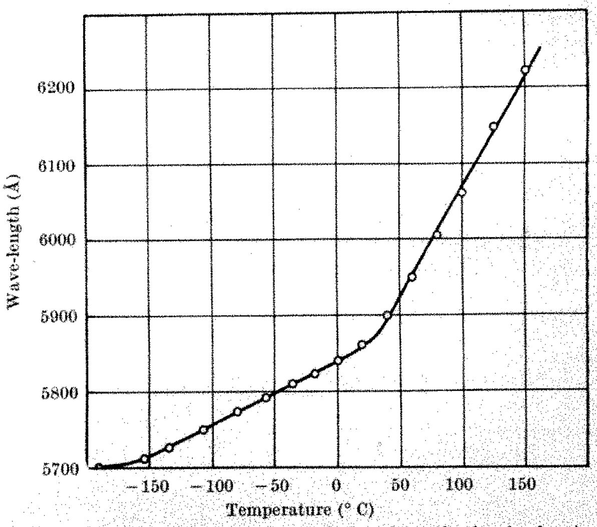

Fig. 2. Temperature dependence of the absorption-band edge in CdS–*Z* polycrystalline material

systematically causes the breakdown of the sample contained in a ring inside the press form; this is due to the increase in volume during the transition from the high-pressure CdS–R to the normal-pressure CdS–Z phases.

Comparatively, Fig. 1 shows the absorption spectra of CdS–W (*a*) and CdS–Z polycrystalline sample (*b*) carried out at room-temperature on the specimen, ground and polished to a thickness of 0·3 mm. From these measurements we obtain, for the CdS–Z polycrystalline sample, a value of 2·10 ± 0·01 eV at an absorption coefficient of $2{\cdot}5 \times 10^2$ cm^{-1}, in full agreement with the photoelectronic indication of a band gap of 2·1 eV recently reported by Lind and Bube[5] for cubic cadmium sulphide powder prepared by thermal decomposition of cadmium methyl mercaptide. According to our measurements, the CdS–Z form has an energy gap of 0·33 eV lower, at room-temperature, than CdS–W; this is an interesting fact since CdS–W and CdS–Z are, from the point of view of energy, quite similar. It should also be noted here that, for CdS–Z glassy specimens obtained after the first pressure treatment of CdS–W, the edge of the absorption band is situated at slightly longer wave-lengths compared with CdS–Z polycrystalline specimens. This edge shift, which corresponds to a decrease in the width of the forbidden band in CdS–Z glassy specimens, may be the result of a slight modification in the short-range order due to the glass formation[6,7].

Fig. 2 shows the plot of the absorption band edge as a function of temperature in the range from liquid air to 150° C; the wave-length of the edge is taken here as the point where the transmission is 0·5 per cent of its value in the non-absorbing region. $d\lambda_{\rm lim}/dT$ presents two slopes in the considered temperature range which corresponds to a shift of the band gap equal to $-3{\cdot}4 \times 10^{-4}$ eV/°C in the range $-150°$ C to 20° C, and to $-9{\cdot}4 \times 10^{-4}$ eV/°C between room-temperature and 150° C. We believe that the possibility of transforming the hexagonal form (CdS–W) into the cubic form (CdS–Z) with a physical process—that is, without changing the nature and the concentration of impurity atoms—offers a useful tool for studying the general properties of the CdS semiconductor depending on the long-range order, since the difference from CdS–W and CdS–Z is of geometrical order rather than of structural.

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P. MANCA
F. RAGA

Istituto di Fisica della Università di Cagliari,
Cagliari,
Italy.

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Anomalous Electron Scattering in Dilute Silver–Manganese Alloys and its Analogy with the Ramsauer Effect

DILUTE alloys of a transitional metal (for example, manganese) in a noble metal (for example, silver) exhibit anomalous properties at low temperatures. Their electrical resistivity in the neighbourhood of liquid hydrogen temperatures shows a minimum, followed by a maximum at a still lower temperature[1]. They exhibit a large negative (electrical as well as thermal) magneto-resistance[2,3]. Magnetic susceptibility measurements[4] suggest an antiferromagnetic transition with indications of 'parasitic' ferromagnetism as well.

The Lorenz parameter (in a zero magnetic field) shows anomalous behaviour[5] at liquid helium temperatures, 'dipping' below the normal Sommerfeld value L_n and tending to that value (L_n) at temperatures of about 1° K or below. This is probably because the pseudo-Weiss internal field arising from the transition to ferro/antiferromagnetism removes the spin degeneracy of the ground-state of the impurity (manganese) ion. The possibility of the manganese ion occupying either of two (or more) closely spaced levels makes it possible for the conduction electrons to undergo 'spin-flipping' inelastic collisions[6,7] with it, besides the normal elastic collisions. Since it is well known that inelastic small-angle scattering can, by virtue of electron transitions 'vertical' with respect to the Fermi surface, contribute considerably more to the thermal resistivity than to the electrical resistivity (while large-angle impurity scattering, even if inelastic, would, to quote Ziman[8] "merely turn a hot electron going east into a cold electron going west and thus has little effect on the heat current"), this mechanism seems to explain the dipping of the Lorenz parameter below the Sommerfeld value. The fact that, close to absolute zero, the Lorenz parameter approaches the value L_n is also understandable since such an inelastic scattering could not occur at absolute zero of temperature. Scattering which would involve loss of energy by the electron is not permitted since there are no lower electronic states available for it, scattering which would involve gain of energy by the electron is also not possible since there are no ions in the excited states at 0° K to transfer energy to the electron.

One would wonder whether, apart from this indirect evidence based on a dipping Lorenz parameter, there is any other evidence pointing to the possibility of the inelastic electron scattering taking place appreciably at small angles. Let us consider, for example, the anomalous scattering of slow electrons by rare gas atoms. Linde[9] has directed attention to the close similarity between the anomalous scattering of conduction electrons at low temperatures, due to magnetic impurities in noble metals and the anomalous scattering of electrons (having kinetic energies in the neighbourhood of the usual Fermi energies) by rare gas atoms, the so-called Ramsauer–Townsend effect[10].

Firstly, anomalous electrical resistivity behaviour is exhibited in a noble metal containing small amounts of metals like chromium and manganese which, in the gaseous state as well as in dilute solution[11], have an electron configuration rather similar to that of a rare gas.

Secondly, in the case of inelastic collisions of electrons with helium atoms, for example, it is found that, at small angles, inelastic scattering considerably exceeds the elastic scattering but drops below it at larger angles. If, in particular, the electrons are slow, electron exchange becomes significant and the experimentally observed[12] large scattering at small angles may be due to polarization effects caused by the interaction of the inelastic and the elastically scattered electron waves[13].

The anomalously high ('giant') thermo-electric power in these dilute alloys at low temperatures is thought to occur if, besides the asymmetry of the inelastic scattering about the Fermi energy of the solvent metal, two criteria are satisfied[14]: (1) the relaxation time for elastic scattering of the conduction electrons is spin-dependent; (2) there is appreciable, but not over-riding, inelastic scattering of the conduction electrons by the magnetic impurity ions.

The former criterion is also necessary to account for the negative (electrical as well as thermal) magneto-resistance, the magnitude of which correlates with the square of the magnetization of these alloys. But it is the latter criterion which is very significant in the present context: elastic scattering should also be present to a significant extent. In fact, the giant thermo-electric power is considered to be a consequence of 'mutual electron drag' arising from the interaction of the inelastic and the elastically scattered electrons, which again conforms to the explanation given[13] for the large small-angle scattering of electrons by the helium atoms

M S. R Chari

Centre for Advanced Physics,
National Physical Laboratory, New Delhi 12.

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CRYSTALLOGRAPHY

Interdiffusion Method for the Preparation of Single Crystals of Certain Hydroxides and Basic Salts

Single crystals of insoluble compounds have been grown[1] by means of slow interdiffusion of solutions containing the constituent ions or molecules By making use of ammonia is one of two vertically interdiffusing solutions, I have grown single crystals, with dimensions ranging from a few tenths of a mm to a few mm, of the hydroxide, the basic nitrates and a basic sulphate, as well as the isomorphous basic selenate, of cadmium. Likewise single crystals of some of the corresponding compounds of divalent manganese and cobalt have been prepared

Cadmium hydroxide was made in the following way By means of a pipette, a 0 5 M solution of cadmium nitrate was carefully introduced at the bottom of a 200-ml beaker previously three-fourths filled with distilled water The beaker was placed in a desiccator together with a second beaker containing 3 M ammonia After a few days many crystals appeared in a ring-shaped zone about 1 cm wide at some intermediate level on the wall of the first beaker Thin hexagonal platelets formed in the upper part of the zone and large hexagonal prisms in the lower part Sometimes needle-shaped crystals of a basic nitrate appeared below the zone of hydroxide crystals By gradually increasing the initial concentration of the cadmium nitrate solution I obtained single crystals of two more basic nitrates

The other compounds already mentioned were obtained with the same or a slightly modified technique Some basic salts, for example, were best obtained when the first beaker was filled with a single homogeneous solution No systematic attempts were made to establish optimal growing conditions for any of the crystals

In contrast to most other diffusion methods, this process requires only a few days' time and can be carried out in a small beaker There is no need for taking rigorous precautions against shocks and vibrations, temperature fluctuations, etc It would seem that this is due to the use of ammonia, which is a weak base and which, furthermore, forms complex ions with the metal ions The complex formation causes an apparent increase in the solubility of the products This increase ultimately led to the dissolution of all crystals already formed if the process was not stopped in time

Hydroxides were obtained from solutions of the nitrates but not from other salt solutions For cadmium this may be traced to the fact that the concentration of the nitrate solution, which is in equilibrium with both the hydroxide and the basic salt, is relatively high[3].

I have determined the lattice parameters of all compounds obtained and the structures of some of them are being examined Although several of the basic salts seem to be new, others were obtained and examined before by Feitknecht[2,3] in his work on basic salts

This method may also apply to hydroxides and basic salts of such metals as lead, zinc, copper and certain trivalent metals

Y M de Haan

Laboratorium voor Technische Natuurkunde,
Technische Hogeschool,
Delft, The Netherlands

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Superlattice Structure in Ga_2Te_3

Many $A^{III}{}_2B^{VI}{}_3$ compounds crystallize in structures based on zincblende or wurtzite Because of the unequal atomic concentrations of the components, some lattice sites which are occupied in zincblende and wurtzite are vacant in these compounds The original work on these compounds showed only crystal structures with a random distribution of the vacancies[1,2] Later, superlattice structures were observed for Ga_2S_3 (refs 3 and 4), In_2Te_3 (ref 5), and In_2Se_3 (ref 6) No superlattice for Ga_2Te_3 has previously been reported

In order to be able to observe a superlattice in Ga_2Te_3, it was decided to form the compound at as low a temperature as practicable Reference to the phase diagram[7] shows a compound $GaTe_3$, with a peritectic decomposition at 429° C. A mixture of 77·5 at per cent tellurium 22·5 at per cent gallium was heated to 450° C (at which temperature it is liquid) in a sealed, evacuated, silica tube The temperature at one end of the tube was then lowered by 5°–10° C for 5 days, and tellurium distilled off X-ray powder patterns from the residue were obtained in an 11 46 cm diameter Debye–Scherrer camera, using copper $K\alpha$-radiation

Seven lines were found which could not be indexed on the zincblende unit cell, containing 4/3 molecules of Ga_2Te_3 with random vacancies If one of these is ascribed to the strong line of tellurium, the remainder can be indexed on the basis of an orthorhombic unit cell, with the following lattice constants· $a=4\,1_7$ Å, $b=23\,6_0$ Å; $c=12\,5_2$ Å If the lattice constant of the zincblende cell is written as a_0, then these are related to a_0 as follows· $a=a_0/\sqrt{2}$ $b=4a_0$ $c=3a_0/\sqrt{2}$ By comparison with the zincblende cell, the orthorhombic unit cell contains 8 molecules of Ga_2Te_3 From the occurrence of a (070) reflexion, the space group must be primitive Assuming that the point group is a sub-group of $\bar{4}3m$ (the point group of zincblende) leaves 8 space groups, from which no further selection can be made

P C. Newman
J A Cundall

Mullard Research Laboratories,
Redhill, Surrey

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CHEMISTRY

Stability and Vaporization Behaviour of Group IV–VI Transition Metal Monophosphides

THE vaporization properties of Group IV–VI transition metal monophosphides were investigated in order to obtain knowledge of their thermodynamic stability and of the possible existence of binary gaseous molecules between phosphorus and the respective transition metal. For this purpose, a Knudsen effusion–mass spectrometer assembly similar to that described by Chupka and Inghram[1] was used. Included in the investigation were the monophosphides of titanium, zirconium, hafnium, niobium, tantalum, molybdenum and tungsten.

The primary phosphorus species observed in the vapour above the condensed phase were P, P_2 and P_4. In the temperature range 1,000° – c. 1,800° K, diatomic phosphorus was the major species. At higher temperatures monatomic phosphorus also became an important vapour component. Tetratomic phosphorus was, under all conditions, either a minor species or was not detectable. In no case has a gaseous species containing both metal and phosphorus been observed. This has been checked especially for the monomeric monophosphides. The concentration of such species in the vapour phase at the temperatures and pressures investigated is at least 100–1,000 times less than that of the major vapour species. With respect to the formation of binary gaseous molecules, the transition metal phosphides appear to behave similar to the corresponding nitrides for which no binary gaseous molecules have been observed.

All phosphides investigated vaporize according to equation (1) by decomposition into phosphorus vapour and a solid phase of lower phosphorus content:

$$\frac{z}{x} MP_{1-\delta} = \frac{z}{x} MP_{1-\delta-x} + P_z \qquad (1)$$

where $0 < x < 1-\delta$ and z is either 1, 2 or 4, δ is approximately 0.04 for TiP and NbP, and 0.08 for α-ZrP. For the other phosphides it is apparently close to zero.

From X-ray diffraction analysis of the residue, it was found that the condensed phase of lower phosphorus content, which is in equilibrium with the monophosphide, is phosphorus-saturated tungsten metal and sub-phosphides of the composition $MoP_{0.7}$ and $TiP_{0.6}$ in the respective systems.

Analysis of the phosphorus ion-intensity as a function of phosphorus content in the condensed phase at constant temperature indicates the presence of a homogeneity range for the TiP phase that extends between $TiP_{0.96-0.946}$ at 1,700° K. No indication for a homogeneity range was found for MoP, WP, and α-ZrP.

The ion-intensities of P_2^+ were correlated to vapour pressures through silver calibration[2]. The ratio in electron multiplier efficiencies for P_2^+/Ag^+ was 3.0. The ionization cross-sections for silver and monatomic phosphorus were obtained from Otvos and Stevenson[3]. For the phosphorus dimer the value of 21.8 was estimated. The possibility of a very low vaporization coefficient was not taken into account. The P_2-dissociation pressures obtained for the phosphorus-deficient boundary composition of the respective phosphide as a function of temperature were used for the calculation of the partial molal free enthalpies of phosphorus dissociation, $\Delta\bar{G}_T^\circ[P_2]$. The results are listed in Table 1 for 1,000° K, 1,500° K and 2,000° K. Also included is the temperature-range over which the experimental pressure measurements were made. For hafnium and tantalum monophosphides the determination of the temperature-pressure relationship was complicated by the apparent presence of a diffusion barrier, and, in the case of tantalum monophosphide, by reaction of phosphorus vapour with the tantalum effusion cell. From the possible observations it can be estimated that, at 2,000° K, the stability of tantalum monophosphide is comparable with that of α-ZrP and the stability of hafnium monophosphide is considerably higher than that of α-ZrP and similar to that of thorium subphosphide ($ThP_{0.7}$) (ref. 4) and uranium monophosphide[5]. The results presented in Table 1 show that the stability of the monophosphides of group IV increases markedly with atomic number. The same appears to be the case for the group V monophosphides of niobium and tantalum, while for molybdenum and tungsten monophosphides the stability trend is reversed. Within a period the stability decreases with atomic number. This decrease is most pronounced in third transition period.

Table 1. PARTIAL MOLAL FREE ENTHALPIES OF P_2-DISSOCIATION FOR SELECTED TRANSITION METAL MONOPHOSPHIDES

Compound	Temperature range of pressure measurement (° K)	$\Delta\bar{G}_T^\circ[P_2]$ in kcal/mole P_2 1,000° K	1,500° K	2,000° K
$TiP_{0.94}$	1,295–1,705	55.8	40.8	25.9
$ZrP_{0.92}$	1,770–2,120		66.8	51.1
$NbP_{0.95}$	1,200–1,450	55.4	34.0	12.7
MoP	1,190–1,450	54.3	28.3	
WP	950–1,250	36.7	17.3	

A comparison of the stability of the groups IV–VI transition metal monophosphides with the corresponding nitrides is attempted on the basis of information reviewed by Storms[6]. Such a comparison is only of relative significance for the groups IV and V compounds because of the strong dependence of the non-metal activity on the composition in a given binary system, and the difficulty of comparing compounds of exactly the same stoichiometry. For the groups IV and VI nitrides the family trends appear to be similar to those found for the corresponding phosphides, but are less pronounced. The trends within a period are also in the same direction as indicated for the phosphides, but are considerably more pronounced. As a result, the group IV nitrides, especially titanium nitride, are of higher stability than the corresponding phosphides; whereas the group V mononitrides appear to be somewhat less stable and the group VI mononitrides significantly less stable than the corresponding monophosphides. The high thermodynamic stability of hafnium monophosphide, α-zirconium monophosphide and tantalum monophosphide, together with their reported resistance to chemical attack, makes these compounds attractive for special high-temperature applications. The rather low stability of WP and the non-existence of a lower tungsten phosphide above 1,000° C make tungsten metal a promising oxygen-free container material for the more stable phosphides.

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K. A. GINGERICH

Department of Chemistry,
University Park, Pennsylvania.

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Molecular Orbital Theory and Experimentally Determined Energy-levels

ONE of the more successful descriptions of the electronic structure of aromatic hydrocarbons has been given by molecular orbital theory. Originally formulated by Hückel[1], it has been developed and modified by many different authors. An account of the molecular orbital theory in its various forms has been given by Daudel,

Table 1. VALUES OF β

β (eV)	Reference
3 12	*a*
3 00	*b*
3 06	*c*
3 1	*a*
2 8	*d*
0 87	*e*
2 48	*f*
2 318	*g*

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g, Ref 11

Table 2 ENERGY-LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS (eV)

	I*	x_1†	$1\ 15\bar{E}_1$*	^1L_a†	E_A
(1) Benzene	9 21	1	4 80	5 96	−0 43
(2) Naphthalene	8 10	0 618	3 75	4 52	0 68
(3) Anthracene	7 37	0 414	2 95	3 31	1 41
(4) Tetracene	7 00	0 295	2 52	2 64	1 78
(5) Phenanthrene	8 09	0 605	3 60	4 24	0 69
(6) 1 2 Benzanthracene	7 45	0 4525	3 02	3 45	1 33
(7) Chrysene	7 80	0 520	3 38	3 88	0 98
(8) Pyrene	7 55	0 4505	3 12	3 72	1 23
(9) Triphenylene	8 09	0 684	3 69	4 12	0 69
(10) 1 2 5 6 Dibenzanthracene	7 80	0 4735	3 11	3 55	0 98
(11) 3 4 Benzpyrene	7 19	0 371	2 82	3 40	1 59
(12) 1 2 7 8 Dibenzanthracene	7 68	0 4915	3 04	3 70	1 10
(13) 1 2 Benzpyrene	7 56	0 497	3 17	3 74	1 22
(14) 1 2 3 4 Dibenzanthracene	7 61	0 499	3 16	3 72	1 17
(15) Picene	7 80	0 502	3 40	3 81	0 98
(16) Perylene	7 03	0 3475	2 53	2 86	1 75
(17) 1-2-4-5 Dibenzpyrene	7 27	0 4215	2 83	3 32	1 51
(18) Coronene	7 44	0 54	3 01	3 621	1 34
(19) Anthanthrene	7 00	0 291	—	2 34	1 78
(20) 1 12 Benzperylene	7 35	0 439	2 90	3 20	1 43

* Compounds 1–18 ref 7 Compounds 19 and 20 hitherto unpublished results

† Ref 9

Moser and Lefebvre[2] There are two relationships given by molecular orbital theory which are of interest The first, concerning the ionization potential I of the molecule, is

$$I=\alpha+x_i\beta \qquad (1)$$

where α is the coulomb integral, β the exchange integral and x_i is a number characteristic of the molecule

The second is that the energy-levels ε_i of the ground-state molecule are symmetrical about α and have the form

$$\varepsilon_i=\alpha+x_i\beta \qquad (2)$$

The energy difference between the highest filled and lowest unfilled orbitals of the molecule is $2x_i\beta$ This figure has been taken to be the first singlet–singlet transition of the molecule[3,4] designated ^1L_a in Platt's notation[5], p in Clar's notation[6] In the simple molecular orbital theory, no account is taken of electron spin and the equating of $2x_i\beta$ to a term of definite spin multiplicity seems doubtful, and preferably one should take some averaged-out value over terms of different multiplicities Dewar[3] has suggested that as the lowest singlet–triplet transition ^3L_a is directly proportional to ^1L_a the use of ^1L_a alone is permissible A simple comparison of ^3L_a terms, designated T_1 in reference 7, with ^1L_a terms given in Table 2 shows that there is no direct relationship between ^1L_a and ^3L_a Values of β obtained by comparing $2x_i\beta$ with ^1L_a and other procedures are given in Table 1

Ionization potentials have been evaluated[4] by using an expression similar to equation (1), namely

$$I=a+b\nu \text{ (eV)} \qquad (3)$$

with $a=4\ 39$, $b=0\ 857$ and $\nu={}^1L_a$.

The value 4 39 is the work function of graphite[8], 'the infinite condensed ring molecule'. As the polycyclic aromatic hydrocarbons increase in size the ionization potentials decrease[7] and it is to be expected that as the molecule increases to infinity, the ionization potential decreases to 4 39 eV

An experimental correlation between the ionization potential and the first excited state E_1 has been found by Birks and Slifkin[7], namely,

$$I=4\ 39+1{\cdot}15\bar{E}_1 \text{ (eV)} \qquad (4)$$

where $\bar{E}_1$ in the original paper was put equal to $\dfrac{T_1+S_1}{2}$

T_1 is the ^3L_a level and S_1 is the ^1L_b (ref 5) or α (ref 6) level This equation holds for eighteen aromatic hydrocarbons to better than 0 05 eV Equation (4) is similar in form to the Huckel equations (1) and (3)

It is apparent that $\bar{E}_1$ or $1{\cdot}15\bar{E}_1$ is a better quantity to correlate with x_i or $x_i\beta$ than ^1L_a or any other state of definite spin multiplicity In Figs 1 and 2 are shown plots of x_i against ^1L_a and x_i against $1\ 15\bar{E}_1$ for 16 aromatic hydrocarbons The hydrocarbons benzene, triphenylene and coronene have not been included in this plot as, being highly symmetrical, their energy-levels, as predicted by molecular orbital theory, are degenerate[9] The best straight lines for the two graphs were calculated by the method of least squares[9,10], ^1L_a and $1\ 15\bar{E}_1$ being treated as the dependent variables The equation for the lines in Fig 1 is

$$^1L_a=5\ 27x_i+1{\cdot}13 \text{ (eV)} \qquad (5)$$

The sum of the squares of the deviations from this line is 0 2347 eV^2. Similar procedures have been applied by other authors to ^1L_a and x_i (refs 9 and 11) The equation for the line in Fig 2 is

$$1{\cdot}15\bar{E}_i=4\ 31x_i+1\ 07 \text{ (eV)} \qquad (6)$$

The sum of the squares of the deviations is 0 1272 eV^2 Hence x_i correlates better with $\bar{E}_1$ than with ^1L_a Equations (6) and (4) give

$$I=5\ 46+4{\cdot}31x_i \text{ (eV)} \qquad (7)$$

Thus Hedges and Matsen's[4] equation used to predict ionization potentials does not give correct results as, contrary to expectation, x_i and E_1 or ^1L_a do not approach zero together

The values of α and β obtained by comparing equation (7) with the Huckel equation (1) are 5 46 (eV) and 2 155 (eV) respectively One explanation for the high value of α is

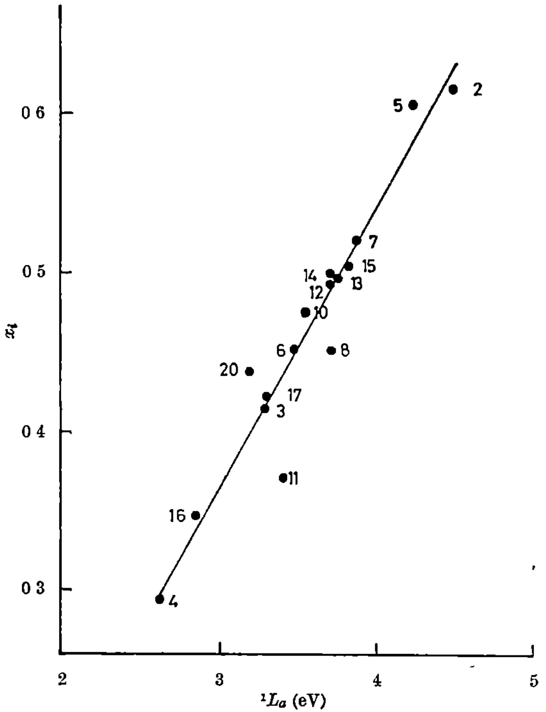

Fig 1 Plot of x_i against ^1L_a

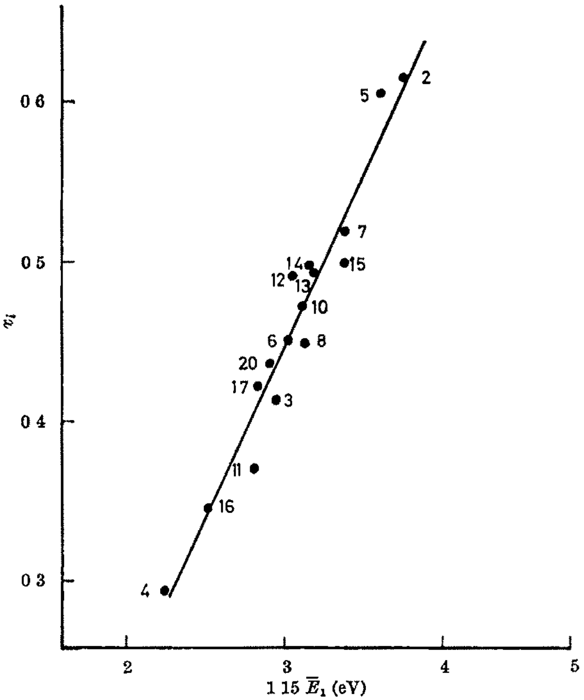

Fig 2 Plot of x_i against 1 15$\bar{E}_1$

that as the hydrocarbon molecules increase in size, the carbon–carbon separation increases[12] and hence α decreases This value of α is an averaged-out value for the relatively small hydrocarbons examined The low value of β obtained is due to the positive intercept on the x_i axis The value of β also increases as the carbon–carbon distance decreases, therefore this value of β should be regarded as an averaged-out value for the sixteen hydrocarbons examined

Another important energy-level of these molecules is the electron affinity E_A Molecular orbital theory[4] gives the relationship

$$E_A = \alpha - x_i\beta \tag{8}$$

By analogy with equation (4) we have

$$E_A = 4\ 39 - 1\ 15\bar{E}_1 \text{ (eV)} \tag{9a}$$

or

$$E_A = 8\ 78 - I \text{ (eV)} \tag{9b}$$

Values of the electron affinity derived in this way are listed in Table 2 No experimental values are available for comparison

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M A SLIFKIN*

Physical Laboratories,
University of Manchester

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* Present address Department of Physics, Rutherford College of Technology, Newcastle upon Tyne

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Humidity and Photochemistry

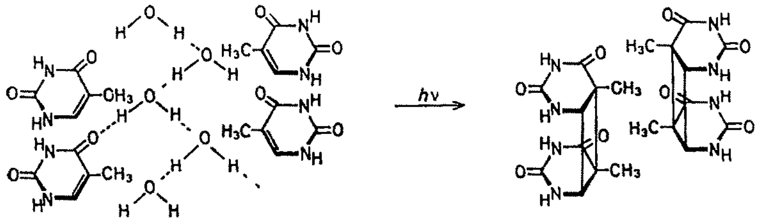
In the course of an investigation of the ultra-violet irradiation of solid films of uracil and thymine[1], certain inconsistencies were observed in the irradiation of thymine films which were unexplainable at the time and therefore not reported Information now available has led to an investigation of the influence of humidity, more properly the vapour pressure of water, on this photochemical reaction, and evidence is presented here that humidity is a factor in this reaction

When a thin film of solid thymine or uracil was irradiated, thymine-dimer or uracil-dimer was isolated[1] These dimers were found to be identical to those obtained when frozen solutions were irradiated[1,2] Thus, I proposed that microcrystalline aggregates of solutes form when solutions are frozen, and frozen solution irradiation may be used as an efficient method for irradiation of solid[1] Stewart has presented evidence to support this hypothesis and has pointed out that two thymine molecules held in a favourable configuration readily undergo the photochemical formation of the dimer[3]

Since thymine and uracil have absorbancy maxima in the 260–270 mμ region and the dimers of these compounds have no absorbancy in this region except for end absorption, the decrease in absorbancy was used as the criterion for dimerization It was found that irradiation of thin films of uracil resulted in a constant decrease of the absorbancy after irradiation However, the results varied in the case of thymine films, the greatest decrease in the absorbancy of the spectrum was 55 per cent, but most of the time it was less and in a few instances there was no decrease at all This inconsistency was difficult to understand at that time

The structure of thymine monohydrate crystal has now been determined, and the stability of the crystalline structure has been shown to be dependent on the water of crystallization When this water is removed, the framework of the crystal collapses[4] This information led to the suspicion that humidity may be the cause of the inconsistency in the results obtained for the irradiation of thymine films Thymine monohydrate crystals as described would be most stable at high humidities and would provide the ideal packing favourable for photo-dimerization, but on the removal of water, which would occur when humidity is lowered, the packing would collapse and dimerization would become difficult In order to test this possibility, irradiation of thymine films was carried out in a 'constant humidity chamber' A small desiccator was used for this purpose The opening of the lid was fitted with a two-hole rubber stopper through which was extended a thermometer (to permit a reading of the beaker temperature) and a model 11 *SC*–1 'Pen-Ray' quartz lamp (Ultra-violet Products, Inc , San Gabriel, California) The beaker containing the thymine film to be irradiated was elevated to a height in the desiccator so that the tips of the lamp and thermometer extended down into the beaker approximately one inch from the film A 100-ml beaker containing a constant humidity solution or phosphorus pentoxide was placed in the bottom of the desiccator and another small thermometer (to permit a reading of chamber temperature) was suspended to the side of this beaker through a rubber stopper fitted into the desiccator plate

The thin films were prepared in 50-ml beakers A 1 0-ml portion of a 1 mM methanolic solution of thymine was pipetted into the beaker, dried quickly on a heated hot plate, and placed in a constant humidity chamber overnight A reference absorption spectrum was recorded of a solution prepared by dissolving one of the films in 10 0 ml. of distilled water Additional films were irradiated for certain time intervals and absorption spectra were recorded of solutions prepared by dissolving each of the irradiated films in 10 0 ml of distilled water. The difference between the unirradiated and irradiated samples



indicates the percentage of dimerization. Table 1 shows the percentage of dimerization under various conditions

Table 1

Time (h)	Humidity (%)* 98	71	30	P_2O_5	Temperature (°C)* In beaker	In chamber
1	15 0	17 3	11 4	7 5		
2	31 0	30 9	21 0	16 3	40–44°	20–24°
3	55 5	55 0	27 0	21 7		

* The constant humidity solutions were prepared according to the *Handbook of Chemistry and Physics*, thirty-second ed (Chemical Rubber Publishing Co , Cleveland, Ohio, 1950), $CuSO_4$ $5H_2O$, 98 per cent, NH_4Br, 71 per cent, NaCl, 30 5 per cent Since humidity is related to temperature, both the temperature in the chamber and in the beaker containing the thymine film were noted

These results indicate clearly that as the humidity increases there is greater photodimerization. This suggests that the water content of the crystals is influenced by the surrounding humidity and that the hydrated form, favoured at high humidity, is the more easily dimerized form Based on this, it is suggested that humidity should be taken into consideration for an intermolecular photoreaction in crystals which contain water of crystallization

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SHIH YI WANG

Department of Biochemistry,
School of Hygiene and Public Health,
The Johns Hopkins University,
Baltimore 5, Maryland

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Photovoltaic Phenomena in Aqueous Solutions

RESULTS of our potentiometric measurements of the photovoltaic effect in the ultra-violet region on platinum and mercury electrodes in aqueous solutions of sulphuric acid, potassium hydroxide and potassium sulphate led us to the conclusion that the observed potential changes of the illuminated electrode are caused by a competitive action of oxidizing and reducing species formed in water under the incident light

A confirmation of this hypothesis has been found on examining the same solutions polarographically under constant irradiation by a high-pressure mercury lamp Whereas no observable change appears on polarographic curves of deaerated solutions of potassium hydroxide and potassium sulphate when exposed to light, a rapid increase of current of the magnitude about 10^{-8} amp (Fig 1) follows the illumination of the dropping mercury electrode in solutions of sulphuric acid On extinguishing the light, the current falls back to its original value The production of current on exposure to light begins to appear at potentials by about 0 6 V more positive than the rise of the reduction current due to electrolytic evolution of hydrogen, and it increases with increasing negative potential of the dropping electrode With increasing concentration of hydrogen ions up to about 1 N the current caused by light increases, further from there it begins to diminish In water–ethanolic solutions of sulphuric acid the photocurrent is smaller by about 1/2 than in aqueous solutions

Qualitatively the same phenomenon was observed with solutions of perchloric, hydrochloric and acetic acids Besides the appearance of this relatively small current on the foot of the wave the actual polarographic reduction wave of hydrogen ions is not affected by illumination. There was found no effect whatever of ultra-violet light on the foot of the polarographic reduction waves of oxygen, Cd^{4+}, Zn^{2+} or Ni^{2+} ions. Nitrous oxide dissolved under atmospheric pressure stimulates the increase of current in acid solutions and introduces an identical effect in solutions of potassium sulphate and potassium hydroxide

The described experimental results could be interpreted on the basis of the effect of the photosensitized decomposition of water on the electrode surface The metallic atoms in the surface of the electrode excited by the incident quanta of light transfer energy to the adsorbed molecules of water. It can be assumed that the dissociation products of water, H and OH radicals, are also adsorbed at the electrode surface, and that their heat of adsorption is greater than that of the water molecule In that case, quanta of lower energy than 118 kcal/mole (that is, light of longer wave-length than 2422 Å) can lead to splitting of the H–OH bond in water (The potential changes in all solutions and the production of photocurrent in acid solutions were observed to a small extent also with ultra-violet light of λ above 3200 Å.) The radicals formed recombine rapidly to water, molecular hydrogen or hydrogen peroxide The occurrence of the reaction products at the electrode surface results in a change of potential of the inert electrode, the extent and the direction of which depend on experimental conditions However, the concentration of the electroactive species is not high enough to cause a measurable polarographic current

In solutions of acids the hydrogen ions adsorbed at the negatively charged electrode combined with photolytically generated hydrogen atoms to form H_2^+ By this reaction the equilibrium between decomposition of water and recombination of its radicals is disturbed, and the total reaction now results partly in formation of H_2^+ and OH, which can both be reduced at the electrode in an extent sufficient to produce a polarographic current

A similar mechanism occurs in the presence of N_2O which turns hydrogen atoms into OH radicals Alcohol, on the other hand, acts as a scavenger of H and OH, changing

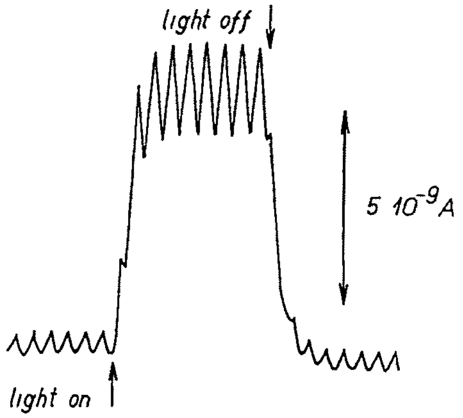

Fig 1 Polarographic current in deaerated 1 M H_2SO_4 at constant potential −0 9 V (versus 1 M Hg_2SO_4 electrode), illumination of the dropping electrode by an ultra-violet lamp denoted by arrows

them into water or molecular hydrogen, and thus suppresses to a certain extent the production of current

A photosensitized decomposition of water could well account for experimental results published by other authors on photovoltaic phenomena in aqueous solutions[1-3] as well as on the acceleration of electrodeposition of hydrogen and oxygen by ultra-violet light[4-6]

M Heyrovský

Polarographic Institute,
Czechoslovak Academy of Sciences,
Prague,
and University of Cambridge

R G W Norrish

Department of Physical Chemistry,
University of Cambridge

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Effect of Geometrical Isomerism on the Behaviour of *cis* and *trans* Hexahydroindanes under Electron Impact

Recently[1] I reported observations on the behaviour of *cis* and *trans* decalines under electron impact It was shown that in these ionized stereoisomers an effect due to geometrical isomerism appears in two different ways First, mass spectra, although qualitatively identical, show important differences between relative abundances of several main fragment ions Relative abundances of molecular ions also differ and indicate a greater stability of ionized *trans* isomer Secondly, from the point of view of energetics two results were obtained (*a*) Ionization potentials measured for both isomers are the same, 9 61 ± 0 02 eV, within experimental uncertainties (*b*) Appearance potentials of the two fragment ions first produced indicate that a lower energy is required to dissociate ionized *cis* isomer into fragments

All these results can be interpreted in a satisfactory manner if it is noticed that the greater stability of ionized *trans* isomer parallels its greater thermodynamic stability which represents about 3 kcal/mole (ref 2) An amount of energy of that order due to steric conformation is released in *cis* decaline when fragmentation occurs

I wondered if a similar behaviour could still be observed in a case where the difference of thermodynamic stability between *cis* and *trans* isomers is smaller—the *cis* and *trans* hexahydroindanes (bi*cyclo*-0,3,4-nonanes) Thermodynamic stability of *cis* isomer is only 1 kcal/mole smaller than that of *trans* isomer[3]

Similar experiments were performed on hexahydroindanes as on decalines (*a*) Mass spectra were recorded for electron energies of a few volts above ionization potential, and relative abundances expressed as a fraction of total ionic current were calculated (*b*) Ionization potentials of both isomers were determined as well as appearance potentials of the two main fragment ions, that is, $C_7H_{12}^+$ and $C_6H_{10}^+$, mass numbers 96 and 82, which are formed in the simplest way as shown in the following scheme

$$\begin{array}{lcccccc} & \begin{array}{l}C_9H_{16}^+\\ m/e\ 124\end{array} & \rightarrow & \begin{array}{l}C_7H_{12}^+\\ m/e\ 96\end{array} & + & \begin{array}{l}C_2H_4\\ m/e\ 28\end{array} \\ \text{and} & & & & & \\ & \begin{array}{l}C_9H_{16}^+\\ m/e\ 124\end{array} & \rightarrow & \begin{array}{l}C_6H_{10}^+\\ m/e\ 82\end{array} & + & \begin{array}{l}C_3H_6\\ m/e\ 42\end{array} \end{array}$$

Appearance potentials of three other important fragment ions, $C_7H_{11}^+$, $C_6H_9^+$ and $C_5H_7^+$, absent in mass spectra at 10 volts but present at 12 volts, have also been determined

Relative abundances of ions are given in Table 1 for 12 and 10 volts electron energies

Table 1 Mass Spectra of *cis* and *trans* Hexahydroindanes at 12 and 10 V (Percentage of Total Ionic Current)

Ion formula	$C_9H_{16}^+$	$C_7H_{12}^+$	$C_7H_{11}^+$	$C_6H_{10}^+$	$C_6H_9^+$	$C_5H_8^+$	$C_5H_7^+$
Mass No	124	96	95	82	81	68	67
12 V							
cis isomer	19 9	31 3	2 3	26 1	8 5	1 0	2 5
trans isomer	28 5	26 3	3 3	22 3	3 7	1 3	6 1
10 V							
cis isomer	48 7	23 1	—	28 2	—	—	—
trans isomer	62 2	17 9	—	20 0	—	—	—

The figures in Table 1 show important differences between relative abundances of molecular ions and main fragment ions in *cis* and *trans* hexahydroindanes and indicate a greater stability of *trans* isomer as in the case of decalines

Table 2 gives results obtained for ionization potentials (*IP*) and appearance potentials (*AP*) in the two isomers under the same experimental conditions as for decalines Ionization efficiency curves have been treated by Warren's method[4]

Table 2 Ionization Potentials of *cis* and *trans* Hexahydroindanes and Appearance Potentials of Main Fragment Ions (eV)

Formula and mass No of the ion		*cis* isomer	*trans* isomer
$IP(C_9H_{16}^+)$	124	10 13 ± 0 03	10 18 ± 0 03
$AP(C_7H_{12}^+)$	96	10 96 ± 0 03	11 16 ± 0 03
$AP(C_7H_{11}^+)$	95	11 71 ± 0 05	11 80 ± 0 05
$AP(C_6H_{10}^+)$	82	10 79 ± 0 03	11 00 ± 0 02
$AP(C_6H_9^+)$	81	11 99 ± 0 05	12 07 ± 0 04
$AP(C_5H_7^+)$	67	12 21 ± 0 05	12 19 ± 0 03

From these results it turns out that, within experimental error, ionization potentials of *cis* and *trans* hexahydroindanes are the same On the other hand, appearance potentials of the two principal fragment ions, $C_7H_{11}^+$ and $C_6H_{10}^+$, are different, the values being smaller for the *cis* isomer than for the *trans* isomer For the other fragment ions investigated, no difference between appearance potentials could be detected

As a consequence, the behaviour of *cis* and *trans* hexahydroindanes under electron impact appears quite similar to that found in *cis* and *trans* decalines Although the difference between thermodynamic stabilities is very small in this case, only about 0 05 eV, there is a noticeable difference between the stabilities of ionized molecules as well as in the energetics of further decomposition of both isomers

The results recorded here can be interpreted in the following way (*a*) Relative thermodynamic stabilities are reflected in the behaviour of ionized isomers in the fundamental state (*b*) An amount of energy of 1 kcal/mole provided by steric conformation is released in *cis* isomer when the molecule breaks down into masses 96 and 82, and used in the breaking process itself

Similar cases of more complex systems are being investigated in this laboratory I thank Prof L D'Or and the Fonds National de la Recherche Scientifique for advice and support

P Natalis

Faculty of Sciences,
University of Liège, Belgium

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Hydrolysis of the Phosphorothioate Ion catalysed by Silicone Grease

When preparing trisodium phosphorothioate from thiophosphoryl chloride and sodium hydroxide at 80° according to Åkerfeldt[1], it was observed that the yields became much smaller when the ground-glass joints of the apparatus used had been treated with silicone grease In fact, in some cases no trisodium phosphorothioate could be isolated from the reaction mixture In these instances liberal amounts of silicone grease had been used in lubricating the glass joints

Thus it was observed that silicone grease in some way interfered with the reaction between thiophosphoryl chloride and sodium hydroxide and/or caused a decomposition of the reaction product, the SPO_3^{3-} ion

A closer examination of this phenomenon has now revealed that silicone grease is a catalyst of the hydrolysis of the phosphorothioate ion in strongly alkaline solution

At 50° and in 1 M sodium hydroxide the rate of hydrolysis constant of the phosphorothioate ion was found to be $2\ 5 \times 10^{-4}$ min^{-1}, as measured in a carefully cleaned glass flask In the presence of 30 mg/100 ml of Dow Corning high vacuum silicone grease the rate of hydrolysis constant increased to $2\ 1 \times 10^{-3}$ min^{-1}, that is, nearly nine times the value obtained with a clean flask. The rates of hydrolysis were followed by determination of liberated orthophosphate according to Gomori[2]

It was further noticed that a strongly alkaline medium is necessary for silicone grease catalysed hydrolysis of the phosphorothioate ion No catalysis is observed, for example, at *p*H 8 0 This is probably due to the fact that in strongly alkaline medium silicone grease does no longer adhere to the walls of the glass flask, but is emulsified in the liquid, thus increasing the catalytic surface tremendously

Mechanistically the hydrolysis of the phosphorothioate ion in alkaline medium is likely to be due to a water attack on that ion[3]. The catalytic action of silicone grease may therefore involve activation of water molecules hydrogen bonded to the surface of the silicone grease. A similar water activation, caused by negatively charged oxygen atoms, is observed, for example, in the hydrolysis of certain S-substituted phosphorothioic acids[4].

STIG ÅKERFELDT

Research Institute of National Defence,
Department 1,
Sundbyberg 4, Sweden.

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Identification of Organic Compounds from Food Odours by Pyrolysis

IN preparing the combined technique of pyrolysis and gas chromatography for the general purpose of identification of compounds in food odours we now have carried out pyrolysis experiments using some aliphatic compounds as test substances In a previous note I reported the detection of the benzene ring in organic compounds by pyrolysis and gas chromatography[1], using a Pye apparatus from which the argon detector was removed and replaced by a flame ionization detector following the method of Murray and Williams[2] A column carrying 30 per cent 'Apiezon *L*' on 'Chromosorb *W*' was used The quartz pyrolysis tube (length, 20 cm , inner diam , 2 mm) was filled with 'Chromosorb *P*' over a length of 10 cm and was connected to the top of the column Nitrogen was used as the carrier gas at a flow of 32 ml /min

50–5,000 μg of the compound in the vapour form and in the presence of a large excess of air was injected through the pyrolysis tube, which was heated to 600° C by an electric furnace

The peaks of the pyrolysis products were characterized using the Kovats index scale, because this index has been shown to be nearly independent of experimental conditions[3]

The gas chromatograph thus was used as a "poor man's mass spectrometer", as first suggested by Keulemans[4]

Fig 1 illustrates the results obtained in the pyrolysis of some C_5 straight chain compounds with one functional group

It is seen that every compound shows a characteristic pyrolysis pattern The reproducibility of the pyrolysis patterns, obtained on repeated injections of the same compound, is good and eliminates the danger of misinterpretation of the results for identification purposes

For a tentative interpretation of these "pyrograms"[5] use was made of the relation between boiling point and Kovats index as reported elsewhere[3]

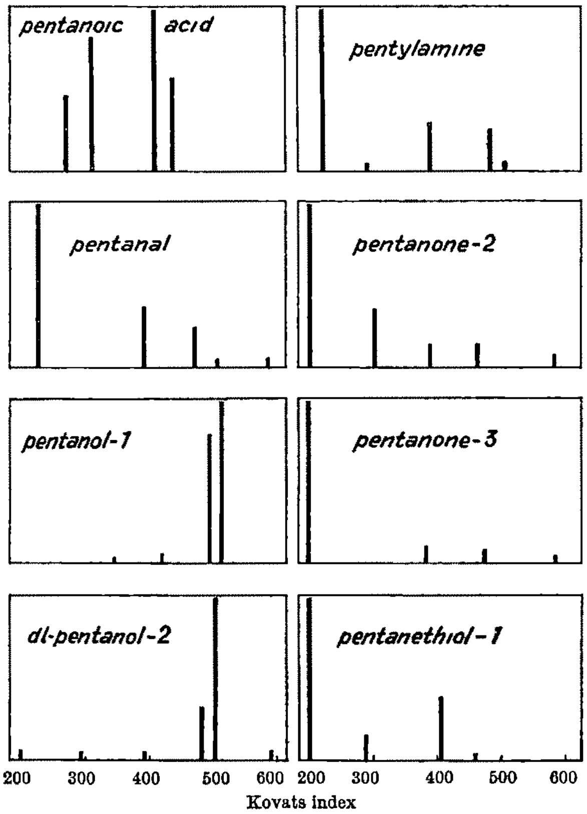

Fig 1 Results obtained in the pyrolysis of some C_5 straight chain compounds. Abscissa, Kovats index units Ordinate, relative peak area as percentage of main peak

The main reaction during the pyrolysis of the alcohol seems to be removal of water from the molecule with the formation of the corresponding olefine The pyrolysis of the acid results in the formation of a C_4-hydrocarbon by decarboxylation (main peak at 404 index units)

The carbon chain in the amine, aldehyde and the ketone is completely disintegrated into smaller fragments

J H DHONT

Central Institute for Nutrition and
Food Research T N O , Utrecht.

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Effect of Reaction of Urea with Jute on the Photochemical Degradation of the Fibre

IN the course of investigations carried out with the view of improving the resistance of jute fibre towards light by chemical modification, it was observed that a remarkable protection against degradation of the fibre by light can be achieved by reacting the fibre with urea in presence of a catalyst at an elevated temperature

Reaction of urea with cotton[1] has been shown to have no appreciable effect on the usual textile properties of the fibre In jute, however, marked changes in both physical and chemical properties of the fibre are noticed, particularly in the susceptibility of the fibre towards photochemical degradation Jute undergoes degradation under the influence of light at a much higher rate than other vegetable fibres[2] leading to loss in tensile strength, increase in alkali solubility and fibre acidity, and a number of other changes in fibre properties[3,4] Treatment with urea arrests these photochemical changes and this is illustrated by a set of experiments described here with results shown in Table 1

Table 1 EFFECT OF LIGHT ON UREA-TREATED JUTE YARN

Treatment	N_2 content (%)	Tensile strength (lb.)			Solubility in 1% NaOH			pH of aqueous extract (10%)		
		Before exposure	After exposure	Loss (%)	Before exposure (%)	After exposure (%)	Rise in solubility	Before exposure	After exposure	Decrease in pH value
Nil (original)	0.36	8.08 ± 0.21	6.03 ± 0.11	25.4	13.1	24.1	11.0	5.8	5.0	0.8
Blank (heated at 140° C)	—	7.99 ± 0.19	5.36 ± 0.19	32.9	13.1	24.3	11.2	5.4	5.0	0.4
Urea with catalyst	4.78	6.13 ± 0.14	5.94 ± 0.15	3.1	26.8	29.9	3.1	5.4	5.4	Nil
Urea without catalyst	2.54	6.18 ± 0.20	4.87 ± 0.20	21.2	20.2	29.5	9.3	5.6	5.2	0.4

The treatment[1] was carried out on de-oiled samples of 8.5-lb. broad loom warp yarn of average mill quality. The yarn was soaked in a 50 per cent (w/v) solution of urea containing 1.0 per cent cupric chloride as catalyst for 1 h, squeezed and air dried. A similar sample was also prepared without using the catalyst and the two samples, along with one untreated to serve as a blank, were placed in an air oven kept at 140° C. After 2 h heating at this temperature, the samples were separately washed with four changes of boiling distilled water for a period of 25 min and air dried. The three samples thus prepared were afterwards exposed in a fadeometer[5] along with a control, for 40 h. Tensile strength, solubility in 1 per cent sodium hydroxide and pH of aqueous extracts of the samples, both before and after light exposure, were then determined and the results given in Table 1.

The results show that under the conditions used for the reaction with urea, the nitrogen content of the fibre is raised from 0.36 per cent to 4.78 per cent and 2.54 per cent, the figures corresponding to the presence or absence of catalyst respectively, with a simultaneous lowering of tensile strength by about 25 per cent. After light exposure all the samples show appreciable reduction in tensile strength varying from 21 to 33 per cent except that treated with urea in presence of catalyst where the reduction in strength is only 3 per cent. Consistent results are also obtained in alkali solubility tests and the pH values of the aqueous extracts, which indicate that very little chemical changes have occurred during exposure of the fibre treated with urea in the presence of catalyst. Moreover, during alkali boiling of the light-exposed portions the resistant fibre did not show any lintering whereas the effect was well marked in all the other samples[6].

From the foregoing observations, it is evident that the photochemical degradation of jute is appreciably checked by reacting the fibre with urea in the presence of cupric chloride as a catalyst. In photochemical degradation of jute, the non-cellulosic components, particularly lignin, play an important part[5]. The reaction with urea probably modifies the lignin, either through cross-linking or otherwise, as a result of which the photo-sensitive property of lignin is minimized, leading ultimately to higher stability of the fibre in light.

H. P. BHATTACHERJEE
A. S. DUTT
W. G. MACMILLAN

Indian Jute Mills Association Research Institute,
Calcutta, 27.

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BIOCHEMISTRY

Density Homogeneity and Estimated Molecular Weight of Interferon

THIS communication presents evidence of the density homogeneity of chick embryo interferon and gives an estimate of its molecular weight as determined by equilibrium sedimentation in a cæsium chloride density gradient.

The source of interferon was pooled allantoic fluids collected from 13-day-old chick embryos 3 days after infection with the *WS* strain of influenza *A* virus[1]. Crude interferon was prepared by centrifugation of allantoic fluid at 65,000*g* for 1 h and recentrifugation of the supernatant fluid at 100,000*g* for an additional hour. This procedure freed the allantoic fluid of all hæmagglutinin and most of the infectious virus. A similar crude interferon was concentrated and purified 10-fold (with respect to original protein concentration) by chromatographic separation on a 'Sephadex *G*-100' column[2]. Both the crude and partially purified preparations of interferon were used in this work.

Interferon was assayed by a modification of the plaque-inhibition method described by Wagner[1]. Monolayer cultures of chick embryo cells were exposed for 1 h to dilutions of crude or partially purified interferon or to fractions of these preparations collected after gradient centrifugation. The cell layers were then infected with an estimated 100 plaque-forming units of vesicular stomatitis virus and overlayered with nutrient agar containing neutral red. Quantitation of interferon activity was based on percentage reduction in number of plaques appearing during 44–48 h on interferon-treated monolayers compared with the number of plaques on simultaneously infected control monolayers. The interferon content of each fraction collected after gradient centrifugation was calculated from a dose-response curve determined concurrently for the unspun interferon.

Equilibrium sedimentation studies were performed as follows. 5-ml. samples of interferon were brought to a starting specific gravity of 1.31 by the addition of powdered cæsium chloride. The samples were centrifuged at 100,000*g* for 65 h in the swinging bucket rotor of a Spinco model *L* preparative ultracentrifuge. Approximately 60 fractions were then collected from the bottom of each centrifuge tube. After determination of its specific gravity, each fraction was dialysed against phosphate-buffered saline to remove cæsium chloride and afterwards assayed for interferon.

The results obtained with crude interferon, shown in Fig. 1, indicate that interferon activity banded at an apparent effective density of 1.30. The interferon appears to be homogeneous as evidenced from the Gaussian nature of its distribution about its effective density (Fig. 2). This graph was constructed by plotting the natural logarithm of the concentration of interferon against the square of the distance of each fraction from the region of maximum concentration of interferon. A Gaussian distribution, when so transformed, will be linear. The experimental results show a high degree of correlation to the linear regression line calculated by the method of least squares.

Meselson and Stahl[3] showed that the standard deviation of the distribution curve for a given macromolecular species in a linear density gradient is inversely proportional to the square root of the molecular weight of the macromolecule. The molecular weight may be calculated from the following equation:

$$\text{Mol. wt.} = \frac{\rho \; R \; T}{(d\rho/dr_0) \cdot \omega^2 \cdot r_0 \cdot \gamma^2}$$

where ρ is the effective density of the macromolecule, R is

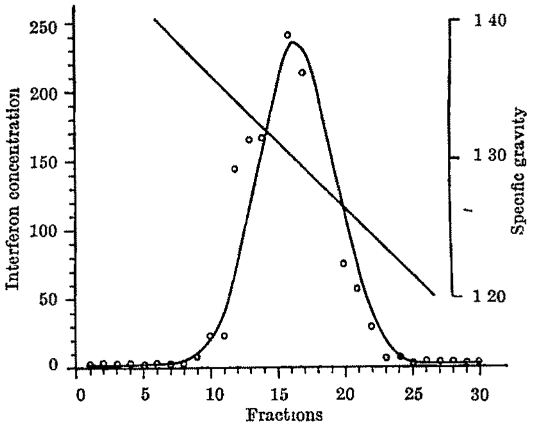

Fig 1 Distribution of interferon after equilibrium sedimentation of a crude preparation in a cæsium chloride density gradient The concentration of interferon in each fraction is represented by the open circles The density gradient is represented by the diagonal line

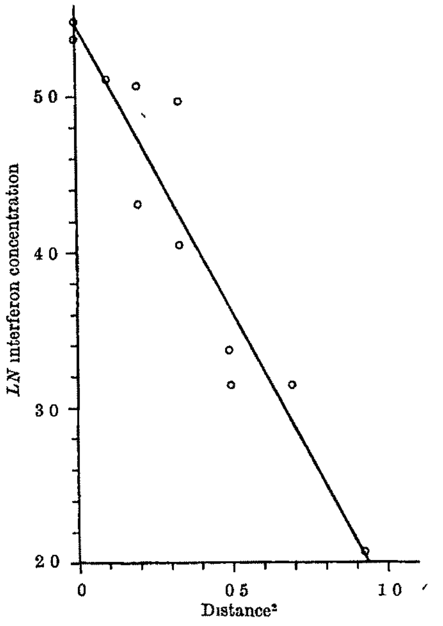

Fig 2 Evidence for density homogeneity of crude interferon The natural logarithm (*LN*) of interferon concentration is plotted on the ordinate against the square of the distance from the locus of maximum interferon concentration on the abscissa The regression line was calculated by the method of least squares

the gas constant, *T* is temperature (° Kelvin), $d\rho/dr_0$ is the slope of the density gradient, ω is the angular velocity, r_0 is the distance from the centre of rotation to the locus of effective density and γ is the standard deviation of the distribution curve of the macromolecule

Using this formula and substituting the experimentally determined value of ρ (1 30) and γ (0 45, the slope of the regression line in Fig 2), a molecular weight of 25,000 for interferon was calculated

Similar results were obtained by equilibrium sedimentation of a partially purified interferon (Fig 3) The effective density of this material was 1 31 and its distribution about its effective density also appeared to be Gaussian as evidenced from the linearity of its transformation (Fig 4) The molecular weight of this partially purified interferon was calculated to be 34,000

This range of values for the molecular weight of chick embryo interferon is in accord with values of 20,000–34,000 daltons determined by Lampson *et al*[4] from sedimentation velocity investigations of a purified interferon from a virtually identical source Purification of interferon is not required by the method described in this communication Furthermore, since each molecular weight calculation is determined by a large number of assay points, the error introduced by the relative lack of precision of interferon assay is minimized

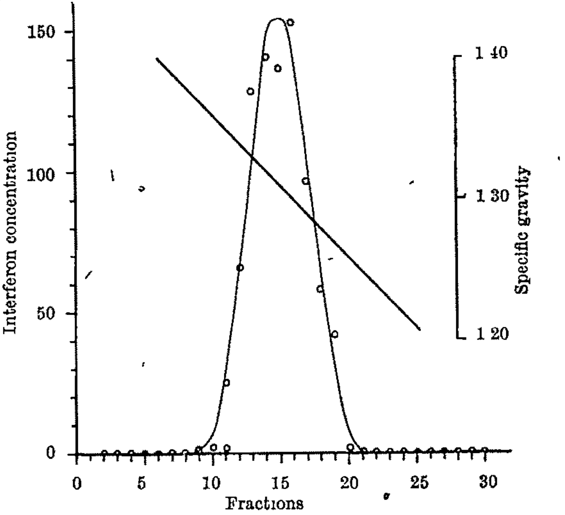

Fig 3 Distribution of interferon after equilibrium sedimentation of a partially purified preparation in cæsium chloride density gradient See legend of Fig 1

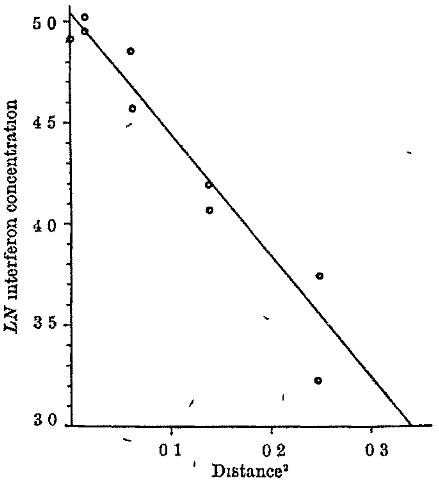

Fig 4 Evidence for density homogeneity of partially purified interferon The method of plotting is identical with that of Fig 2

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Note added in proof Since this manuscript was submitted, Z Rotem and P A Charlwood (*Nature*, 198, 1066, 1963) have determined the molecular weight of chick, mouse and monkey interferons by sedimentation in sucrose density gradients, in conjunction with proteins of known sedimentation properties They concluded that the sedimentation coefficients of the three interferons were very close to that of lysozyme (1 9 S), and estimated a molecular weight range of 13,000–25,000 daltons

LEO E KREUZ
ALLAN H LEVY

Department of Microbiology,
Johns Hopkins University School of Medicine,
Baltimore, Maryland

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Origin of Methanol and Dimethyl Sulphide from Cooked Foods

A SURVEY of the volatiles produced from a range of cooked vegetables showed that both methanol and dimethyl sulphide were usually present as major components[1]. When a commercial sample of instant coffee powder was examined, however, no methanol and only small amounts of dimethyl sulphide were detected, although other common volatiles of low boiling point which are engendered from amino-acids[2] were conspicuously present. It appeared likely, therefore, that both methanol and dimethyl sulphide were produced from vegetables on cooking by some system which was not present in the coffee powder.

One obvious possible origin of methanol is pectin, which was suggested as the source in tea[3], although in this case it was believed to be produced by prior enzyme action (cf. ref. 4). We have now shown that when purified pectin preparations are boiled in phosphate buffer (5 mg/ml. 0·1 M, *p*H 6·5), they hydrolyse to yield methanol in comparable amounts as are formed from cooked vegetables. Methyl esters of simple carboxylic acids are not hydrolysed to any detectable extent under these conditions, and it appears probable that most of the methanol observed from cooked foods arises from the non-enzymatic hydrolysis of pectin.

Although dimethyl sulphide is known to be produced by heating a solution of methionine[2,5] either alone or in presence of sugars and other natural oxidants, the amounts formed are relatively small[2]. Obata and Mizutana[5], however, reported that heating methionine in the presence of plant material known to contain pectin increased the yield of the sulphide. We have now shown that heating methionine and purified pectin at 100° in buffer solutions (*p*H 6·5) at concentrations of 1–5 mg/ml. produces quantities of dimethyl sulphide together with dimethyl disulphide, methane thiol, acrolein and methanol, of the same order as obtained from certain cooked vegetables. It appeared likely that dimethyl sulphide is produced by a mechanism similar to that suggested by Lavine *et al.*[6] for the reaction of methionine with methanol in the presence of strong acids. However, the use of methanol alone, methanol and galacturonic acid, or methyl esters of simple carboxylic acids instead of pectin failed to increase the amount of dimethyl sulphide produced over that obtained from methionine alone. Methyl donors, such as choline and betaine, only showed trace activity. That pectin was acting as a methyl donor was shown by an examination of the decomposition of ethionine. When heated with ninhydrin[2,7] this compound gives mainly ethane thiol, but when pectin is present ethyl methyl sulphide is also produced. The influence of *p*H and other metabolites on the transmethylation reaction has still to be examined. It appears likely, however, that although dimethyl sulphide may be produced enzymatically[4] or from other sources[8], part at least comes from the reaction described, and this may be of importance in enhancing the flavour of manufactured foods.

J. C. CASEY
R. SELF
T. SWAIN

Low Temperature Research Station,
Downing Street,
Cambridge.

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Independence of the Formation of Extracellular Protease on the Amino-acid Level in the Cells of *Bacillus megatherium*

SYNTHESIS of extracellular protease in micro-organisms is repressed in the presence of free amino-acids in the medium[1-3]. However, the formation of intracellular enzyme is not influenced by amino-acids. The quantitative relationship between the amount of amino-acids present in the medium, their level in the cells and inhibition of enzyme formation was investigated during short-time incubation.

The culture grown up overnight on a solid synthetic *C*/*G*[4] medium was intensively aerated for 2 h in the same liquid medium at 35° C. The cells (0·75 mg dry wt./ml.) were washed and incubated at 35° C in the *C*/*G* medium containing different amounts of casamino-acids and calcium chloride (1 × 10⁻³ M) for the stabilization of the enzyme. After incubation for 1 h on a shaker (100 strokes/min, amplitude 6 cm) 100 μg/ml. of chloramphenicol was added. The proteolytic activity was determined in supernatant after centrifugation of the cells. No accumulation of the enzyme occurred in the cells during incubation with amino-acids. The free amino-acids were extracted from the washed cells by boiling for 20 min in the distilled water[6] and colorimetric determinations were carried out[7] (Fig. 1). In some experiments the centrifuged cells were not washed before boiling but the found amino-acid levels were almost identical.

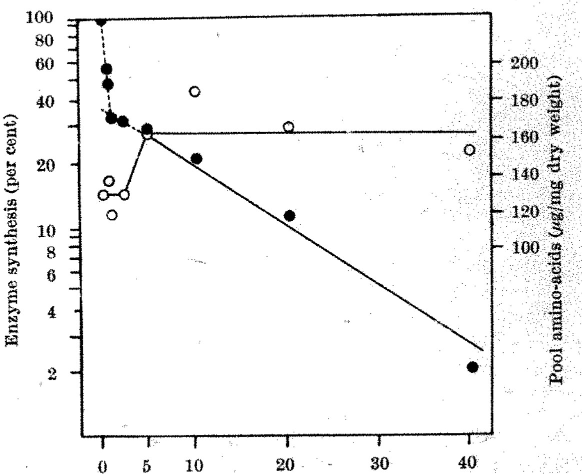

Fig. 1. Relationship between the content of amino-acids in the pool and protease repression. Growing culture of *B. megatherium* was incubated for 1 h on a shaker at 35° in synthetic medium (ref. 4) with Ca^{++} and the aforementioned concentration of casamino-acids. The formation of enzyme was stopped by addition of chloramphenicol

Chloramphenicol inhibited enzyme formation by 95–100 per cent; actinomycine *D* (2·5 μg/ml.) by 90–95 per cent. This shows that, under the conditions of these experiments, the enzyme was synthesized and not only released from the cells. The enzyme formation decreased logarithmically with the increasing amino-acid concentration in the medium. When the concentration of amino-acids was lower than 5 mg/ml., the repression approximated to 60 per cent. During incubation of the cells in the medium containing less than 1 mg casamino-acids/ml. the amino-acids were mostly consumed. The shape of the curve is, therefore, probably deformed in this region.

As is evident from the curve, there are two factors acting in the repression of protease formation by amino-acids, the nature of which is still unknown.

When the cells were incubated with 40 mg/ml. casamino-acids, enzyme formation was decreased by 98 per cent, but the amino-acid level in pool increased by 40 per cent only. When incubated with amino-acid concentration causing 70 per cent repression of protease, the content of free amino-acids in the cell was not increased at all. The hypothesis can therefore be suggested that amino-acids bring about protease repression only when entering the cell, probably during the transport through the cytoplasmic membrane. This suggestion corresponds with Gorini's[8] finding on repression of enzymes synthesizing arginine by exogenous arginine. Should this idea about the repression of synthesis of bacterial protease be confirmed, the concept on the role of cytoplasmic membrane in the control of cell metabolism will be supported[9].

It is possible to repress the protease formation in the synthetic medium by addition of individual amino-acids. Our preliminary experiments give evidence that, for example, threonine might play an important part in enzyme repression. In 1×10^{-3} M concentration it inhibits protease formation by about 70 per cent, but it has no influence either on the activity of the enzyme or on incorporation of ^{35}S-methionine into the cell proteins.

J. CHALOUPKA
P. KŘEČKOVÁ
L. ŘÍHOVÁ

Institute of Microbiology,
Czechoslovak Academy of Sciences,
Prague.

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Changes in the Constitution of Myelin caused by Ions

IN an investigation of conditions which produce *in vitro* changes of myelin similar to those which occur in Wallerian degeneration (with formation of ovoids and spheres) the importance of the presence of a sufficient amount of calcium in the medium became apparent. A similar observation was made years ago by Nageotte[1]. The effects of high concentrations of calcium or of sodium ions on the constitution of myelin were therefore examined. Central and peripheral myelins homogenized with equal amounts of either 1 M sodium chloride or 0·5 M calcium chloride were extracted by water or by chloroform/methanol (2 : 1) and the extracts and residues were chemically analysed. Comparable experiments were performed by histochemical means on teased and cryostat-sectioned fresh central and peripheral nervous tissue.

It has been found that in the presence of excess sodium chloride in the medium an 'oil in water' emulsion is formed, in which the solubility characteristics of the droplets are determined by the polar ends of the phospholipids and proteins. In these circumstances, about 50 per cent of the cerebroside, of the phospholipids and of the cholesterol of myelin can be extracted by water together with a part of the myelin protein and mucopolysaccharide. Myelin homogenized with calcium chloride breaks down into a 'water in oil' emulsion in which the outer coat of the droplets is hydrophobic. Extraction of such an emulsion with water yielded some protein and polysaccharide and only small amounts of the various lipids. The difference between treatment with sodium chloride and treatment with calcium chloride is schematically demonstrated in Fig. 1. The effect of the ions is obviously due to the fact that calcium salts of phospholipids are more lipophilic, whereas the sodium compounds are more hydrophilic and therefore more attracted into the aqueous phase[2–4].

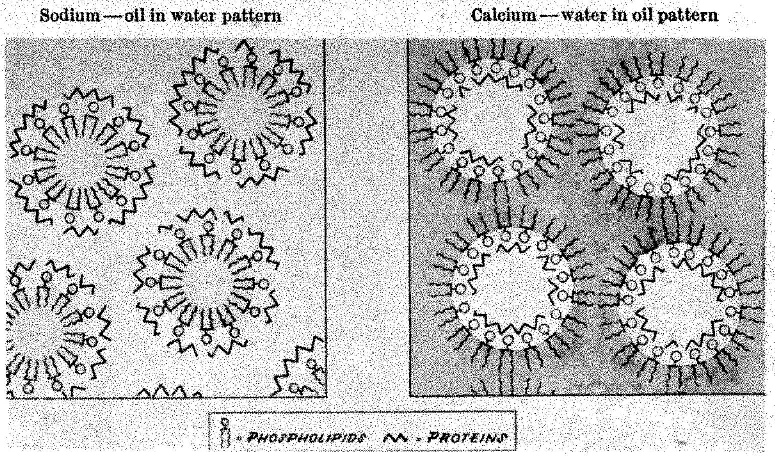

Fig. 1

Full results regarding these experiments, including findings which bear on the problem of the chemical morphology of myelin, will be published elsewhere. It is the purpose of the present communication to show the implications of these observations to a number of problems.

(1) The myelin sheath represents a cell membrane rolled on itself many times. It may be assumed that also *in vivo* ions and other factors capable of affecting the hydrophilia of polar lipids have a similar effect on the cell membrane and possibly also on membranes of various organelles to that which has been presently shown to occur *in vitro* in the myelin sheath. Bending of a phospholipid layer in the direction towards a 'water in oil' emulsion as shown in Fig. 2 (Ca) will produce an increased density of charges on the surface, an occurrence which might possibly be related to the phenomena of transmission of impulses. More marked bending over a large area of the membrane may help to explain phenomena associated with phagocytosis. A complete change of an area of a cell membrane into an 'oil in water' emulsion as shown in Fig. 3 (Na) will produce aqueous channels through which the cytoplasm communicates with the outside.

In an equilibrated area the lipid and aqueous layers are parallel and the inside of the cell does not communicate with the outside. Change into a 'water in oil' pattern (Fig. 3, Ca) will increase the impermeability to aqueous constituents, will render the wall more rigid and might allow lipid-soluble substances to cross the cell wall.

Recently, Luzzati and Husson[5] and Stoeckenius[6] have shown that emulsions of phospholipids in water assume under certain conditions a hexagonal pattern instead of

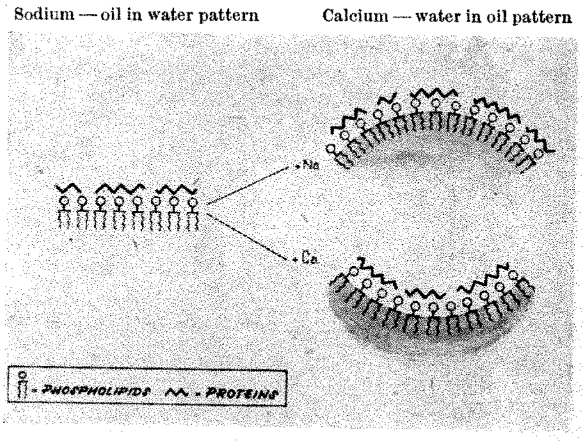

Fig. 2

the lamellar arrangement. A similar pattern was shown by Dourmashkin, Dougherty and Harris[7] and by Husson and Luzzati[8] to be present in the walls of various cells and viruses which underwent certain treatments. The hexagonal patterns described obviously represent 'water in oil' emulsion systems.

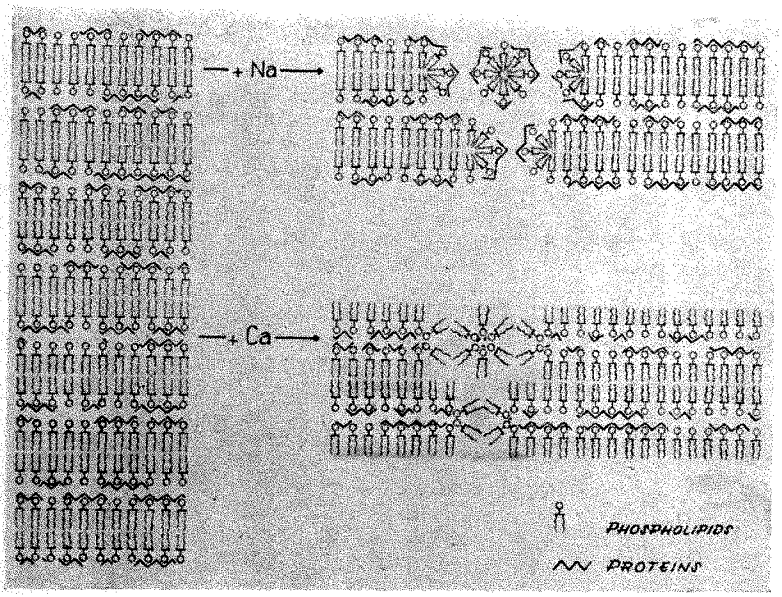

Fig. 3

(2) The lysosomes first described by De Duve are heterogeneous cellular organelles which behave like little bags which contain mainly hydrolytic enzymes with an acid *p*H optimum surrounded by a lipoproteic barrier which prevents free access of the respective substrates[9]. The lysosomes were considered to overlap in their characteristics mitochondria and microsomes[10]. It is suggested that the lysosomes and the 'smooth-surfaced vesicles' are derived from the endoplasmic reticulum, and possibly also from the mitochondria or even the cell membrane, by a change of these structures into 'water in oil' systems in which the membrane is folded into a globular body surrounded by polar lipids with the hydrophilic groups directed inwards. Enzymes which are normally situated on the outer surface of membranes and which belong, therefore, to the spaces between cells or between the two layers of internal reticulum, are enclosed in bags made by the inverted membranes. The enzymes may be regarded as belonging to the outer surface of cells and to spaces in continuity with this surface (to which the cavities between the layers of the endoplasmic reticulum and between the mitochondrial layers probably belong) which have been artefactually or *in vivo* enclosed in bags mainly lined by the polar groups of phospholipids.

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M. Wolman
H. Wiener

Department of Pathology,
Government Hospital,
Tel-Hashomer, Israel.

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Preparation of Phosphatidylserine

Several methods for the preparation[1,2] or quantitative isolation[3] of phosphatidylserine are now in existence. An investigation of some of the factors involved in the chromatography of phospholipids[4,5] revealed the role of H+ ion exchange in the conversion of the salt form to the acidic form of phosphatidylserine on a silicic acid column. The recognition of this phenomenon, which was also indicated by experimental results published afterwards[6], led to the development of a so-called silicic acid–'Hyflo Super Cel' column in the sodium form. These columns have been used successfully for the preparation of phosphatidylserine of high purity from ox-brain in conjunction with a shortened version of the method of Folch[7] for the isolation of phosphatidylserine-rich fractions.

The columns were prepared by treating a mixture of silicic acid (Mallinckrodt, *A.R.*, 100 mesh) and 'Hyflo Super Cel' in the proportion of 2 : 1 (w/w) with excess 1 N hydrochloric acid (vol. in ml. equivalent to approx. 15 times the weight of silicic acid) followed after filtration on a Buchner funnel by excess 1 M sodium bicarbonate in which the mixture was allowed to remain for 5 min. After filtration, the mixture was washed 5 times with distilled water which had been boiled until the *p*H had risen to 6·8. This is important since water which is slightly acid, for example *p*H 5·0, reconverts ionizable groups on the adsorbent from the Na+ form into the H+ form, resulting in the elution of some phosphatidylserine with cloroform/methanol 4 : 1 (v/v) together with phosphatidylethanolamine and other impurities. After having been washed, the mixture was spread in a shallow layer in an oven at 120° and left for 16–24 h. It was afterwards stored *in vacuo* over phosphorus pentoxide and used when required.

Ox-brain obtained freshly from the slaughter house and transported to the laboratory in ice was extracted with acetone, ethanol and petroleum ether (40°–60°) as described by Folch[7]. The latter extract was evaporated to dryness and the residue dissolved in a minimum volume of chloroform from which crude cephalin in a yield of 17 g/kg (wet wt.) of brain was precipitated with 5 vols. of ethanol (1 h at −10°). A phosphatidylserine-rich fraction (fraction III) was obtained from the crude cephalin by chloroform–methanol fractionation according to Folch[7]. The yield was 4·5 g fraction III/kg (wet wt.) of brain.

Columns were prepared from the treated silicic acid–'Hyflo Super Cel' mixture which had been washed with chloroform/methanol 4 : 1 (v/v) as described by Rathbone[6]. 58 g of the mixture gave a preparative column of suitable dimensions (26 cm × 2·3 cm) although smaller columns were frequently used. Fraction III was washed with 0·1 M sodium chloride and again with upper phase containing sodium chloride in the biphasic system of Folch, Lees and Sloane-Stanley[8]. The material derived from the lower phase was dissolved in a suitable volume of chloroform/methanol 4 : 1 (v/v) (approx. 3 mg P/ml.) and applied to the column at a loading of approx. 0·5 mg P/g of column filling. Chloroform/methanol 4 : 1 (v/v) (3·2 column volumes) eluted phosphatidylethanolamine, inositol phospholipids, cerebrosides and other impurities. Phosphatidylserine was eluted with chloroform/methanol 3 : 2 (v/v) (3·2 column volumes). The later fractions eluted with this solvent contained impurity. Thin-layer or paper chromatography of the fractions indicated those containing only phosphatidylserine, and these were pooled and the solvent was removed under reduced pressure in an atmosphere of nitrogen. The residue was dissolved in ether and centrifuged to remove particles and the lipid was finally stored in chloroform under nitrogen. The final yield of pure phosphatidylserine was approx. 3 g/kg wet wt. of brain.

Phosphatidylserine eluted from columns gave one spot staining with ninhydrin and protoporphyrin when chromatographed on silicic acid impregnated paper[9]. Traces of a ninhydrin-staining breakdown product appeared on storing, however, even at −10° under nitrogen. Chemical analysis of the phosphatidylserine was as follows: serine/P = 0·98; ethanolamine/P = 0·0; fatty acid/P = 2·01; absolute phosphorus content 3·82 per cent (theoretical 3·92 per cent). Determination of the fatty acids present by gas chromatography showed that oleic acid constituted 39·6 per cent of the total acids and stearic acid 52·4 per cent. C_{20} acids comprised 5·1 per cent and C_{16} acids 2·6 per cent. Since lysophosphatidylserine prepared by the action of snake venom phospholipase A on phosphatidylserine[10] contains mainly stearic acid and since phospholipase A removes the β-fatty acid of L-α-diacylglycerophosphatides[11,12], it is clear that a positional asymmetry of fatty acids exists in phosphatidylserine similar to that found in other phosphatides.

L. Rathbone
P. M. Maroney

Department of Chemical Pathology,
Guy's Hospital Medical School,
London, S.E.1.

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Comparison of *in vivo* and *in vitro* Actions of Various Ox Pituitary Growth Hormone Fractions

Discovery of an *in vitro* action of a hormone is of intrinsic value in determining the detailed mechanism of its action—a situation well illustrated in recent years in the case of insulin. Pituitary growth hormone is known to have an action *in vitro* on isolated muscle preparations—to stimulate uptake of glucose[1,2], incorporation of amino-acids into protein[3,4] and accumulation of a non-utilizable amino-acid[5] by diaphragm from the hypophysectomized rat, and to influence the metabolism of adipose tissue[6]. Whether these *in vitro* actions of the hormone play any part in bringing about its growth-promoting action has not been clarified[2,4,5,7-9]. The purpose of this communication is to show that by column chromatography ox growth hormone can be resolved into three fractions which, though all retaining potency *in vitro*, possess markedly differing growth-promoting activities.

Growth hormone, prepared by a modification of the Wilhelmi procedure[10] in which the initial extraction of the lobes is at *p*H 5·5, was chromatographed on columns of DEAE-cellulose (made by the method of Peterson and Sober[11]) in a borate buffer 6·5 mM $Na_2B_4O_7$ and 5·5 mM hydrochloric acid, *p*H 8·7. Part of the protein was eluted as a retarded peak in this buffer and the remainder with a sodium chloride gradient applied in two steps, the first increasing the molarity of sodium chloride to 0·1, the second to 0·5. Under these conditions, 80–90 per cent of the protein applied to the column was eluted as three main fractions: (*A*) eluted in the starting buffer (30–40 per cent); (*B*) eluted by the first gradient (15–25 per cent); (*C*) eluted by the second gradient (15–30 per cent). The proportion of each fraction varied with the original growth hormone preparation used, and in some cases evidence for heterogeneity within the fractions was suggested by the presence of more than one chromatographic peak. On rechromatography each fraction was eluted under the expected conditions. Examination of the starting material and the various fractions by starch-gel electrophoresis (using the method of Ferguson and Wallace[12], but with a slightly higher *p*H (8·8) at which all the main electrophoretic components move towards the anode) showed that chromatography had led to a separation of the electrophoretic components of growth hormone and that fractions *B* and *C* at least still contain several components. Fractions from two chromatographic runs were tested for growth-promoting activity in hypophysectomized rats, the increase in weight over 10 days of groups of five rats, each injected daily, being measured. The *A* fraction (Table 1) was found to possess growth-promoting activity similar to that of the unfractionated growth hormone. The faster running portion of the *B* fraction, though not quite so effective as *A*, also possessed growth-promoting activity, whereas B_2 and *C* fractions did not bring about any stimulation of growth when supplied at low doses. Injected in larger quantities B_2 showed about 10 per cent and *C* 3 per cent of the growth-promoting capacity of unfractionated growth hormone.

Table 1. Promotion of Growth of Hypophysectomized Rats by Various Fractions of Growth Hormone

Fraction	Dose (μg/day)	Increase in weight (mean ± *S.E.*) (g)	Increase in weight corrected for controls (mean ± *S.E.*) (g)
Saline control	—	−4·9 ± 0·9	—
Unfractionated	15	+10·3 ± 5·1	+15·2 ± 5·1
(1) *A*	15	+10·4 ± 3·7	+15·3 ± 3·8
(1) B_1*	15	+1·9 ± 1·7	+6·8 ± 1·9
(1) B_2*	15	−4·6 ± 2·0	+0·3 ± 2·2
(1) *C*	15	−0·9 ± 1·9	+4·0 ± 2·1
Saline control	—	+0·5 ± 0·7	—
Unfractionated	15	+10·8 ± 0·5	+10·3 ± 0·9
(2) *A*	15	+11·1 ± 1·8	+10·6 ± 1·9
(2) B_1	15	+9·7 ± 1·3	+9·2 ± 1·5
(2) *C*	15	+0·8 ± 1·8	−1·3 ± 1·9
Saline control	—	−2·2 ± 0·7	—
Unfractionated	5	+5·9 ± 0·9	+8·1 ± 1·2
Unfractionated	15	+10·5 ± 2·2	+12·7 ± 2·3
Unfractionated	45	+17·6 ± 1·5	+19·8 ± 1·7
(1) B_2*	150	+11·7 ± 2·0	+13·9 ± 2·2
(2) *C*	150	+4·9 ± 1·3	+7·1 ± 1·5

*Fraction (1) *B* showed some separation into two peaks and these were tested separately.

Uptake of glucose by diaphragm was measured by the disappearance of glucose from the medium[13], incorporation of glycine into protein as previously described[14] and release of free fatty acids from the epididymal fat pad was estimated by Dole's method[15]. Each fraction of a third chromatographic run was found at a concentration of 50 μg/ml. to stimulate uptake of glucose and incorporation of glycine into protein of diaphragm from the hypophysectomized rat (Table 2). A similar effect was seen when the concentration was reduced to 5 μg/ml. Moreover, the fractions tested in Table 1 and found to possess little growth-promoting activity were found at 1 μg/ml. to stimulate uptake of glucose and in two cases amino-acid incorporation (Table 2). A significant *in vitro* action of *A* at 0·1 μg/ml. was not observed. Each of these fractions at 25 μg/ml. was found to stimulate release of fatty acids by fat pads from normal rats (Table 3).

Thus the *in vitro* activity of fractions B_2 and *C* appears to be more or less as great as for *A* and B_1 (much more than 20 per cent, Table 2) despite their growth-promoting activity being very substantially less (much less than 10 per cent in the case of *C*, Table 1). On the other hand, the retention of *in vitro* activity by fractions *A* and B_1

Table 2. EFFECT OF VARIOUS FRACTIONS OF OX PITUITARY GROWTH HORMONE ON THE UPTAKE OF GLUCOSE AND INCORPORATION OF GLYCINE INTO PROTEIN BY ISOLATED DIAPHRAGM FROM HYPOPHYSECTOMIZED RATS

Fraction added to incubation medium	Uptake of glucose (mg/g wet wt./h)	Radioactivity in protein (c.p.m./mg)
No addition	3·17±0·13	16·4±0·35
(3) *A* (50 μg/ml.)	4·95±0·31	21·9±1·3
(3) *B* ,,	5·20±0·64	22·8±2·6
(3) *C* ,,	5·50±0·58	21·4±2·0
No addition	3·12±0·18	14·3±1·4
(3) *A* (5 μg/ml.)	5·10±0·28	17·4±1·6*
(3) *B* ,,	4·35±0·28	17·3±1·8*
(3) *C* ,,	5·18±0·46	17·8±1·9*
No addition	2·60±0·13	11·1±0·69
(1) B_2 (1μg/ml.)	4·28±0·27	14·1±1·00
(1) *C* ,,	3·31±0·12	11·0±0·66*
(2) *C* ,,	3·39±0·15	12·7±0·42
No addition	2·47±0·29	11·3±0·44
(3) *A* (0·1 μg/ml.)	3·08±0·58*	10·9±0·82*
(3) *A* (1 μg/ml.)	4·40±0·41	15·0±1·43

Each figure is the mean ± *S.E.* of the mean of six observations. Except for (*) the value of *P* for the stimulation due to each fraction is at least <0·05. Incubation was for 1·5 h, initial concentration of glucose was 2·5 mg/ml.

Table 3. EFFECT OF VARIOUS FRACTIONS OF OX PITUITARY GROWTH HORMONE ON THE RELEASE OF UNESTERIFIED FATTY ACIDS BY EPIDIDYMAL FAT PADS OF NORMAL RATS

Fraction added to incubation medium	Fatty acids released (μmole/g tissue/3 h)
No addition	5·2±0·51
(3) *A* (25 μg/ml.)	12·4±1·00
(3) *B* ,,	10·0±0·81
(3) *C* ,,	11·5±1·20

Each figure is the mean ±*S.E.* of the mean of six observations. The value of *P* for the stimulation due to each fraction was <0·001. Incubation was for 3 h in a medium containing 5 per cent ox serum albumin.

which also possess good growth-promoting activity does not provide any evidence of the possibility of a separation of the agents responsible for the *in vivo* and *in vitro* actions[8,15,16], though it is not completely excluded. Although the *in vitro* actions of growth hormone remain a fascinating problem in mechanism it is doubtful whether at the present time they provide a very helpful guide to the means by which growth hormone promotes growth.

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K. L. MANCHESTER*
M. WALLIS

Department of Biochemistry,
University of Cambridge.

* Present address: Department of Biochemistry, University College, London.

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Control of Cellular Differentiation in Plants by the Distribution of Enzymes in a Lipid-bound System

IN analysis of the sequence of enzyme differentiation it was found that several enzymes are lipid-bound and that tissue differentiation is determined by the distribution of enzymes in a lipid-bound system. During an investigation of unsaturated lipids and the hæmatin enzymes in the endodermis and phloem of plants it was found that there is a functional relation between unsaturated lipids and the enzymes which determine differentiation and development[1]. Lipotropic agents applied to growing plants changed the pattern of enzyme distribution with resultant changes in tissue differentiation. Ethylene, in concentrations ranging from saturation in water to only a few parts per million, induced changes in lipoprotein. Associated with these changes it was found that the location of the lipids, and associated enzymes that give rise to the endodermis, were changed from normal distribution to new locations.

Ethylene at high concentrations induced a change in unsaturated lipid material in the endodermis and phloem, and negative reactions were obtained for cytochrome oxidase, peroxidase, and some dehydrogenases and esterases. Leaves of narcissus bulbs grown in an ethylene atmosphere had a redistribution of sudanophilic and osmophilic lipids. Lipid materials either moved to or were induced to form in the outermost cells of the leaf under the lower epidermis; and there was an associated change in the distribution of enzymes, particularly the hæmatin enzymes. A redistribution of lipids and enzymes to one side of the leaf appeared to be responsible for the asymmetrical growth associated with ethylene.

By growing plants in lipotropic substances (ethylene, etherized water) the endodermis and hypodermis, notable for their localizations of unsaturated fatty acids and peroxidase[2], were blocked in their normal topographic development. Under ethylene, the lipid–hæm systems that normally impregnate the walls of the endodermis are deposited elsewhere and apparently move outward to the surface of the plant. In roots and stems of castor beans, changes in enzyme pattern and tissue differentiation occurred in the endodermis adjacent to phloem; the endodermis between the vascular bundles contained some unsaturated lipids and associated peroxidase and cytochrome oxidase.

Lipid-bound enzymes of the phloem are apparently in the mitochondria of the sieve tubes and companion cells, but there appears to be a lipid–hæm peroxidase system free in the sieve tubes and not localized in mitochondria. It has been postulated that phospholipids hold functional groups of enzymes[3]; unsaturated lipids apparently hold in close association groups of enzymes that determine the differentiation and location of the endodermis in plants.

The general effect of ethylene and etherized water on plant tissues has been observed[4-7] and an interpretation is now presented of the influence of ethylene on the distribution of lipids, lipid-bound enzymes and resultant tissue differentiation. The breaking of cambial and bud dormancy by ethylene has been supposed to affect the concentration and distribution of auxin; but detection of unsaturated lipids, and microtitration to determine iodine numbers, reveal changes in distribution of unsaturated lipids. Ethylene must be applied prior to the appearance of lipid carbonyl compounds and in advance of normal lipid condensation and polymerization in protoplasts, walls and cellular membranes. The heat-stable lipid-bound peroxidase system of the endodermis may be changed in its differentiation if ethylene is applied in the formative stages of tissue development.

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D. S. VAN FLEET

Department of Botany,
University of Georgia,
Athens,
Georgia.

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Human Red-cell Phosphogluconate Dehydrogenases

SINCE the advent of starch-gel electrophoresis various enzymes, including serum cholinesterase[1], erythrocyte glucose-6-phosphate dehydrogenase[2] and erythrocyte acid phosphatase[3], have been demonstrated to exist in human beings in different genetically determined modifications. We report here a procedure for the detection of human red-cell 6-phosphogluconate dehydrogenase (6-PGD) by starch-gel electrophoresis, and the existence of this enzyme in different genetically determined forms.

Blood (0·2 ml.) is added to water (0·4 ml.) and after 10 min the mixture is spun at 2,000*g* for another 10 min. Clear hæmolysate (50 µl.) is applied to each 12 mm × 4 mm insert of Whatman No. 17 filter paper. Electrophoresis[4] is carried out for 4 h at a potential gradient of 10 V/cm in a starch-gel, made in 0·01 M phosphate buffer at *p*H 7·0, and placed horizontally between two cooling plates. 6-PGD activity is detected by a modification of a method described for glucose-6-phosphate dehydrogenase[5]. An 'overlay', made up in 10 ml. of 0·1 M *tris* buffer at *p*H 8·0 containing agar (100 mg), nicotinamide adenine dinucleotide phosphate (2·0 mg), sodium-6-phosphogluconate (10 mg), phenazine methosulphate (0·4 mg) and the tetrazolium salt 'MTT' (2·0 mg), is poured at 45° over the sliced starch-gel and after setting is incubated at 37° for about an hour. The enzyme is located as a bright blue zone.

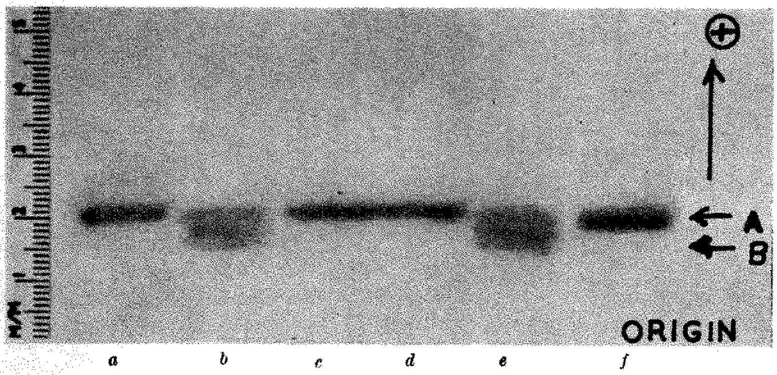

Fig. 1. Starch-gel electrophoresis of 6-PGD in human hæmolysates in 0·01 *M* phosphate buffer at *p*H 7·0

The normal result for red-cell 6-PGD is shown in Fig. 1, *a*, *c*, *d* and *f*, and consists of a single sharply defined band, *A*, which moves about 2·0 cm towards the anode. In 10 instances out of 150 random blood samples from adults, mostly of European descent, we detected the atypical band pattern shown in Fig. 1, *b* and *e*, where the sharp band *A*, although still present, stained to about half the intensity of the normals, and was accompanied by an equally sharp band *B*, which ran somewhat more slowly, and which likewise stained with about half the intensity of the normal *A* band. When bloods showing this variant 6-PGD were examined at intervals over a period of several weeks they were always found to have the same electrophoretic pattern, indicating that the possession of these two bands is a constant characteristic of the red cells of such individuals.

The presence of heparin in concentrations necessary to prevent clotting interfered with the normal electrophoretic behaviour of 6-PGD, but if the red cells were washed before hæmolysis this 'heparin effect' disappeared.

Examination of the families of several of the variant individuals demonstrated that the trait is genetically determined and is probably a heterozygous condition for a gene pair, with simple Mendelian inheritance of an autosomal nature. One such pedigree is shown in Fig. 2. With an incidence of about 1 in 15 one would expect that 1 person in $30^2 = 900$ would be homozygous for the atypical variety of 6-PGD. So far we have not found such an individual.

The total red-cell 6-PGD activity was determined quantitatively[6] in several cases for the heterozygous condition and was found not to differ significantly from the

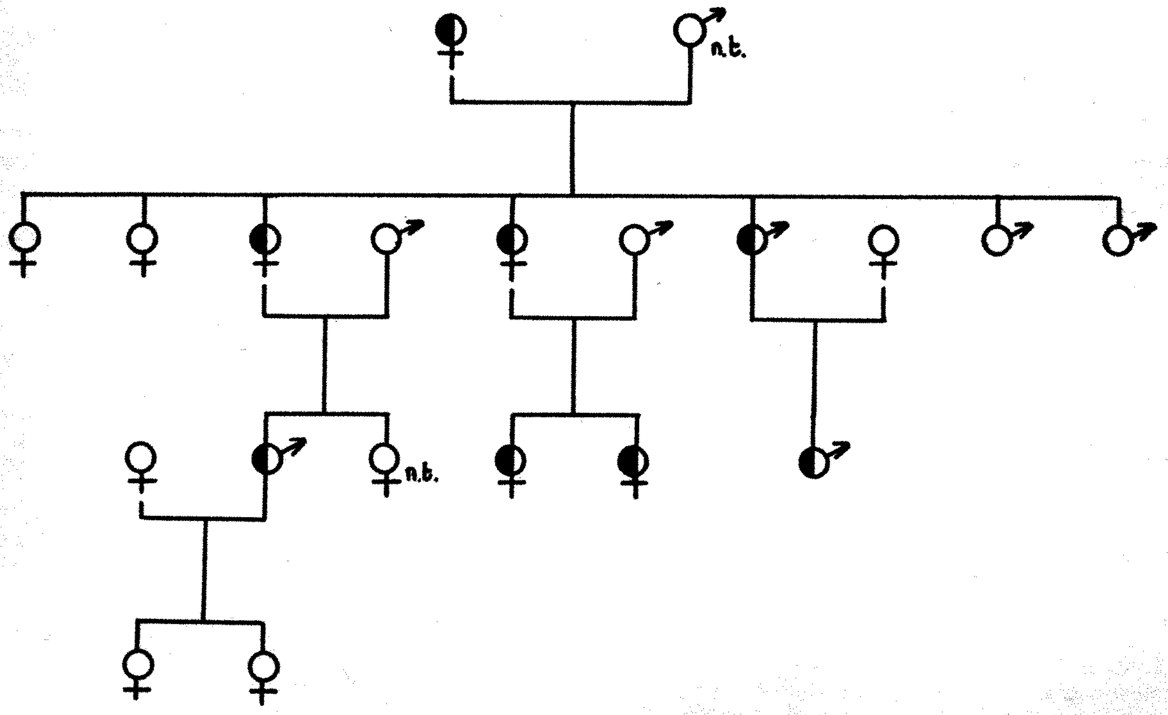

Fig. 2. Pedigree of a family in which the 6-PGD variant occurs. ◐, *A* plus *B* bands; ○, *A* band only; *n.t.*, not tested

total activity in individuals with the normal homozygous state.

R. A. Fildes
C. W. Parr

Department of Biochemistry,
The London Hospital Medical College,
London, E.1.

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Identification of *p*-Hydroxyphenoxymethyl Penicillin as a Metabolite of Phenoxymethyl Penicillin

When urine of normal adults to whom phenoxymethyl penicillin (penicillin V (200 mg)) had been administered was chromatographed[1], a second inhibition zone in addition to the penicillin V zone was observed (Fig. 1). The R_F value of this product was identical with that of *p*-hydroxyphenoxymethyl penicillin, obtained from a sample of crude penicillin V[2]. This metabolite represents about 10 per cent of the activity found in the urine.

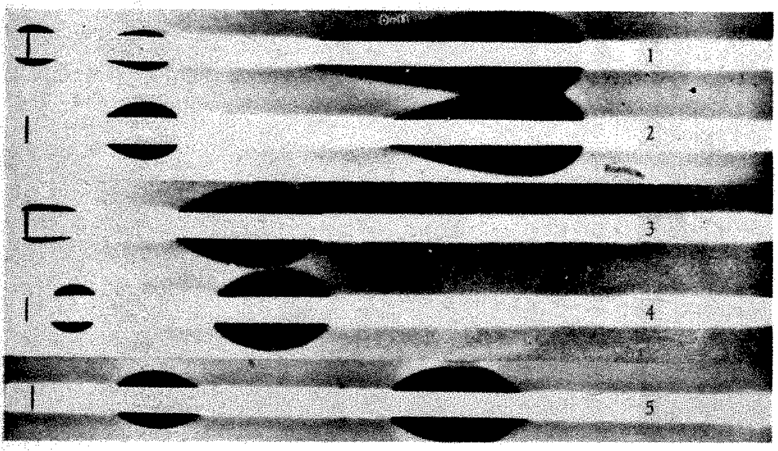

Fig. 1. Bioautography of paper chromatograms of urine after oral administration of penicillin V and phenethicillin. Whatman No. 1 paper, buffered with 10 per cent sodium citrate at *p*H 5·5 (Nos. 1 and 2) or *p*H 6·0 (Nos. 3, 4, 5). Developing solvent: ether saturated with 28 per cent ammonium sulphate. R_F values of penicillin V: 0·60 ± 0·06 at *p*H 5·5 (Nos. 1 and 2), 0·32 ± 0·04 at *p*H 6·0 (Nos. 3 and 4); *p*-hydroxypenicillin V: 0·13 ± 0·02 at *p*H 5·5 (Nos. 1 and 2), 0·05 ± 0·01 at *p*H 6·0 (Nos. 3 and 4); phenethicillin: 0·49 ± 0·05 and metabolite: 0·17 ± 0·02 at *p*H 6·0 (No. 5)

To confirm the structure of this substance, pooled urine samples were extracted with butyl acetate, after acidification to *p*H 2·0 with phosphoric acid. The penicillin was extracted from the organic solvent into bicarbonate solution and re-extracted into ether after acidification. The ether solution was applied on a column of silica gel, equilibrated with 2 M phosphate buffer of *p*H 6·2. The column was developed with ether containing increasing amounts of methanol (0·5–3 per cent), as described by Behrens *et al.*[3]. The column was cut into 1-in. sections, which were eluted with phosphate buffer of *p*H 7·0. The top section contained the metabolite and some penicillin V. The product eluted from the top of the column was hydrolysed by refluxing in 3 N hydrochloric acid for 24 h, and the side-chain acid was extracted with ether. By circular chromatography using system *E* of Högström[4], phenoxyacetic and *p*-hydroxyphenoxyacetic acid could be identified. There was also an indication of the presence of the *ortho*-acid on the paper chromatogram (Table 1).

Further confirmation of the structure of the side-chain acid was obtained by gas chromatography of the methyl esters. The acid was treated with methyliodide (7 m.mole) and 0·5 M sodium methylate (2 m.mole) for 5 h at 65° in a sealed tube. In this way phenoxyacetic acid was transformed into the methyl ester, and the hydroxyphenoxyacetic acid into the methyl ester of the methoxy acid. These esters could be separated by gas chromatography on a column of 'Reoplex 400' (20 per cent) at 180° (ref. 8). By this method the presence of *p*-hydroxyphenoxyacetic acid was confirmed, and a small peak of *o*-hydroxyphenoxyacetic acid was detected.

Table 1. Paper Chromatography of Phenoxyacetic Acids

Acid*	R_F†	Colour‡
o-$HOC_6H_4OCH_2COOH$	0·41	Violet
m-$HOC_6H_4OCH_2COOH$	0·23	Red-violet
p-$HOC_6H_4OCH_2COOH$	0·15	Blue
$C_6H_5OCH_2COOH$	0·37	—

* The hydroxyphenoxyacetic acids were prepared by reaction of the appropriate dihydric phenol with chloroacetic acid[5,6].

† Butanone-2 : water : diethylamine, 921 : 77 : 2 (ref. 4). The acids were detected with bromophenol blue indicator or by diazo reagent.

‡ Colour obtained by reaction with diazotized *p*-nitroaniline[7].

The predominant active metabolite of penicillin V in human beings is *p*-hydroxy isomer. In the urine of some persons who had received penicillin V, we found a second metabolite (probably the dihydroxy derivative), which remained at the start on the paper chromatogram (Fig. 1, lanes 1 and 3).

A more polar biologically active metabolite was also observed when the urine of persons who had received α-phenoxyethylpenicillin (phenethicillin) was examined (Fig. 1). Although the structure of this metabolite was not established, it seems very likely that hydroxylation of the benzene ring had also occurred.

The presence of active metabolites in the urine after administration of the following penicillins has been described: benzylpenicillin (penicillin *G*)[9,10], penicillin V, phenethicillin and propicillin[10], phenoxybenzyl penicillin (phenbenicillin)[10,11], 3,4-dichloro-α-methoxybenzyl penicillin[10,12], oxacillin and cloxacillin[10,13].

The hydroxylation of aromatic compounds in the organism is well known, and metabolites of this type have been described for such drugs as phenobarbital, diphenylhydantoin, and phenylbutazone[14]. These transformation products are often conjugated with glucuronic acid, and possibly penicillin V is transformed into a substance of this type that is inactive. Another inactive degradation product could be penicilloic acid, as was demonstrated with benzylpenicillin[15].

H. Vanderhaeghe
G. Parmentier
E. Evrard

Rega Institute,
University of Louvain, Belgium.

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PHYSIOLOGY

A Serum Protein present in Pregnant Women

SMITHIES[1] has briefly mentioned a protein band which he found in the starch-gel electrophoretograms of sera from about 10 per cent of women who were either in late pregnancy or had recently delivered. In the work reported here, a much higher frequency has been found for what is probably the same protein band. In some runs in a borate gel at $pH = 8{\cdot}5$, under essentially the same conditions as described by Smithies[2], the protein of the investigation reported here migrated at a rate slightly slower than the first haptoglobin band of Hp 2–2 sera to which hæmoglobin had been added (Fig. 1). In other runs, the two were not separated. Accordingly, the typings for presence and absence of the band in Hp 2–2 sera are not included in the data given here although it was undoubtedly present in the majority of such sera.

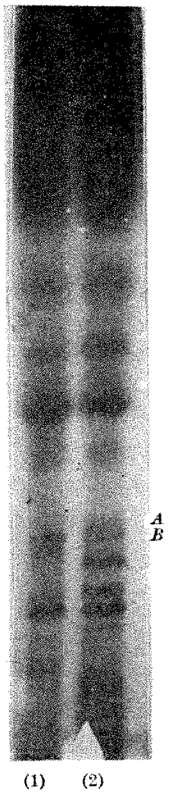

Fig. 1. Anode at the top of photograph. Starch-gel electrophoresis of two sera from pregnant women at 4–5 V/cm for 14 h in borate buffer (0·0092 M sodium hydroxide, 0·023 M boric acid $pH = 8{\cdot}5$) and staining with amido black 10*B*. (1) Haptoglobin type 1–1; (2) haptoglobin type 2–2; (*A*) first haptoglobin band of Hp 2–2; (*B*) pregnancy protein

The sera of 226 pregnant European women were examined and of these 141 were Hp 1–1, Hp 2–1 and Hp 0. The period of pregnancy has been divided into four seventy-day intervals and the frequency of occurrence of the band in each is shown in Table 1.

When the frequencies in the first and second intervals were pooled, χ^2 for heterogeneity was non-significant ($\chi^2_2 = 4{\cdot}767$, $0{\cdot}10 > P > 0{\cdot}05$).

Great variation in the concentration of the protein in different sera as judged by the intensity of staining with amido black 10*B* (Gurr) was observed. It is possible that more sensitive methods would detect it in sera from all pregnant women. In a group of 107 control sera from female blood donors 75 were Hp 2–1 or Hp 1–1 and one of these possessed a very faint band. The remainder did not possess a perceptible band.

Table 1. FREQUENCY OF THE OCCURRENCE OF A SPECIFIC SERUM PROTEIN AT INTERVALS OF PREGNANCY

Presence (+) or absence (—)	Interval of pregnancy (1) 0–70*	(2) 71–140	(3) 141–210	(4) 211 to end of pregnancy	Total individuals	% frequency
+	2	37	43	42	124	86
—	2	7	2	6	17	14
Total	4	44	45	48	141	100

* Days

The band was observed in three other individuals: (1) an Australian Aboriginal woman known to be pregnant, (2) a New Guinea native woman of adult age, for whom no information about pregnancy was available, (3) a European man whose carcinoma of the prostate was being treated with dienœstrol (50 mg twice a day).

Hirschfeld and Söderberg[3], using immunoelectrophoretic methods, have detected proteins in sera from pregnant women which were not present in control sera. It is possible that the protein reported here is identical with one of these proteins. It also seems likely that this protein is analogous to that found by Heim[4] and Beaton *et al.*[5], using starch-gel electrophoresis, in the serum of neonatal, tumorous and pregnant rats. Darcy[6] has demonstrated by immunological methods increased concentrations of a normal serum protein in the same classes of rats, and Heim[5] suggested that this protein and the one he detected by starch-gel electrophoresis are identical. The presence of the one and increased concentrations of the other appear to be concomitant with tissue growth and lactation in the rat.

The results presented here, however, do not exclude the possibility that the human protein is induced by increased levels of œstrogen; it has been demonstrated that the administration of œstrogen can induce the formation of specific proteins in the liver of the chicken embryo[7].

I thank Prof. L. W. Cox, Department of Obstetrics and Gynæcology, Queen Elizabeth Hospital, Adelaide, and Dr. H. B. M. Lewis of the Red Cross Blood Bank, Adelaide, for supplying sera. I also thank Prof. J. H. Bennett and Dr. M. J. Mayo for advice and Mr. I. R. Goodwins for the photography of Fig. 1.

D. W. COOPER

Department of Genetics,
University of Adelaide.

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Action of Prostaglandin E_1 on Tissues which respond to Bradykinin

PROSTAGLANDIN E_1 (9-keto, 11α, 15, dihydroxy-prost-13-enoic acid, PGE_1) was the first of several prostaglandins to be isolated by Bergström *et al.*[1,2]. It is a member of a chemically new group of vasodilator substances. Other naturally occurring vasodilators such as bradykinin, acetylcholine and histamine have effects on several types of tissue in addition to smooth muscle. For example, bradykinin increases capillary permeability, produces pain on application to a blister base and releases adrenaline from the adrenal medulla[3,4]. The actions of PGE_1 on these and other biological preparations have been investigated using bradykinin as a standard for comparison. The results are summarized in Table 1.

The vasodilator activity of PGE_1 was confirmed on the flow of blood in the hind-limb of a cat. Like bradykinin, it increased capillary permeability as shown by intradermal injection into guinea pigs pre-treated with Pont-

Table 1. THRESHOLD DOSES OR CONCENTRATIONS OF PROSTAGLANDIN E_1 AND BRADYKININ REQUIRED TO PRODUCE EFFECTS ON THE BIOLOGICAL PREPARATIONS USED IN THIS INVESTIGATION

Response	Preparation	PGE_1	Bradykinin
Vasodilatation	Cat hind limb	200 ng	20–100 ng
Increased capillary permeability	Guinea pig skin	100 ng	1–10 ng
Pain production	Human blister base	No effect (> 100 µg/ml.)	0.1 µg/ml
Adrenaline release	Cat adrenal medulla	No effect (> 7.5 µg/kg)	0.15 µg/kg
Reduced air entry	Guinea pig lungs	No effect (> 50 µg/kg)	4–10 µg/kg
Contraction of isolated smooth muscle	Rat uterus	60 ng/ml	0.5 ng/ml
	Guinea pig ileum	8 ng/ml	10 ng/ml
	Rat duodenum	(Contraction) 250 ng/ml	(Relaxation) 5 ng/ml

amine sky blue. A dose of 1 µg (in 0.1 ml.) produced a maximal effect. The intensity of bluing with PGE_1 was less for a given area of lesion than with bradykinin and the very intense bluing produced by bradykinin was never observed with PGE_1. Estimates of relative activity were therefore difficult to make but PGE_1 was at least 10–100 times less active than bradykinin.

PGE_1, unlike bradykinin (0.1 µg/ml.), did not cause pain on application to human blister bases in concentrations from 0.1 to 100 µg/ml. Retrograde injections of bradykinin (2 µg) into the cœliac artery of the 'Nembutal' anæsthetized cat released the equivalent of 1 µg of adrenaline from the adrenal medulla as detected by the denervated nictitating membrane. PGE_1 did not cause any detectable adrenaline release (< 0.2 µg) in doses up to 20 µg.

PGE_1 also differed from bradykinin in that it did not reduce air entry into the lungs of the guinea pig as measured by the Konzett and Rossler method. Like bradykinin, however, it caused contraction of the isolated rat uterus and the isolated guinea pig ileum; it further contracted the isolated rat duodenum, which is relaxed by bradykinin.

I thank Prof. S. Bergstrom of the Karolinska Institute, Stockholm, for the prostaglandin E_1, and Dr. E. D. Nicolaides, of Parke, Davis and Co., Ann Arbor, for the synthetic bradykinin used in these experiments.

E. W. HORTON

Miles-Amos Research Laboratories,
Stoke Poges,
Buckinghamshire.

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Mechanism for concentrating Iodine in Marine Algae

THE fact that various marine algae are able to concentrate iodine against a gradient induced many workers to compare this mechanism with the uptake of iodine by the thyroid gland in mammals[1,2]. The reported results were incongruent in so far as some suggested that this mechanism may be similar[3], others, that it is different from the one operating in the thyroid gland[4].

All these experiments were performed on living tissue, which by virtue of their metabolic activities may have influenced to some extent these results. We have therefore examined the concentration mechanism on non-viable material.

The leaf-like fronds of seaweed, *Caulerpa* of the order Siphonales, cast ashore at the beaches of the Mediterranean (Tel Aviv), were collected during August and September. This material was then washed in filtered sea water, dried at room temperature and, after shredding, pulverized to a fine powder in a pharmacological mill of the Willy type. In order to compare with thyroid tissue the ability of these dried and pulverized algae to concentrate iodine, we used the method adopted in elucidating the role of different anions on the uptake of thyroid glands in our previous work[5,6]. 100 mg of the algae tissue powder dispensed in dialysis bags were submerged in beakers each containing 5 ml. of the medium solution. This medium solution consisted of different molar concentrations of $KClO_4$–KBF_4 and potassium iodide dissolved in sea water. The control medium consisted of sea water only. 0.1 ml. of carrier-free iodine-131 solution (Radiochemical Centre, Amersham, England), giving a count rate of 100,000 c.p.m. in a commercial well-type scintillation counter (Nuclear, Chicago), were added to each beaker.

All the experiments were run in duplicate and the counts did not differ by more than 2–5 per cent per experiment. The beakers were incubated at a temperature of 28° C for 120 min, then the bags containing the pulverized tissue were removed, adequately washed and assayed for the radioactivity in the well, after their volume had been made up to 2 c.c. with sea water. These measurements represent the tissue (T).

0.1 ml. of each of the medium solutions, again made up to a volume of 2 c.c. with water, were measured for their radioactivity in the well and represent in the results the medium (M).

The results are summarized in the table as T/M ratios, defined as the

$$\frac{\text{c.p.m. of } {}^{131}\text{I}/0.1 \text{ g pulverized tissue}}{\text{c.p.m. of } {}^{131}\text{I}/0.1 \text{ ml. medium}}$$

It is obvious from the results that all anions used reduced the T/M in comparison to the controls without these anions. The amount of reduction varies with the molar concentration, increasing with decreased concentration, reaching a maximum and then decreasing again.

The molar concentrations for maximal reduction in uptake of iodine-131 in the algae was for iodide 10^{-5}, for fluoroborate 10^{-6} and for perchlorate 5×10^{-7}, being very similar to those found by Wolff and Maurey in their experiments with animal tissues[7].

Table 1. DEPRESSION OF THE T/M BY DIFFERENT ANIONS

T/M of control = 12.1 ± 1.6

Molarity	T/M with $KClO_4$	T/M with KBF_4	T/M with KI
10^{-2}	9.5 ± 0.45	10.3 ± 1.15	0.25
10^{-3}	9.3 ± 0.4	10.4 ± 1.15	3 ± 2
10^{-4}	8.7 ± 0.65	9 ± 0.45	4 ± 1
10^{-5}	9 ± 0.5	9.2 ± 0.6	6.1 ± 0.5
10^{-6}	8.4 ± 0.8	7.5 ± 1.6	6.6 ± 0.3
5×10^{-7}	7.5 ± 0.9	9.1 ± 1.8	
10^{-7}	7.8 ± 0.7	11 ± 1.85	

The figures represent the mean and standard deviation.

Our previous assumption, therefore, that ionic size is of importance in the uptake mechanism seems to be verified with the additional restriction that probably more parameters of the ion-like univalency, proper size and structural properties are of importance. The ionic size itself is, as Wolff pointed out, an operative one and depends on the conditions under which it is measured, its hydration state being of utmost importance. There seems to be an optimal concentration which determines the operational ionic volume in such a gradient and which varies for different ions. This may partly explain the failure of Tong to account for any depression of the T/M in his algae experiments with potassium perchlorate in only a 10^{-3} molar solution.

As the inhibition of iodine uptake may not be identical with the concentration mechanism, we undertook also experiments with labelled $K^{36}ClO_4$. The preliminary results of these showed again a concentration of the $K^{36}ClO_4$ ion in the algae. Further experiments, which are in progress, show also an increase of this uptake under the influence of bovine thyroid-stimulating hormone (Organon), adding again to the similarity of the uptake mechanism in the algae and thyroid glands[8].

These results indicate that by all the criteria used the concentration mechanism of iodine in algae and thyroid glands resemble each other or may be even reduced to an identical fundamental property of the specific tissue, not necessarily connected with its vital organization in the living cell

Z Lewitus
H. Schreiber

Department for Isotopes and Thyroid Physiology at
Rogoff's Clinical Research Institute,
Beilinson Hospital, Israel

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Lung Lining in Bird, Reptile and Amphibian

The lungs of mammals are lined with a film of lipoprotein, which has the function of reducing the tension of the sharply curved alveolar surface[1] A similar film was also found to exist in the pigeon and the frog[2] Miller and Bondurant[3] and Klaus *et al* [4] have, however, concluded as a result of a study of the surface properties of saline extracts of minced lung from turtle, frog, and pigeon that a distinctive surface-active material exists in mammalian lungs only We have, therefore, re-examined the question

The species used were the chicken (*Gallus*), slow-worm (a legless lizard, *Anguis fragilis*), and clawed toad (*Xenopus laevis*) The lungs of these were compared with those of mammals; a summary of the findings is given in Table 1 The methods used have been described elsewhere[2,5,7]

Small bubbles, squeezed from a fragment of mammalian lung into a hanging drop of air-saturated water, usually contract somewhat and then remain stable The ratio of final (stable) to initial surface area is called the stability ratio, for bubbles initially between 36μ and 60μ in diameter the mean stability ratio is about 0 7 Ordinary bubbles of this size, for example those formed in blood, contract and disappear in a few minutes In our experiments we found that bubbles squeezed from fragments of the bird, reptile, and amphibian lungs were as stable as those from mammalian lungs, the smallest of them were stable enough and small enough to show Brownian motion

When bubbles (say 100μ in diameter) from mammalian lung are immersed in de-aerated water under a glass slide, they first flatten as the air dissolves and the surface tension falls, and then suddenly resume at an almost spherical shape, in the latter process, the equatorial diameter is reduced by about 10 per cent The phenomenon is known as 'clicking', and repeats itself rhythmically as the bubble dissolves, it demonstrates[6] that the bubble surface has a free energy in excess of the product of its area and surface tension Bubbles from bird lung behaved in de-aerated water exactly like those from mammalian lung; those from the slow-worm showed less prominent 'clicking', while those from toad lung did not 'click', but contracted steadily

Table 1 PROPERTIES OF BUBBLES FROM THE LUNGS OF MAMMAL, BIRD, REPTILE AND AMPHIBIAN, COMPARED WITH THOSE OF ORDINARY BUBBLES

	Bubbles from lungs of				Bubbles in blood, etc
	Mammal	Chicken	Slow-worm	Toad	
Stability ratio of bubbles from fresh lung (means of batches of 30)	0 60–0 87	0 74 0 78	0 73 0 80	0 81 0 82	0 0
Brownian motion	+	+	+	+	–
Clicking (per cent decrease in equatorial diameter)	10%	10%	2%	nil	nil
Stability ratio of bubbles from lung collapsed once and re-inflated (means of batches of 30)	0 60–0 87	0 73 0 80	0 16*	0 06 0 29 0 39	0 0
Stability ratio of bubbles from lung washings (means of batches of 10)	0 5–0 9	0 6 0 7	0 0	0 1 0 1	0 0

* Mean of batch of five bubbles

A mammalian lung may be made to collapse to an air-free state by exposure to a vacuum followed by sudden restoration of atmospheric pressure If it is then re-inflated, it retains much of the air forced into it, and fragments from it give stable bubbles like those from fresh lung[2] We found that when chicken lung was collapsed, and air was injected into the tissue, fragments from the lung when squeezed into air-saturated water gave bubbles as stable as those from mammalian lung The slow-worm and toad lungs, on the other hand, when collapsed and re-inflated, retained little air, showed a strong tendency to collapse again spontaneously, and yielded bubbles much less stable than those from fresh lungs

If washings from mammalian lungs are carefully freed from any bubbles they may contain, and are then frothed by blowing air from a needle into the liquid[7], they form bubbles with stability ratio usually in the range 0 5–0 9 Injection of saline into the bird lung tissue, and recovery of the saline by squeezing, produced washings resembling in this respect those from mammalian lung Washings from the slow-worm and toad lungs, however, provided few stable bubbles

It is evident from these findings that a surface film capable of reducing to a large degree the tension of the surfaces of the fine air spaces of the lung exists in the bird, reptile and amphibian, as well as in mammals The lung lining film of the toad is, however, less elaborate than that of the slow-worm, which is in turn less elaborate than that of the bird or mammal The failure to re-form a complete film after collapse and re-inflation, and the poor stability of bubbles from lung washings, in the toad and slow-worm show that these have only a small reserve of the saline-dispersible 'lining complex' from which the lining film is formed; the smallness of this reserve would explain the weakness of the surface film on the extracts of amphibian and reptile lung studied by other workers[3,4] Our experiments provide no evidence as to whether the bird has a greater or smaller supply of complex than has the mammal

The curvature of the surfaces of the air spaces in reptile and amphibian is less than in mammal or bird, and it is not unexpected that their supply of surface-active agent should be less than in the warm-blooded classes In the mammal, the alveoli would collapse if the surface film were absent, or, if the lung were held open by air pressure, transudation from the blood capillaries into the alveoli would occur In the bird, collapse of the lung may in part be prevented by its more rigid structure (though it can, as stated here, be collapsed artificially), to prevent transudation, however, a surface film is even more necessary than in the mammal, because the curvature of the walls of the air capillaries of the bird is even sharper than that of the walls of the mammalian alveoli

R. E Pattle
D A W Hopkinson

War Office,
Chemical Defence Experimental Establishment,
Porton Down,
Salisbury, Wilts

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PHARMACOLOGY

Effect of 'Tremorine' and some Anti-Parkinson's Disease Drugs on Acetylcholine in the Rat's Brain

Giarman and Pepeu[1,2] have demonstrated that atropine sulphate and scopolamine hydrobromide decrease total brain acetylcholine in the rat. Both drugs are effective against Parkinson's disease and prevent the tremor, rigidity and the parasympathetic stimulation induced by the administration of 'Tremorine' (1,4-dipyrrolidino-2-butyne)[3].

In the present work the effects of 'Tremorine' and of some anti-Parkinson's disease drugs on brain acetylcholine have been investigated. Adult male rats 150–200 g were killed by decapitation at the time stated in each experiment. The brain (excluding cerebellum, olfactory lobes and pituitary) was removed quickly and total acetylcholine was extracted by the method of Smallman *et al.*[4] The acetylcholine content of the extracts was estimated on the isolated eserinized rectus abdominis muscle of the frog. The values for acetylcholine are expressed in terms of acetylcholine chloride. The effect of the various drugs used was carefully examined on the rectus abdominis in order to compensate for any influence on the response of the muscle to acetylcholine. All the drugs were administered to the rats intraperitoneally in 0·1–0·4 ml.

Table 1 shows that 'Tremorine' induces a marked rise of total brain acetylcholine which closely follows the onset of the symptoms. This finding is in agreement with the observation of Stern *et al.*[5] that 'Tremorine' stimulates brain cholineacetylase. Pretreatment of the rats with either atropine sulphate or caramiphen hydrochloride prevents the onset of the tremor, rigidity and parasympathetic stimulation induced by the injection of 'Tremorine' and completely prevents the increase of brain acetylcholine. 'Tremorine' has been shown to be a weak inhibitor of the cholinesterase of the erythrocytes and intestinal mucosa[6]. Atropine sulphate, on the other hand, does not prevent the increase of brain acetylcholine caused by the injection of eserine sulphate, which is presumably due to the inhibition of brain cholinesterases (Pepeu, unpublished work). Also diethazine, an anti-Parkinsonism drug of the phenothiazine type, prevents the onset of the symptoms caused by 'Tremorine'. Diethazine diminishes but not always abolishes the increase of brain acetylcholine due to 'Tremorine'. In fact 50 per cent of the rats in the group treated with diethazine and 'Tremorine' had a brain-level of acetylcholine higher than 3 µg/g. Nevertheless, all the rats had very little or no symptoms. Ahmed *et al.*[7] have shown that of the established anti-Parkinson's disease drugs diethazine is the least potent antagonist of acetylcholine on the isolated guinea pig ileum. Moreover, we have noted that caramiphen, like atropine, decreases brain acetylcholine but diethazine does not, as may be seen in Table 2. The rats injected with diethazine are slightly sedated whereas those injected with caramiphen show an increase of the spontaneous motility.

The results reported show a difference between the anti-Parkinson's disease drugs of the atropine type and those of the phenothiazine type. They do not give a definite evidence that the increase of brain acetylcholine is responsible for the symptoms induced by 'Tremorine'. Since 'Tremorine' also increases brain histamine in the rat[8] and depletes brain norepinephrine[9], the mechanism of action of this drug seems to be very complex and its action on brain acetylcholine only one of its aspects.

Table 1

Drugs	No of animals	Dose (mg/kg)	Time (min)	ACH (µgm/gm ± *S E*)	Changes (%)	Remarks
None	26	—	—	2·80 ± 0·08	—	—
'Tremorine'	4	30	4–10	3·66 ± 0·74*	+ 30	Beginning of the symptoms
'Tremorine'	12	30	20–40	4·07 ± 0·64*	+ 45	Full symptomatology
Atropine plus		5	20			
'Tremorine'	8	30	20	2·61 ± 0·20	—	No symptoms
Caramiphen plus		20	20			
'Tremorine'	5	30	20	2·73 ± 0·32	—	No symptoms
Diethazine plus		40	20			
'Termorine'	10	30	20	3·08 ± 0·40	+ 10	No or mild symptoms

*These values differ significantly from the controls at a level of $P < 0·001$

Table 2

Drugs	No of animals	Dose (mg/kg)	Time (min)	ACH (µgm/gm ± *S E*)	Remarks
None	26	—	—	2·80 ± 0·08	
Caramiphen hydrochloride	6	20	40	2·13 ± 0·14*	Mild excitation
Diethazine hydrochloride	6	40	40	2·85 ± 0·22	Sedation

* This value differs significantly from the controls at a level of $P < 0·02$

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GIANCARLO PEPEU

Institute of Pharmacology,
University of Pisa,
Italy

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Phenylquinone Writhing Test: Interpretation of Data

THE antagonism of phenylquinone (2-phenyl-1,4-benzoquinone)-induced writhing in mice has been recommended for investigating weak analgesic activity of new compounds[1,2], but the non-specificity of this test has been emphasized by Hendershot and Forsaith[3]. Many compounds not considered to be analgesics, for example, parasympathomimetics, sympathomimetics and central nervous system stimulants, protect mice against the writhing induced by phenylquinone. During the routine pharmacological screening of new compounds, we observed an apparent correlation between *in vitro* anti-5-hydroxytryptamine activity and the ability of a compound to antagonize phenylquinone-induced writhing. Therefore, to determine more precisely the value of this test in a routine screening programme, it was decided to investigate in more detail the writhing response and its modification by compounds and procedures known to affect the activity and metabolism of biogenic amines.

The *in vitro* anti-5-hydroxytryptamine activity of compounds was investigated using the isolated œstrus rat uterus preparation. Dose-response curves to 5-hydroxytryptamine (5HT) were obtained in the presence or absence of varying concentrations of antagonist and the concentration of antagonist required to reduce the sensi-

Table 1 COMPARATIVE *in vitro* ANTI-5HT AND *in vivo* ANTI-PHENYLQUINONE ACTIVITIES OF LSD, HOMOCHLOROCYCLIZINE, PROMETHAZINE AND MEPYRAMINE

Compound	Anti-5HT activity		Anti-phenylquinone activity	
	Concentration to reduce uterine sensitivity 10 times (µg/ml)	Relative potency	ED_{50} (95 per cent confidence limits) (mg/kg)	Relative potency
LSD	0 01	1	0 019 (0 012–0 030)	1
Homochlorcyclizine	0 25	0 04	6 80 (4 50–10 9)	0 0028
Promethazine	0 1–0 2	0 05–0 1	12 0 (8 60–16 8)	0 0016
Mepyramine	> 2 5	< 0 004	133 (80 6–220)	0·0001

Table 2. EFFECT OF REPEATED INTRAPERITONEAL INJECTIONS OF PHENYLQUINONE ON THE WRITHING RESPONSE IN MICE

Day	1	2	3	4	5
Writhes to phenylquinone (percentage of control)	100	54	11	3	0

2 mg/kg phenylquinone was injected once daily on each of five successive days to a single group of mice, on each day a fresh group of animals was injected with phenylquinone to serve as control

tivity of the preparation by a factor of 10 was calculated Compounds were also investigated for their ability to antagonize phenylquinone-induced writhing, essentially by the method of Hendershot and Forsaith[3] Compounds were administered orally, dissolved or suspended in 5 per cent gum acacia, to groups of 6 starved male albino mice (Tuck strain) weighing 18–22 g One hour later, unless otherwise stated, each animal received an intraperitoneal injection of phenylquinone, 2 mg/kg The total number of writhes in a group of 6 animals was counted for the 20-min period immediately following the phenylquinone injection A new aqueous solution of phenylquinone was prepared using gentle heat before individual experiments. The solution was kept in an amber glass bottle, stoppered when not in use, in order to avoid decomposition of the phenylquinone The ED_{50} of a compound (the dose required to reduce by 50 per cent the number of writhes induced by phenylquinone treatment alone) was calculated by the method of Litchfield and Wilcoxon[4] To improve the precision of the determinations, duplicate experiments were performed

d-Lysergic acid diethylamide (LSD), homochlorcyclizine and promethazine are known to antagonize the effects of injected or released 5HT, mepyramine is less active in this respect The comparative activities of these compounds in antagonizing phenylquinone-induced writhing in mice together with their *in vitro* anti-5HT activities are summarized in Table 1 Eckhardt, Cheplovitz, Lipo and Govier[5] have suggested that the release of 5HT may be a factor in the phenylquinone writhing response It was thought that if the release of 5HT was implicated, then repeated intraperitoneal injections of phenylquinone to an animal would deplete the intestinal tissues of 5HT and decrease the number of writhes obtained at each injection A group of 6 mice was injected with phenylquinone on each of 5 successive days, each day a fresh group of animals was injected with phenylquinone to serve as controls The results of this experiment, summarized in Table 2, show that repeated injections of phenylquinone caused the expected reduction in the number of writhes We also found that the 5HT content of the small intestine of animals given 5 injections of phenylquinone was only 60 7 per cent that of untreated animals, in a further experiment a single injection of phenylquinone reduced the 5HT content of intestinal tissue to 76 3 per cent that of untreated animals in 1 h The 5HT was determined by a spectrofluorometric method[6] It is not known whether the depletion of 5HT by phenylquinone is a specific drug effect, but although treated animals exhibited diarrhœa, autopsy did not reveal obvious irritation or damage to the intestinal tract

The effect of phenylquinone was next investigated in mice sensitized to 5HT by adrenalectomy since Higginbotham[7] has shown that there is up to a 25-fold increase in sensitivity to 5HT in these animals We have found that the sensitivity of mice, adrenalectomized 5 days previously, to injected phenylquinone was markedly increased, a 69 per cent increase in the writhes being observed

Next, the effects of hydrazine and non-hydrazine monoamine oxidase inhibitors (MAOI) were investigated in the phenylquinone test, since these compounds are known to increase tissue concentrations of 5HT In view of the results obtained with 5HT antagonists, MAOI's were expected to potentiate the writhing response Surprisingly, tranylcypromine, phenelzine and iproniazid administered orally 4 h prior to the phenylquinone injection showed definite antagonistic activity to the writhing response (Table 3), and their potencies in this respect closely followed their activities as inhibitors of monoamine oxidase Harmine and pargyline also inhibited the writhing response This result need not necessarily preclude involvement of 5HT in the writhing response since MAOI's might inhibit its release from peripheral stores

Table 3 ANTAGONISTIC EFFECTS OF MONOAMINE OXIDASE INHIBITORS ON THE PHENYLQUINONE WRITHING RESPONSE IN MICE

Compound	ED_{50} (95 per cent confidence limits) (mg/kg)
Tranylcypromine	3 70 (2 21–6 18)
Phenelzine	7 70 (3 50–16 9)
Iproniazid	112 (62 2–201)

As a result of these tests two additional classes of compounds, 5HT antagonists and MAOI's, have been shown to be potent inhibitors of phenylquinone-induced writhing in mice This adds to the type of compounds already known to be active in this test. One might, therefore, ask if the test has any predictive value in the pharmacological evaluation of new compounds Certainly, a positive result can no longer be regarded as indicating a high probability of weak analgesic or anti-inflammatory activity However, we believe the test is of value as a simple primary screening test to detect a diversity of known pharmacological types These have to be characterized more fully by other tests

R T BRITTAIN
D N LEHRER
P S J. SPENCER

Research Division,
Allen and Hanburys, Ltd.,
Ware,
Hertfordshire

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Production of 6-Aminopenicillanic Acid by Dermatophytes

FOLLOWING the isolation of 6-aminopenicillanic acid (6-APA) by Batchelor *et al* [1,2], a number of papers dealing with its formation both by fermentation and by microbial hydrolysis have been published[3] Cole and Rolinson[4] recently reported on production of 6-APA by certain species of *Cephalosporium* and *Emericellopsis*

As we showed some years ago that *Trichophyton* strains were able to produce penicillins[5,6], dermatophytes were examined for production of 6-APA For this purpose, 70 dermatophyte strains were examined The experiments were carried out in shaken cultures in precursor-free media The quantity of 6-APA was measured by a fast biological method[7] and was identified also by paper chromatography. Each test was repeated three times in duplicate. A proto-

Table 1. Production and/or Formation of 6-Aminopenicillanic Acid by Dermatophytes (Units/ml. in Terms of Benzylpenicillin)

Strains	Activity of fermentation broth	6-APA in fermentation broth	6-APA in acetonic extract of myc.	Penicillinacylase activity of for penicillin *G*		penicillin *V*	
				broth	mycel.	broth	mycel.
Epidermophyton interdigitale	1·5	0·2	6·0	6–20	20–50	80–600	300–900
Trichophyton gypseum	0·6	0·9	0·5	0·7–1	1–5	9	30–50
T. gypseum	0·0	0·06	0·0	6	2–10	20–100	80–150
T. gypseum	0·3	0·2	0·4	1	1–6	20–50	20–60
T. mentagrophytes	5·0	±	0·0	1–4	4–8	60–150	300–400
T. interdigitale	1·5	±	±	1–2	80–150	10–30	50–200

type is shown in Table 1. All values in Table 1 represent benzylpenicillin units per millilitre.

The first section of Table 1 shows the quantity of 6-APA produced through fermentation, and the second indicates the penicillinacylase activity of the same strains. The first column shows the total antibiotic activity of the fermentation fluid, the second shows the 6-APA content free in the broth, and the third one discloses the amount of 6-APA in the concentrated acetonic extract of the washed mycelium[8].

As may be seen, dermatophytes are capable of synthesizing 6-APA in various amounts, just like other penicillin-producing moulds are.

Furthermore, the penicillinacylase activity of the fermentation broth and separately that of the washed mycelium of dermatophytes was examined. The values reveal that dermatophytes are able to produce both endo- and exo-cellular penicillinacylase, and that they form 6-APA from phenoxymethylpenicillin more readily and abundantly than from benzylpenicillin. Mycelium generally has a higher activity than fermentation fluid. This is generally characteristic of moulds. Production of penicillinacylase by dermatophytes can be increased by administering the adequate acid.

The presence of penicillinacylase activity in dermatophytes is thus shown, and that 6-APA could be a product of dermatophytes is suggested. This may be the reason of the ineffectiveness of penicillins in the treatment of fungal infections caused by dermatophytes.

J. Uri
G. Valu

Department of Pharmacology,
University Medical School,
Debrecen 12, Hungary.

I. Békési

Antibiotic Research Institute,
Hungarian Academy of Sciences.

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Increased Concentrations of Blood Lipids in Guinea Pigs poisoned by Sarin

In the course of routine withdrawal of blood samples from guinea pigs treated with the anticholinesterase sarin (isopropyl methylphosphonofluoridate) it was noticed that samples taken from some animals were milky in appearance. This visible hyperlipæmia was found to be more prevalent in the female, and this communication describes the experiments which led to this finding.

Male and female guinea pigs from the local colony (500–800 g) were injected subcutaneously with sarin (1,500 µg/kg, that is, approximately 30 LD_{50} doses) and treated intramuscularly with an oxime, 2-hydroxyiminomethyl-*N*-methyl pyridinium methane sulphonate (P2S) 30 mg/kg, and atropine 1 mg/kg. The cholinesterase reactivator P2S and atropine were administered to ensure that a reasonable number of survivors (approximately one-third of the injected animals) would be obtained despite the high dose of sarin.

Because the majority of the survivors of sarin poisoning were spastically paralysed and incapable of, or disinterested in, feeding or drinking, both the survivors and the controls were deprived of feed and water during the experimental period.

Three days after the injection of sarin, the animals were killed and blood samples were drawn from the heart (chest opened after blow on the neck). The blood samples were allowed to clot and spun in a centrifuge at 1,900*g* for 20 min and the entire supernatant was collected for analysis. Phospholipid concentrations determined as lipid phosphorus[1,2] were used as indexes of the degree of lipæmia.

Cholesterol was also estimated in the earlier stages of the experiment, but since the rise in total cholesterol paralleled the increase in lipid phosphorus, they were discontinued.

As illustrated in Fig. 1, the difference in lipid content between samples of blood drawn from poisoned animals and controls was evident to the eye. As the samples cooled to room temperature, large masses of lipid material precipitated from solution. In the sample drawn from the female guinea pig with hyperlipæmia illustrated in Fig. 1 the lipid phosphorus was 30·6 mg per cent whereas that of the control was 3·4 mg per cent.

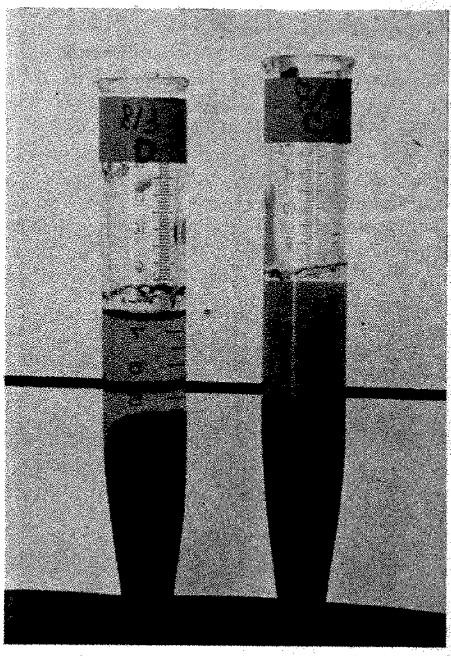

Fig. 1. Hyperlipæmia in a female guinea pig treated with sarin (1,500 µg/kg)

Left test-tube, lipid phosphorus = 3·4 mg per cent; right test-tube, lipid phosphorus = 30·6 mg per cent

Notice that the black line drawn behind the test tubes cannot be seen behind the hyperlipæmic sample. The photograph is shown to emphasize the point that the lipid phosphorus measured is only one component of a general hyperlipæmia

Referring to Table 1, the subcutaneous administration of sarin, 1,500 μg/kg, to female guinea pigs caused a significant increase of lipid phosphorus, the mean being 16·6 mg per cent in the survivors as compared with 3·0 mg per cent in female controls. This increase was more prevalent in females, the mean concentration of lipid phosphorus being the same in male guinea pigs whether sarin-poisoned or controls. Only three males had definite raised lipid phosphorus (15·0, 6·6 and 5·8 mg per cent) out of 29 survivors poisoned with sarin.

Table 1. LIPID PHOSPHORUS CONCENTRATIONS (MG PER CENT) OF SARIN-TREATED AND UNTREATED MALE AND FEMALE GUINEA PIGS

Females				Males		
Controls – treated with P2S and atropine		Experimental – treated with sarin, P2S and atropine		Controls – treated with P2S and atropine	Experimental – treated with sarin, P2S and atropine	
2·5	2·9	1·9	6·0	2·6	1·2	3·0
3·3	4·4	59·6	7·8	1·7	1·8	3·0
3·1	2·3	56·8	6·1	2·2	1·1	3·6
3·1	3·1	6·0	8·9	2·3	1·7	2·6
2·6	2·5	7·2	3·7	2·8	1·2	1·7
2·1	3·9	32·0		2·8	6·6	15·0
4·1	3·5	2·0		1·5	5·8	1·4
3·4	4·1	1·4		2·4	2·4	2·2
1·7	3·8	57·0		2·4	3·8	2·4
2·5	2·7	14·1		2·8	1·9	3·5
4·8		3·9		1·9	1·2	2·4
4·4		1·4		2·3	2·7	1·5
2·5		34·4		1·9	3·4	4·9
3·3		2·5		2·0	2·1	
3·1		30·6		1·6	1·0	
3·1		4·5		2·9	4·2	
$n = 26$		$n = 21$		$n = 16$	$n = 29$	
$\overline{X}_{FC} = 3·0$		$\overline{X}_{FE} = 16·6$		$\overline{X}_{MC} = 2·3$	$\overline{X}_{ME} = 3·0$	
$S_{FC}^2 = 0·95$		$S_{FE}^2 = 393·9$		$S_{MC}^2 = 0·21$	$S_{ME}^2 = 7·2$	

n = number of animals ; $\overline{X}$ = means ; S^2 = variance.

Difference in weight loss during the three days offered no explanation of the sex difference, loss of weight being approximately the same for both sexes (21·4 and 23·0 per cent respectively : Table 2). Attempts to correlate loss in weight and degree of hyperlipæmia in the case of individual animals gave negative results. Also, it is not sufficient to say that the acute effects of sarin poisoning caused the observed increase in blood lipids since only three male guinea pigs showed elevated blood lipid phosphorus. Animals which showed no hyperlipæmia were as spastically paralysed as those with increased blood lipids. All surviving animals showed some degree of paralysis, but it varied greatly in intensity, ranging from a few showing very little paralysis of their limbs to a few which could not right themselves (involvement of all four limbs).

Table 2. MEAN PERCENTAGE LOSSES IN WEIGHT OF SARIN-TREATED AND UNTREATED MALE AND FEMALE GUINEA PIGS (LISTED IN TABLE 1)

Females		Males	
Controls – treated with P2S and atropine	Experimental – treated with sarin, P2S and atropine	Controls – treated with P2S and atropine	Experimental – treated with sarin, P2S and atropine
16·7	21·4	20·2	23·0
[95% confidence limits 9·9–23·5]	[95% confidence limits 14·6–28·2]	[95% confidence limits 15·2–25·2]	[95% confidence limits 15·6–30·4]

A possible explanation of sarin-induced hyperlipæmia is inhibition of esterases (including cholinesterases) concerned in lipid metabolism. Gerebtzoff[3] expressed the opinion that there is "some vague relationship between cholinesterases and lipid metabolism". To explain the sex difference observed, it is necessary to postulate either that the esterases differ in the two sexes or that they are rendered susceptible to inhibition by sarin by some factor peculiar to the female. Kritchevsky *et al.*[4] have stated that there are differences in cholesterol metabolism between male and female rats which can be influenced by gonadectomy and œstrogenization. Also, they found that only some of the rats oxidized cholesterol at some fixed rate whereas another group of rats within the population oxidized cholesterol at a higher rate. The results presented by Kritchevsky *et al.*[4] are similar to those presented here in that females show a greater frequency of increased lipid concentration after sarin treatment than males and also within one group, say the females, some show no increase in lipid phosphorus whatsoever whereas others exhibit hyperphospholipidæmia to a marked degree (range of lipid phosphorus 1·4–59·6 mg per cent).

A reference should be made to the fact that sarin is an established neurotoxic agent in chickens[5], causing demyelination and axonal degeneration. Increase in certain blood lipids may possibly reflect biochemical lesions as yet unknown, which have neurotoxic effects. Guinea pigs are said to be resistant to paralysis with neurotoxic compounds[6], but demyelination occurs in rats which are also said to be resistant[7]. There is, however, to our knowledge, no reference in the literature to any difference in degree of neurotoxicity based on sex.

G. S. FUKUYAMA
P. A. ADIE
E. RUTH HUGHES

Physiology Section,
Defence Research Board of Canada,
Suffield Experimental Station,
Ralston, Alberta.

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HÆMATOLOGY

Formation of Ferrihæmoglobin of Isolated Human Hæmoglobin Types by Sodium Nitrite

THE sensitivity of hæmoglobins to oxidation by potassium ferricyanide and sodium nitrite ($NaNO_2$) has been examined extensively[1,2]. During treatment of oxyhæmoglobin with $NaNO_2$, both components are oxidized through a combination of different reactions, the total reaction being summarized as follows: $NaNO_2 + 2R\text{–}Fe^{++}O_2 + H_2O \rightarrow NaNO_3 + 2R\text{–}Fe^{+++}OH + O_2$ (ref. 2). The rate of ferrihæmoglobin formation by the action of $NaNO_2$ can be calculated from the sigmoid curve representing the relation between reaction time and per cent of ferrihæmoglobin and is expressed as the time required for 50 per cent oxidation of the hæmoglobin (*T* 1/2). It has been demonstrated that the hæmoglobin of cord red blood cells, which is composed mainly of fœtal hæmoglobin (Hb-F), is particularly sensitive to treatment with $NaNO_2$. Since the structural difference between Hb-F and Hb-A is located in one of the two types of polypeptide chains, Hb-F being composed of 2 α-chains and 2 γ-chains ($\alpha_2\gamma_2$) and Hb-A of 2 α-chains and 2 β-chains ($\alpha_2\beta_2$), the possibility is present that $NaNO_2$ will react differently with human hæmoglobin types of other structural composition.

The following human hæmoglobin types have been investigated: Hb-A; Hb-S ($\alpha_2\beta_2^{6\text{val}}$, ref. 3); Hb-C ($\alpha_2\beta_2^{6\text{lys}}$, ref. 4); Hb-F; Hb-Bart's (γ_4, ref. 5); Hb-A_2 ($\alpha_2\delta_2$); Hb-A_2' a variant of Hb-A_2 (ref. 6); Hb-Lepore, composed of normal α-chains and of 'β' chains being related to the β-chain of Hb-A and the δ-chain of Hb-A_2 (ref. 7); Hb-A, GSSG, a mixed disulphide of Hb-A and glutathione with the following formula $\alpha_2\beta_2(SSG)_2$ (ref. 8); Hb-M-Kankakee, an abnormal methæmoglobin composed of abnormal α-chains and normal β-chains (ref. 9). The Hb-Bart's and Hb-M-Kankakee were isolated by preparative CM-cellulose chromatography (ref. 10); all other hæmoglobin components by preparative DEAE-cellulose chromatography (ref. 10) at 4°–10° C within 5 h. The isolated oxyhæmoglobins were dialysed against distilled water at 4° C for 24 h The methæmoglobin formation rates were investigated 26–30 h after the isolation following the technique described by Betke *et al* [2] The concentration of each solution was adjusted in such a way that the optical density read 0 800 ± 0 005 at 500 mμ using the Beckman *DU* spectrophotometer and 1-cm quartz cuvettes The solution was kept at room temperature for 1 h and diluted with an equal volume of a 0 2 M sodium phosphate buffer at *p*H 7 00 ± 0 02 The formation of ferrihæmoglobin was measured, after introducing 0 05 ml of a 0 025 M NaNO$_2$ solution with a micro-syringe (accuracy 1 per cent) into 3 0 ml of the hæmoglobin–phosphate buffer mixture, by determining the increase of optical density at 500 mμ at certain time intervals The oxidation was found completed when no further increase in the optical density readings at 500 mμ was observed, which was confirmed by the determination of the spectra between 430 and 700 mμ using the Beckman *DK*-2 spectrophotometer The molar ratio between hæmoglobin (calculated on 1/4 of the molecular weight at 68 000) and NaNO$_2$ was 1 5 9 In some experiments 0 1, 0 075, and 0 0375 ml of NaNO$_2$ solutions were added to the hæmoglobin solution The rate of ferrihæmoglobin formation of the isolated buffered Hb-M-Kankakee (initial optical density readings 0 780 and 0 785) was measured by determining the decrease in optical density readings at 576 mμ (Beckman *DU*, 1-cm quartz cuvettes)

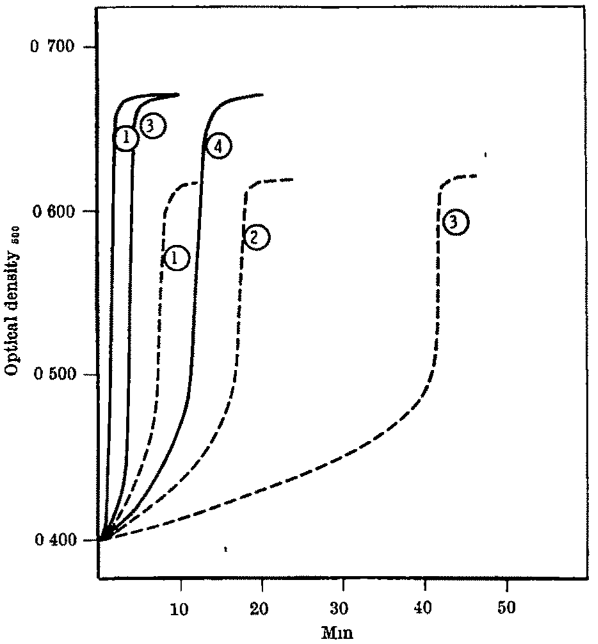

Fig 1 Curves representing the relation between the reaction time and the increase in the production of ferrihæmoglobin 1, 0 1 ml, 2, 0 075 ml, 3, 0 05 ml, 4, 0 0375 ml M 0 025 sodium nitrite ——, Hb-A$_0$, – – –, Hb-A$_2$

From the ferrihæmoglobin formation curves in Fig 1, it is evident that isolated Hb-A$_2$ is more resistant to NaNO$_2$ oxidation than Hb-A$_0$ When the *T* 1/2 values of isolated Hb-A$_2$, Hb-A$_2$′ and Hb-Lepore were compared with the *T* 1/2 values of Hb-A$_0$ fractions, which were isolated concurrently, a 7–11-fold decrease in the rate of ferrihæmoglobin formation was found The β-chain abnormalities, Hb-S and Hb-C, did not differ from Hb-A The *T* 1/2 values established for Hb-F ($\alpha_2\gamma_2$), Hb-Bart's (γ_4) and Hb-A-GSSG were 0 41, 0 23 and 0 61 times the *T* 1/2 value of Hb-A$_0$ respectively It seems, therefore, that hæmoglobin types composed of α-chains and normal or abnormal δ-chains are oxidized by NaNO$_2$ at a low rate, while the oxidation of normal Hb-A or hæmoglobin components with abnormal β-chains is intermediate between those of Hb-A$_2$ and the γ-chains containing Hb-F Alteration of the β-chains of Hb-A by the introduction of one glutathione residue into each β-chain increases the ferrihæmoglobin formation rate Hb-Bart's is oxidized at an extremely high rate

Another feature of the sigmoid oxidization curves (Fig 1) is an initially slow increase in the formation of ferrihæmoglobin, while a steep rise was observed when about 50 per cent of the available ferrous iron of the hæmoglobin was oxidized These observations may indicate that during the action by NaNO$_2$ the ferrous iron of the hæm groups of the δ- or β- or γ- chains is oxidized first (at different rates), followed by an extremely rapid oxidation of the ferrous iron of the hæme groups of the α-chains, which are commonly present in Hb-A, Hb-F and Hb-A$_2$ This hypothesis has been supported by investigations of the sodium nitrite oxidation of isolated Hb-M-Kankakee The oxidation of the unaltered ferrous iron of the hæm groups of β-chains was found to proceed slowly, the curves being hyperbolic rather than sigmoid in shape

The final increase occurring after the logarithmic rise in optical densities of Hb-A$_2$, Hb-A$_2$′ and Hb-Lepore at 500 mμ by the action of NaNO$_2$ was always considerably less than those observed for Hb-A and its variants and for Hb-F (Fig 1) No additional increase was seen after the addition of excess oxidant The visible spectra of the ferrihæmoglobin derivatives of Hb-A$_2$, Hb-A$_2$′ and Hb-Lepore and of Hb-A, measured at a *p*H of 7 0, offered the explanation for these observations since notable differences were present The millimolar extinction values for the isolated Hb-A$_2$ at the wave-lengths 630, 565, 530 and 500 mμ were 3 15, 5 85, 8 25 and 8 65 and for the isolated Hb-A$_0$ 3 8, 4 95, 7 65 and 9 35 respectively The spectra of the oxy-, reduced, CO–, and cyanmethæmoglobin derivatives of Hb-A$_2$ and Hb-A were identical It is noteworthy that similar spectral deviations were observed when Hb-A$_2$ and Hb-A$_0$ were oxidized by potassium ferricyanide

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HOWELL MARTIN
TITUS H J HUISMAN

Departments of Biochemistry and Pathology,
Medical College of Georgia,
Augusta, Georgia

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Role of Liver in the Inactivation of Erythropoietin

THERE is still considerable uncertainty as to the site or sites of inactivation of erythropoietin (ESF) Jacobsen *et al* [1] found that plasma extracts obtained from rabbits treated with acetylphenylhydrazine (APH) showed definite ESF activity when tested in hypophysectomized rats These APH-treated rabbits had evidence of liver damage In plasma extracts obtained from animals without evident liver damage, no erythropoietic activity was observed Prentice and Mirand[2,3] placed normal rats under low oxygen tension (8–12 per cent) and did not detect ESF in their plasma When the experiment was repeated on rats treated with carbon tetrachloride, ESF was found in the plasma When plasma obtained from animals treated

Table 1 EFFECT OF LIVER PERFUSION ON ACTIVITY OF ESF*
(Activity 3 h iron-59 bioassay in starved rats)

	Groups	% Dose in plasma	^{59}Fe γ/ml plasma	% Dose in erythrocytes	% Dose in tibiæ
1	Control perfusate (30 min)	44 6	2 5	0 23	1 4
			Perfusion 1		
2	ESF incubated with Ringer solution (30 min)	2 2	1 2	6 4	4 3
3	90-min perfusion	3 1	1 3	3 1	4 3
4	160-min perfusion	4 5	1 8	2 4	4 4
5	ESF incubated with saline solution	4 5	1 5	2 7	4 8
			Perfusion 2		
6	2-min perfusion	8 0	2 1	2 1	3 8
7	240-min perfusion	9 0	1 5	1 5	4 1
8	360-min perfusion	7 6	1 1	1 8	4 3
9	ESF incubated with saline solution	6 1	1 1	2 0	4 3

* ESF Armour Lot No *K*, from APH sheep plasma

Bioassay values represent mean of at least 6 rats per group

Groups 2-5 each rat received a volume of perfusate to which 4 2 cobalt units of ESF had been added, groups 6-9 a volume of perfusate to which 2 8 cobalt units had been added

Liver and spleen were also analysed and showed similar patterns

with APH was injected directly into the portal vein, no erythropoietic response was observed When such plasma was injected into the jugular vein, a definite effect was obtained From this observation they concluded that liver has a role in the inactivation of ESF

Burke and Morse[4] perfused livers from normal rats with blood obtained from rats treated with cobaltous chloride They assayed for ESF by measuring uptake of iron-59 by red blood cells *in vivo* by fasting rats, and significant decrease in ESF activity was observed within 3 h of perfusion When similar experiments were performed with carbon tetrachloride poisoned livers, no reduction in ESF-level was observed They therefore concluded that the liver participates in the removal of ESF from the blood

Stohlman and Brecher[5] showed that plasma obtained from APH-treated rabbits was active whether or not there was liver damage Later, Stohlman[6] showed that in animals where the bone marrow was damaged by irradiation the ESF-levels were higher than in controls and it was concluded that bone marrow may be the site of utilization and/or inactivation of ESF. These conflicting results have led us to re-examine this problem in the isolated perfused rat liver system where known amounts of exogenous ESF were added to the perfusate

Rat livers were perfused by the method of Miller *et al* [7] Plasma was separated and the buffy coat removed The red blood cells were washed with Ringer's solution, diluted to 2 3 times their volume with Ringer's solution and 25 mg heparin and 140 mg glucose added The total volume of the perfusate was 100 ml

The liver was perfused for 30 min 20 ml of perfusate were then removed and kept inside the perfusion chamber as a control (Table 1, group 1) A sheep plasma ESF preparation was added to the perfusate in amounts of 3 cobalt units/ml and samples were taken at the times indicated in Table 1 The red blood cells were separated and the supernatant was refrigerated until the bioassay was performed The same concentration of ESF present in the perfusate was incubated with either 0 8 per cent sodium chloride solution or red blood cell Ringer's solution and kept for the duration of the experiment in the perfusion chamber at 38° C (Table 1, groups 2, 5, 9)

Samples from the perfusate and the controls were tested by bioassay in rats by the method of Hodgson *et al* [8] Fasting rats were used and on the third and fourth day of fasting they were injected with aliquots of the supernatant of the perfusate and killed on the fifth day. Three hours prior to death, iron-59 (0 1 μc /rat) previously incubated with homologous plasma was given intravenously Blood, tibiæ, spleen and liver were removed, processed and counted in a well-type scintillation counter.

It is clearly shown that there are no differences in ESF activity between the samples obtained after perfusion and the controls incubated with either Ringer's or saline solutions. The validity of the assay is demonstrated by the difference between the control without added ESF and the control to which ESF has been added

If the estimated biological half-life of ESF is 8 h and if the liver had a role in the conjugation of this hormone, then certainly an effect should have been observed

Our results are not in agreement with the findings of Burke and Morse[4] It must be noted that in their experiments blood with a high endogenous titre of ESF was used as perfusate, whereas in the present experiments ESF was added to the red blood cell Ringer's mixture

On the basis of these findings, it is concluded that exogenous ESF is not inactivated by the perfused rat liver The reasons for the differences between the behaviour of endogenous and exogenous ESF are not apparent

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SIEGMUND FISCHER*
PAUL S ROHEIM

Department of Medicine,
Albert Einstein College of Medicine,
Yeshiva University,
New York 61

* Permanent address Department of Virology, School of Medicine, University of Chile, Santiago

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Electrophoretic Patterns of *Macaca mulatta* and *Macaca nemestrina* Hybrid Hæmoglobin

VARIATIONS in the electrophoretic mobilities of hæmoglobin have been described in many species of sub-human primates[1–6]. A colony of 524 *Macaca mulatta* and 13 *Macaca nemestrina* was surveyed for hæmoglobin types by filter paper electrophoresis Two animals within the group had physical characteristics which combined features of both *M. mulatta* and *M nemestrina* These animals were purchased from the Miami Rare Bird Farm, Kendall, Florida, as part of a group of *M nemestrina*

Capillary blood of all animals was used for determination of the hæmoglobin type using 0 0125 M veronal buffer at *p*H 8 6 (ref 7) Venous blood was drawn from any animal with an abnormal pattern, and the method adopting 0 019 M barbital buffer at *p*H 8 6 was used[7]

The hæmoglobin of *M. mulatta* had the electrophoretic mobility characteristic of human hæmoglobin type A (refs 3 and 4), while the mobility of the hæmoglobin of *M nemestrina* was distinctly greater No variation within either group could be detected The hæmoglobin patterns of the suspected hybrids had both the slow component of the *M mulatta* and the more rapid component of the *M nemestrina* Fig 1 is a photograph of the electrophoretic patterns obtained from three representative animals

A *M. nemestrina* female and a *M mulatta* male were mated in the laboratory, and a premature, but viable, hybrid was obtained This animal had a hæmoglobin pattern the same as that of the two suspected hybrids Both the suspected hybrids were females and both had been mated successfully, one to a *M mulatta* and the other to a *M nemestrina*

It is of interest that the hybridization of the two species occurs readily in the laboratory, and it might be suspected

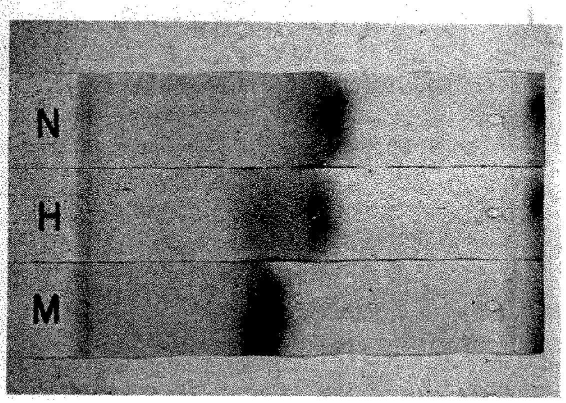

Fig. 1. Electrophoretic patterns of hæmoglobins of : (*N*) *Macaca nemestrina*; (*H*) hybrid; (*M*) *Macaca mulatta*

that the two females which were purchased were the results of natural or unplanned interspecific mating. The desirability of producing a hybrid between species of primates was pointed out by Jacob and Tappen[2]. Investigations of hybrids would help to elucidate the genetic mechanism of hæmoglobin transmission. At the present time, the difference between the hæmoglobins of the two species is used for measuring the viability of bone marrow transplants in irradiated animals.

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L. I. GOTTLIEB *
Primate Center and J. L. VAN LANCKER
Department of Pathology,
University of Wisconsin, Madison.

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* Postdoctoral Fellow of the American Cancer Society.

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Acetylcholinesterase Inactivation of Enzyme-treated Erythrocytes

THE molecular architecture of the red cell membrane formed mainly by lipids and proteins is not known; but it is possible to obtain information about its surface structure with the aid of agents which act on components located at the outer surface of the erythrocyte. This approach has been used to show the role of sialic acids in the electrokinetic behaviour of erythrocytes in an electrolyte medium[1].

Treatment of red cells with proteolytic enzymes has been widely used in blood-grouping techniques since Morton and Pickles[2] made the observation that trypsinized erythrocytes were agglutinated in saline suspension by incomplete Rh antibodies. However, the mechanism by which these enzymes exert their serological effect is not understood, although it has been demonstrated that this kind of treatment causes decrease in the electrophoretic mobility of erythrocytes[1], associated with the release of *N*-acylated neuraminic acid-containing mucoids[1], destruction of various blood group receptors[3], reduction of *in vivo* survival[4] and lysis in response to *p*H changes[5]. Prager *et al.*[6] have demonstrated that diisopropyl phosphoryl trypsin and *p*-chloromercuribenzoate-inactivated papain were bound to the red cell surface but did not possess any serological effect, therefore suggesting that the proteins must act enzymatically in order to affect agglutination.

Among the few enzymes recognized in the red cell membrane, acetylcholinesterase is one of the most investigated and previous studies have shown that the activity of this enzyme is reduced in new-borns affected with ABO hæmolytic disease[7]. A similar defect has been described in erythrocytes of patients with paroxysmal nocturnal hæmoglobinuria[8] and one of the features of this disease is the hæmolysis caused by acidified normal serum. Yachnin *et al.*[5] have shown that normal erythrocytes were made susceptible to lysis in response to changes in *p*H, by treatment with proteolytic enzymes, neuraminidase or sodium periodate; however, the effect of these agents on the red cell acetylcholinesterase was not examined. This communication gives the results of such an investigation.

Blood obtained from normal adult individuals was centrifuged and the erythrocytes washed 3 times with 20 volumes of 0·15 M sodium chloride solution. To one volume of packed cells, four volumes of buffered enzyme solution prepared according to Morton[9] were added and incubated for 30 min at 37° C. Cells incubated with buffer were used as controls. At the end of the incubation period cells were washed 3 times with ice-cold saline and a 50 per cent cell suspension was prepared. No noticeable hæmolysis was observed during the manipulation of the cells. Measurements of acetylcholinesterase activity were performed with the acetylthiocholine method[10].

Table 1. ERYTHROCYTE ACETYLCHOLINESTERASE INACTIVATION BY PROTEOLYTIC ENZYMES

Enzyme	Concentration (mg/ml.)	Inactivation (%)
Crude trypsin *	1	87
Cryst. trypsin †	10	36
	1	15
Crude papain †	1	94
Cryst. papain †	1	100
Cryst. γ-chymotrypsin †	1	78
Crude bromelin ‡	1	86
Soluble lipase ‡	10	3
Lysozyme †	10	0
Neuraminidase §	20,000 U/ml.	0

To 0·2 ml. packed cells 0·8 ml. of buffered enzyme solution was added. After 30 min at 37° C cells were washed three times with 20 vol. of ice-cold saline solution and activity was measured as indicated in text. Percentage of inactivation related to controls incubated without enzyme.

* Nutritional Biochemicals Corporation, Cleveland, Ohio.
† Sigma Chemical Co., St. Louis, Mo.
‡ Mann Research Laboratories, New York.
§ Gift from Dr. G. L. Ada, Melbourne, Australia.

Table 1 indicates the percentage of inactivation of acetylcholinesterase of intact erythrocytes as compared with the controls incubated with buffer, and it can be noticed that the concentrations of proteolytic enzymes correspond to those routinely used in the detection of incomplete antibodies. When red cell ghosts, prepared by osmotically induced hæmolysis or by freeze-thawing, were incubated with proteases a similar inactivation of the enzyme was observed. From experiments in which solubilized acetylcholinesterase was mixed with trypsin and initial rates measured for 2 min or when trypsin was added at various time intervals to complete assay systems, it was possible to infer that this proteolytic enzyme did not interfere with the measurement of the acetylcholinesterase activity. This possibility was also ruled out when the activity was measured by a modification of the electrometric method of Michel[11]. No significant acetylcholinesterase activity could be detected in the supernatant solution after enzymatic treatment.

Studies of the time course of the enzymatic acetylcholinesterase inactivation over a 60-min period at 37° C revealed an exponential loss of activity with first-order kinetics, as can be observed from Fig. 1. This observation is compatible with the indication that proteases do not act merely by binding to the red cell membrane but by an active proteolytic process. In experiments in which red cells were agglutinated with their ABO iso-antibodies, no protection against proteolytic inactivation of acetylcholinesterase could be detected. Treatment of erythrocytes for 24 h with highly purified cholera vibrio neuraminidase[12] did not inactivate acetylcholinesterase, suggesting that *N*-acetyl neuraminic acid, an integral part of the human erythrocyte surface, was not associated with

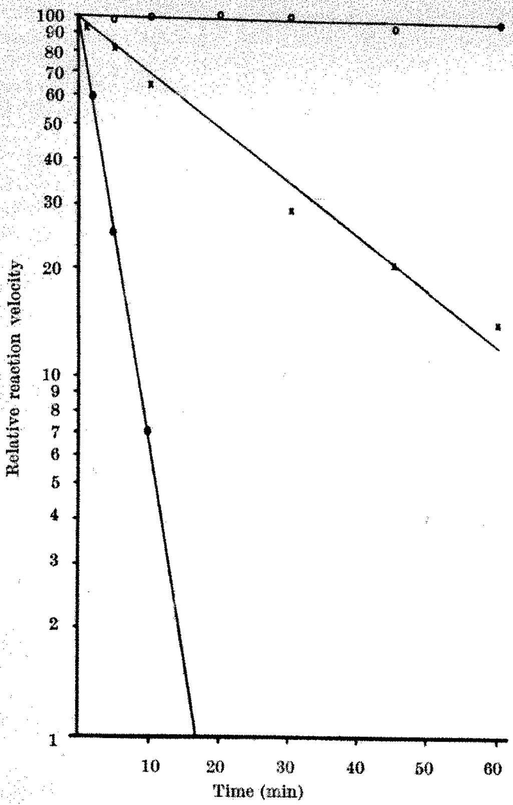

Fig. 1. Effect of time and concentration on erythrocyte acetylcholinesterase inactivation by bromelin. To 0·1 ml. of a 50 per cent cell suspension 0·4 ml. of bromelin in 0·1 M phosphate buffer, *p*H 6·0, was added, tubes incubated at 37° C for time indicated, immediately washed three times with 20 vol. ice-cold 0·15 M sodium chloride solution and stored as 50 per cent cell suspension at 4° C. Activity was measured as indicated in text. ○, 0·1 mg/ml.; ×, 1 mg/ml.; ●, 10 mg/ml.

the activity of the enzyme. Lysozyme and lipase (10 mg/ml.) were equally ineffective.

The activity of intracellularly located enzymes, such as lactic dehydrogenase, glucose-6-phosphate dehydrogenase, acid phosphatase and inorganic pyrophosphatase as well as the stromal enzymes alkaline phosphatase and adenosine triphosphatase, was not affected by treatment of whole red cells with proteolytic enzymes.

This work was supported by grant *AM*–01387–07 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U.S. Public Health Service.

Note added in proof. Since this paper was submitted for publication, Firkin *et al.* (*Australasian Ann. Med.*, **12**, 26; 1963) have reported acetylcholinesterase inactivation by trypsin and chymotrypsin.

FRITZ HERZ
EUGENE KAPLAN
JOSEPH H. STEVENSON, JUN.
Departments of Pediatrics, Sinai Hospital, and the
Johns Hopkins University, School of Medicine,
Baltimore, Maryland.

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PATHOLOGY

Streptokinase and Buerger's Disease

BUERGER originally described thromboangiitis obliterans as primarily a thrombotic process occurring in arteries and veins[1]; later he postulated that the thrombosis arose from an angiitis[2]. Impressed with the thrombotic aspects of the disease, our laboratory undertook an investigation of clot formation and dissolution in Buerger's disease. Clots obtained from patients diagnosed as suffering from Buerger's disease were exposed to varying concentrations of streptokinase ('Varidase'; Lederle Laboratories) as were clots obtained from normal patients. While the blood clots obtained from normal patients were found to be resistant to high concentrations of streptokinase, similar concentrations of streptokinase completely dissolved the clots obtained from cases with thromboangiitis obliterans

In recent years the entire concept of thromboangiitis obliterans as an entity has come under attack[3]. The findings described here would lend support to the concept of considering Buerger's disease a distinct entity.

Seven male patients (aged 36–46; average, 42), who had previously had amputations for thromboangiitis obliterans, were evaluated; none of these cases was in an acute phase of the disease. Only two cases had had an amputation within one year of the time of the investigation; the others had had an amputation 1–16 years previously.

Fifty-nine controls were used consisting of 28 normal individuals with an average age of 35 and 31 patients admitted to the hospital for various states of disease. The average age of the patient controls was 54 years. Of the 31 patients, there were six patients who had undergone lower extremity amputations for atherosclerosis.

The method of testing was as follows: Tubes containing concentrations of streptokinase beginning at 100 units and increasing to 2,500 units were prepared; 1 c.c. of freshly drawn patient's blood was then added to each test tube. The volume was kept constant. The blood and streptokinase were mixed and then allowed to remain undisturbed for 20 min, during which time clotting occurred. Afterwards the tubes were incubated at 37° C for 70 min. Thus a total of 90 min elapsed from the time of collection of the blood sample until completion of the test. Each clot was observed for the length of time necessary for complete lysis; however, if this did not occur during the 70 min of incubation, no further observations were made.

It was found that 50 per cent of the control clots ceased to lyse when the concentration of streptokinase reached 1,500 units. None of the control clots lysed when exposed

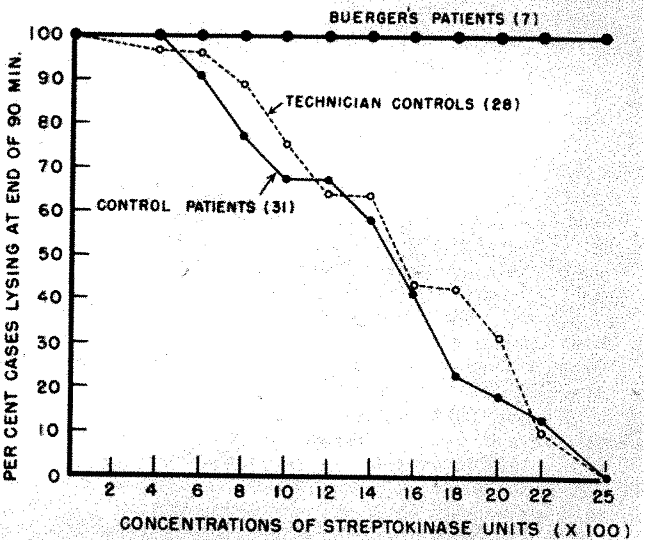

Fig. 1. Percentage of cases lysing their clots at the end of 90 min plotted against increasing concentrations of streptokinase

to 2,500 units of streptokinase for 70 min of incubation. The clots of all seven cases of thromboangiitis obliterans lysed when exposed to 2,500 units of streptokinase in less than 90 min (Fig. 1).

That streptokinase has an optimal concentration for fibrinolysis has been known but not adequately explained. The possibility of an inhibitor preventing clot lysis at higher concentration has been suggested[4]. Investigations are being carried out to explain further this mechanism in the normal individual and the difference noted in cases of Buerger's disease. The unusual behaviour of blood clots obtained from cases of thromboangiitis obliterans suggests that an abnormality in clot formation or lysis may be the ætiological factor in Buerger's disease.

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NORMAN ENDE

Clinical Laboratory, Veterans Administration Hospital,
and Department of Pathology,
Vanderbilt University School of Medicine,
Nashville, Tennessee.

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Lactic Dehydrogenase in *L*1210 Cells

PROMPTED by the striking difference in lactic dehydrogenase activity observed by Woodliff[1] in sensitive *L*1210 leukæmic cells compared with those resistant to amethopterin and 6-mercaptopurine we have examined lactic dehydrogenase-levels in sensitive *L*1210*Z* and a strain (*L*1210*C*59) resistant to both amethopterin and 6-mercaptopurine and partially resistant to cytoxan.

Tumour-bearing mice were kindly supplied by Dr. A. Goldin and Mr. S. R. Humphreys and maintained as already described[2]. The mice used were $B6D2F_1$ ($C57BL/6J$♀ × $DBA/2J$♂). Local tumours (average weight 250 mg) from the site of injection and infiltrated spleens (500 mg, 5 times normal weight) were removed 9–12 days after inoculation and stored at – 15° two weeks prior to preparation of the enzyme extracts. These extracts were prepared by homogenizing the tissues in 3 volumes of chilled 0·1 M sodium phosphate buffer, *p*H 7·0, followed by centrifugation at 60,000*g* for 30 min. Protein content was measured spectrophotometrically[3].

Each 1-ml. incubation contained DPNH 0·00013 M, sodium pyruvate 0·002 M, sodium phosphate, *p*H 7·0, 0·1 M, and 14 μg protein; read in a Beckman *DU* spectrophotometer at 32°. The Δ absorbance for a control incubated without pyruvate was 0·004/min.

As shown in Table 1, we could reveal no difference in lactic dehydrogenase activity in extracts of sensitive and resistant cells.

Table 1. LACTIC DEHYDROGENASE IN TUMOUR CELL EXTRACTS

	Initial Δ absorbance/min (340 mμ)
Sensitive local tumour	0·062
Resistant local tumour	0·058
Sensitive spleen	0·052
Resistant spleen	0·052

Extracts of local tumours have been analysed by starch electrophoresis[4] by Dr. L. A. Costello of Brandeis University and shown to be predominantly the muscle (*M*) type. Heart (*H*) type was barely detectable. M_3H_1, M_2H_2, M_1H_3 were present in trace amounts which decreased in the order named. There was no difference in the pattern obtained from sensitive or resistant cells.

The difference between our results and those of Woodliff may be explained by: (1) different strains of tumour were used; (2) Woodliff used the ascites form of the tumour; (3) the cytochemical technique used by Woodliff to measure lactic dehydrogenase may reveal a difference in staining properties of the cells unrelated to the amount of enzyme present.

We conclude that lactic dehydrogenase in extracts of the drug-sensitive and drug-resistant *L*1210 cells examined is quantitatively and qualitatively the same.

R. L. KISLIUK *
G. ZEL
H. A. WALTER, JUN.
G. F. NORBERT
U. F. HUBLITZ
R. P. D'AMICO

Department of Pharmacology,
Tufts University School of Medicine,
Boston 11, Mass.

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* Leukæmia Society Scholar.

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Distribution of Endogenous and Parenterally Administered Porphyrin in Viable and Necrotic Portions of a Transplantable Tumour

TETRAPHENYLPORPHINESULPHONATE (TPPS) is a synthetic, non-naturally occurring porphine derivative which accumulates to a greater extent in the tumours than in the other tissues of tumour-bearing rats after parenteral administration[1]. Because of its optical and solubility properties it can be differentiated from, and analysed in the presence of, endogenous porphyrin. These properties made possible quantitative study of two aspects of the tumour localization of porphyrin that had been seen grossly with other parenterally administered porphyrine: the greater apparent accumulation in the necrotic portions of the tumours[2,3], and the absence of apparent red fluorescence in some tissues despite high porphyrin concentrations[4].

Quantitative analyses of endogenous porphyrin and TPPS were done by an extraction procedure and spectrophotometric assay developed for this purpose and described previously[1]. This assay utilizes the complete separation of the major absorption peak of TPPS at 438 mμ from that of endogenous porphyrin at 407 mμ in dilute acid. Three zones of the Walker carcinosarcoma 256, grown 18 days, were separated by dissection: (1) sub-capsular tissue, comprising about 2 mm depth from the surface; (2) viable tissue of a homogeneous grey-white colour; (3) non-hæmorrhagic necrotic zone in which small discrete, pearly white areas, identified histologically as regions of necrosis, merge towards the centre of the tumour.

In Table 1 is shown the distribution of endogenous porphyrin in the three zones of control tumours, and of TPPS in tumours from rats that received 5·0 mg TPPS intravenously 24 h before killing. The concentration of TPPS was considerably higher than that of the endogenous porphyrin in all zones of the tumour. TPPS was most highly concentrated in the necrotic area, as was the endogenous porphyrin. TPPS concentration in the viable portion of tumour was also high, while that of the endogenous porphyrin was low, about that found in 12-day-old non-necrotic tumours[5]. The difference between

Table 1. ENDOGENOUS PORPHYRIN AND TPPS IN SUB-CAPSULAR VIABLE AND NECROTIC ZONES OF WALKER 256 CARCINOSARCOMA

		Control (6 animals) mg endogenous porphyrin*/gm net weight	Experimental (6 animals) mg TPPS/g net weight
Sub-capsular	mean ± *S.D.*	5·6 ± 3·3	22·5 ± 5·9
Viable	mean ± *S.D.*	2·3 ± 1·7	34·5 ± 10·6
Necrotic	mean ± *S.D.*	16·0 ± 6·3	52·1 ± 13·8

*Calculated on the basis of $\left(E_{1\,\text{cm.}}^{1\%}\right) = 4\cdot9 \times 10^3$ (ref. 6)

TPPS concentration in viable and sub-capsular zones is not marked.

The finding that the parenterally administered TPPS and the spontaneously appearing endogenous porphyrin were both most concentrated in the necrotic zone suggests that they may both be accumulated from the circulation rather than endogenous porphyrin having arisen *in situ* from broken-down hæm. The high concentration of TPPS and the low concentration of endogenous porphyrin in the viable portion of the tumour represent the major difference in the pattern of their distribution. While there was no significant removal of endogenous porphyrin from the circulation into the viable portion of these tumours, such a mechanism could explain the concentration of parenterally administered hæmatoporphyrin[5], as well as the spontaneous accumulation of natural porphyrins that is known to occur in non-necrotic tumours and in the viable portion of necrotic tumours.

The far greater apparent fluorescence of the necrotic portions seemed out of proportion to the differences in TPPS concentration found on analysis. This is seen in Fig. 1, which shows the appearance under ultra-violet light of a tumour which grew for 24 days. The rat received 5 mg TPPS 24 h before killing. The brightest fluorescence occurred in the discrete, pearly white, necrotic foci that merge towards a central area of hæmorrhagic necrosis in which no fluorescence is apparent. In control tumours of this age a faint red fluorescence was seen in areas of non-hæmorrhagic necrosis.

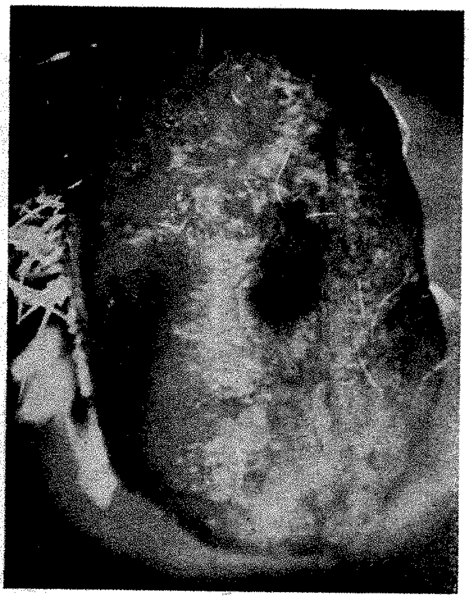

Fig. 1. Black-and-white reproduction of the colour photograph taken under ultra-violet light of the cut surface of the Walker-256 carcinosarcoma. White areas represent red fluorescing areas in the original

We have found that the liver, spleen and kidney exhibit no red fluorescence despite TPPS concentrations as high as 39 μg/g, while much lower concentrations conferred brilliant red fluorescence on tumours, and especially their non-hæmorrhagic areas. The same effect occurred when TPPS was added to whole homogenates and 100,000 × g supernatant solutions of these tissues. Blood was shown to act as a non-fluorescent absorber of the wave-lengths that excite TPPS fluorescence. Decreasing the excitation path-length increased the instrumentally measurable fluorescence, and in the limiting case of zero path-length, quantitative measurement of TPPS can be made even in turbid homogenates of liver, spleen and kidney from the fluorescent emission of the surface of the cuvette[1]. The redder the tissue, the less apparent was the red fluorescence visually. A possible explanation of this is that the filtered ultra-violet light used contained some red light for which the redder tissues have a higher reflectivity than do the less red ones; thus, the observer must make a small intensity discrimination rather than a definite hue discrimination.

These effects may account in part for the emphasis placed on the concentration of porphyrin in necrotic portions of tumours which arose from estimation of relative tissue concentration on the basis of visible red fluorescence.

JAMES WINKELMAN
JOSEPH E. HAYES, JUN.

Laboratory of Technical Development,
National Heart Institute,
National Institutes of Health,
Bethesda 14, Maryland.

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GERONTOLOGY

Age Differences in Choice Reaction Time and Cardio-vascular Status among Pilots

ATTENTION has recently been directed to the conflict of evidence existing in those data on sensory-motor performance in relation to age to which the mathematics of information theory could be reasonably expected to apply in a straightforward manner[1]. Specifically, in the formula for choice reactions[2]: $RT = a + b \log_2 n$ (where n is the number of equivalent choices), the well-known slowing of response with age is sometimes reported as an increase in the constant a, and at other times as a rise in b. In an attempt to resolve these contradictory data on choice, discrimination and movement times, it has been suggested that the effective duration of signals may determine the outcome, producing an increase with age in the intercept when signals are brief, but an increase of the slope when they are relatively long. Whereas the latter result leads logically to interpretations in terms of reduced 'channel capacity' with age, the former has prompted speculations about the possibility of a change in the level of 'neural noise'[3,4]. These ideas derive from the statistical theory of signal detection[5], and as compared with classical psychophysics, their novelty consists in postulating that the brain is a rather 'noisy' communication channel, so that a decision about sensory input is based on information in some way distorted by random neural activity within the central nervous system itself. Hence, the decision mechanism must 'test hypotheses', so to speak, to distinguish between the states of 'noise alone' and 'noise plus signal'. Yet, apart from emphasizing that the latency of a response must be mainly due to the time necessary to collect a statistically reliable sample for a correct decision, it is not clear what properties of the channel are being specified by either type of theory.

Work in progress here may eventually throw some light on this dilemma. Preliminary results, obtained from some four-dozen commercial, military and test pilots, ranging in age from the twenties to the sixties, reveal several interesting trends in performance at a conventional choice reaction task under conditions of what one might call 'information overload'. The aim is to gauge 'spare channel capacity' by the introduction of subsidiary tasks and measurement of their effects on performance of the main task[6,7]. The rationale of this approach is two-fold. In the first place, one must assume that any realistic estimate of the total mental load on the pilot cannot be based solely on observation of what he is doing at any particular moment during the execution of his skill, but must also take into account all other possible decisions which he might have to make at very short notice[8–10] In the second place, it should be borne in mind that the human operator of modern control systems, particularly in aviation, has become progressively less concerned with continuous manual monitoring and more and more concerned with the interpretation of a large variety of signals for action, which may originate from different sources simultaneously or in rapid succession[8,10] In so far as control in this sense must involve transfer of information, the emphasis is then on continuous readiness for quite complex judgements and decisions, many of which have to be made within very restricted time limits if they are not to become antiquated in relation to a rapidly changing display To the extent that a decision preceding any action is the choice of one from a set of alternatives, certain average properties of choices, such as the increase in response latency with increase in input entropy, become relevant in this context, since they must, in some sense, reflect the 'channel capacity', that is, the rate at which the brain can gain information[2]

The basic situation is the familiar one in which the subject is presented with a sequence of signals, visual and auditory, is required to identify them and then to operate some appropriate control with the maximum of speed and minimum of error The inter-signal intervals vary randomly between 0 5 sec and 5 sec, in runs of some 300 in length, each signal having an equal probability of occurrence in ensembles of three, five and eight alternatives, so that at any moment the subject is uncertain which signal is likely to occur and when A detailed record of response latencies, in msec, and of error rates is obtained on the *Setar*[11] and further quantitative analysis carried out with the aid of a Bendix *G*-15 computer The subsidiary tasks utilized are of two main types, with certain variations on each theme

(1) A short-term memory sub-task, which requires the subject to watch a succession of symbols projected on to the centre of the main display, and to recall the symbol presented two stages earlier, single symbols, letters and numbers, or pairs of symbols are used, the presentation lasting in each case some 4 sec, the subject may be asked for recall each time the display changes, or only on certain occasions, on the receipt of a verbal command, also he may be required to carry out both the main and the subsidiary tasks under 4-c/s flicker exposure (this extension constituting an attempt to investigate the possible effects of 'flicker-vertigo'),

(2) The other type of subsidiary task requires the subject, in addition to executing the manual responses in the main task, to 'name' each signal by three very simple code words, describing the successive characteristics of display and control, something approaching a 'stress' situation is imposed by arranging for a delay of 0 18 sec in the auditory monitoring of the subject's speech[12]

In addition, the cardio-vascular system of each individual participating in the investigation is subjected (by Dr R Proper, of the Lovelace Clinic) to a very detailed assessment based on ballistocardiogram stroke volume, plethysmograph and pulse-wave velocity, recorded before and after exercise[13]

It will be seen from Table 1 that, dividing the sample at the age of forty, the average rates of gain of information range from just over five to just over four for the younger group, and from just under five to just under four binary decisions per second for the older group It should be noted that at these rates greater than 95 per cent accuracy is maintained in the basic task, after some preliminary practice, and only about 10 per cent error is made in the subsidiary tasks It is clear from the data that the principal differences between the two age groups are to be found in the values of the constant *a* There is no evidence of any substantial reduction in rate with age until the subjects' maximum capacity is reached under conditions of information overload It is only then, particularly with the short-term memory sub-task, and less so with delayed auditory feedback in the verbal sub-task, that the slope constants show any increase (There is also some evidence in the data, suggestive rather than conclusive that the central refractory phase may be lengthened when the memory task is added to the main choice reaction A suitable technique for more detailed recording is being developed) Even so, the amount of this increase is far from being impressive and basically the curves are displaced upward, changing the intercept *a* Furthermore, amplifying the background stimulation by exposure to intermittent flashes of light when the memory task is attempted seems to reduce the slope, without however, in the case of older subjects, lowering the intercept (attenuating the 'noise'-level?) This finding is in line with some of the results of central refractoriness[14,15]

It is of interest that a trend in the combined cardiovascular and psychological data can also be discerned Ranking the efficiency of cardiac output and the magnitude of reduction in the speed of decision under conditions of information overload, a negative correlation $\tau = 0\ 518$ is obtained, and found to be significant at better than $p = 0\ 001$ level (the normal deviate value is 4 08) (ref 16)

If these essentially preliminary results of an investigation still in progress can be accepted at their face value, they suggest that

(*a*) In a sample of individuals representing the profession of piloting aircraft, the practice of which requires making high-speed decisions, age differences in the rate of gain of information are less impressive than might be expected from other data in the field of gerontology[17] This raises once again the question of occupational transfer effects in investigations of ageing and suggests that investigators should be cautioned to test those primary skills which constitute the over-learned repertoire of adult professional life[18]

(*b*) Pilots over 40 years of age are relatively more susceptible than the younger to the effects of information overload particularly if this involves short-term recall when some other activity intervenes during the period of retention It is not clear, however, whether the conclusion that, to this extent, they may be said to possess less 'spare mental capacity' than the young is really forced on us by the data The paramount difficulty is still that of deciding on an appropriate measure of loss In so far as skill proficiency can be gauged outside the flying situation itself, the routine aspects of their skill are, on the present evidence, unlikely to be affected by ageing However, their responses to sudden emergencies under conditions of information overload may conceivably be expected to become less adequate as they grow older Needless to say, further research is needed to discover the limits within which these assumptions are justifiable

Table 1 REGRESSION ANALYSIS OF INDIVIDUAL PERFORMANCE RECORDS RESPONSE TIME (RT) $= a + b \log_2 n$ MEAN VALUES OF THE INTERCEPT AND SLOPE CONSTANTS PER SUBJECT ($t(b) = t$ TEST ON THE SLOPE CONSTANT)

	Younger Group (Range 20–39, mean age 31 4) $N = 25$	Older Group (Range 40–67, mean age 48 8) $N = 23$
Main Choice Reaction Task Alone	$RT = 0\ 233 + 0\ 090 \log_2 n$ $t(b) = 11\ 27$ Rate $= 5\ 15$ bits/sec	$RT = 0\ 280 + 0\ 085 \log_2 n$ $t(b) = 10\ 26$ Rate $= 4\ 94$ bits/sec
With Short-Term Memory Sub-Task	$RT = 0\ 368 + 0\ 086 \log_2 n$ $t(b) = 4\ 12$ Rate $= 4\ 17$ bits/sec	$RT = 0\ 430 + 0\ 099 \log_2 n$ $t(b) = 4\ 08$ Rate $= 3\ 69$ bits/sec
With Short-Term Memory Sub-Task and Under Flicker Exposure	$RT = 0\ 356 + 0\ 082 \log_2 n$ $t(b) = 4\ 81$ Rate $= 4\ 31$ bits/sec	$RT = 0\ 431 + 0\ 077 \log_2 n$ $t(b) = 4\ 01$ Rate $= 3\ 88$ bits/sec
With Verbal Sub-Task	$RT = 0\ 219 + 0\ 117 \log_2 n$ $t(b) = 11\ 41$ Rate $= 4\ 69$ bits/sec	$RT = 0\ 252 + 0\ 110 \log_2 n$ $t(b) = 9\ 21$ Rate $= 4\ 69$ bits/sec
With Verbal Sub-Task and Delayed Aural Monitoring of Speech	$RT = 0\ 260 + 0\ 105 \log_2 n$ $t(b) = 7\ 80$ Rate $= 4\ 71$ bits/sec	$RT = 0\ 299 + 0\ 117 \log_2 n$ $t(b) = 7\ 78$ Rate $= 4\ 18$ bits/sec

(c) Although the slowing with age, observed in the face of additional information challenge, is equivalent to reducing the rate of transmission of information, it does not unequivocally suggest a reduced capacity for discrimination and choice It may mean, for example, that the forms of encoding and decoding operations performed by the brain are adjusted to the characteristics of the available channel according to the nature of prevailing input and the weight assigned to other inputs Until more plausible theoretical conceptions are brought to bear on the problem at issue, the factor limiting decision and short-term storage processes among the older adults appears to be in the nature of an increased 'noise-level' in the central nervous system Moreover, assuming that the agreement between the findings from psychological and cardiovascular tests is not misleading, it is possible to speculate further that a reduced rate of cerebral blood flow may contribute to the hypothetical random activity in brain cells

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Jacek Szafran

Department of Experimental Psychology,
Lovelace Foundation,
Albuquerque,
New Mexico

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RADIOBIOLOGY

Transmission of Ultra-violet Rays in Quartz Fibres

Ultra-violet rays cannot penetrate deeply into tissues, consequently, investigations of their biological effects have been limited to unicellular forms, cells in tissue culture, and the surface cells of organs and organisms In my apparatus designed for conducting ultra-violet photons to the deeper cells of the brain, penetration of the tissue is achieved with quartz fibres which act as conductors for the energies under consideration

These quartz fibres are made by heating a small section of a clear fused quartz rod 4 mm in diameter in the hottest part of a gas-oxygen flame When the quartz becomes white hot, it is pulled into a fibre as it is moved into the cooler part of the flame The fibre is then broken Each resulting piece of quartz has a large end, which is equal in diameter to the original rod, a tapering portion about 10 mm long, and a terminal fibre 10–40 mm long With a little practice, one can make fibres having tips less than 10μ in diameter The tip of the fibre can be pushed into the brain of a chick embryo with comparatively little disruption of the tissue

The large end of the quartz rod is fixed in a hole in the housing of a low-pressure mercury arc lamp, a source which emits much of its energy at 2537 Å The behaviour of ultra-violet photons is observed by placing the rod and its terminal fibre next to the concave surface of a fragment from an ordinary fluorescent lighting tube The phosphor contained on the inner surface displays a white fluorescence when it absorbs photons in the 2537 Å line Transmission of these photons is demonstrated by placing a filter between the mercury arc source and the large end of the rod, the filter transmits the 2537 Å line plus some blue light Using micromanipulation apparatus, the fibre is brought into contact with the phosphor Under magnification, the blue light visible in the fibre is readily distinguished from the whole light which indicates excitation of the phosphor

Most of the rays which enter the quartz escape from it in the tapering portion; although their angle of incidence on the quartz-air interface is greater than the critical angle for ultra-violet in these media, repeated internal reflexion rapidly reduces this angle of incidence Some of the rays, however, enter the terminal fibre, and are conducted to its tip The emerging cone of energy is fairly well collimated after its passage through the fibre, and its physical effects can be delineated

Hinrichs[1–3] and Hinrichs and Warrick[4] used a pointed quartz rod to direct radiation from a mercury arc against small surface areas of chick embryos and fundulus hearts It is possible that much of the energy conducted in their rods was in the ultra-violet range It must be pointed out, however, that a fair amount of radiant heat is emitted by a mercury arc lamp, such energy is conducted in quartz by internal reflexion, and may have caused the damage observed by Hinrichs and Warrick in the target tissues Using a suitable phosphor as a target, it is possible to demonstrate the transmission of ultra-violet rays in quartz fibres

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J Baskerville Hyde

Department of Anatomy,
Meharry Medical College,
Nashville 8, Tennessee

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Multiple Anomalies occurring in a New-born Spanish Goat following Prenatal Gamma-radiation

A unique and complex combination of abnormalities occurred in a Spanish goat (genus *Capra*) following prenatal irradiation The dam, a vigorous 3-year-old, was subjected to radiation from a cobalt-60 source[1] on the 30th day of pregnancy Dosage was 300 r administered at 100 r /h

Following a normal gestation period of 150 days, the doe gave birth to a female kid weighing 4 3 lb Average weight for irradiated single kids in the flock was 5 2 lb

A number of aberrations were immediately apparent in the animal Functionally the most obvious was the animal's lack of muscular control and inability to rise or stand It was quite vigorous vocally and emitted sounds normal for its species and age The back was held in a rigid straight line, and all limbs were extended backward and parallel with the spinal column The head was drawn downward with the jaw near the chest, which gave a pronounced arched appearance to the neck

Table 1 ELECTROCARDIOGRAPHIC, ELECTROENCEPHALOGRAPHIC RESULTS AND RESPIRATORY RATE OF THREE GOATS

Animal and No	Electrocardiogram			Respiratory rate per min	Electroencephalogram
	Amplitude *QRS* complex (mV)	Amplitude *T* wave (mV)	Heart rate beats per min		
Irradiated abnormal No 27	1 11	0 37	180–240	77	High voltage, 2–4 c/s activity with some secondary waves
Irradiated apparently normal No 25	0 88	0 17	170	55	Low voltage, 8–12 c/s activity
Control No 26	0 66	0 18	170	40	Low voltage, 22–30 c/s activity

A number of morphological defects were apparent, the most obvious being a short, underdeveloped left foreleg and a rudimentary left forefoot There was microcephaly associated with a short upper jaw and a deviation of the mandible to the left There was also a cleft-palate condition and the left ear was shortened The eyes were defective with anophthalmia on the left and microphthalmia on the right associated with a narrow iris In addition to the defects already noted, X-ray observations revealed an underdeveloped left scapula and a prominent dorsal arching of the thoracic spinal column with apex most pronounced at the sixth vertebra

Nourishment of the animal presented a problem For the first three days of life it was held to the mother's nipple, and milk was expressed by hand into its mouth It was apparently unable to suckle because of the cleft palate and deformed mouth A bottle-feeding routine was adopted, utilizing milk obtained from the kid's mother The animal initially lost weight, weighing only 3 8 lb on the 4th day of life Successive weights were as follows 13th day 4 4 lb , 21st day 4 9 lb , and on the 30th day 5 6 lb At six days of age it was able to hold its head erect, and a few days later was able to rise on its hind legs, but could never rise on its hind legs and hold its head erect simultaneously At no time was it able to obtain co-ordinated muscular control The goat preferred to lie on its right side When turned on the left side it usually became very excited and regained right-side posture by violent movements of limbs and head Before death the animal developed a mild diarrhœa It died from pneumonia on the 34th day of life

Clinical and physiological examinations showed rectal temperature slightly above normal, ranging from 104 0° to 104 6° F At 30 days of age the white blood cell count was 12,500/mm^3, the red blood cell count was 13×10^6/mm^3, hæmoglobin was 8 gm/100 ml and hæmatocrit was 25 6 per cent , all these values were within the normal range for young goats Heart rate varied from 180 to 240 per min Respiratory rates varied from 40 to 75 per min The pupil of the microphthalmic eye did not react to light , however, injection of atropine caused wide dilatation after 20 min The eyelids of the absent left eye responded to touch The animal would not respond to acoustic stimuli, but would respond to vibrations such as those produced by persons walking in the area

Recordings of electrocardiograms, electroencephalograms and pneumographs of the abnormal individual plus those of an apparently normal kid (irradiated with 300 r on day 30 *in utero*) and of a control kid (all born on January 2, 1963) show widely dissimilar results (Table 1) Tachycardia, high amplitude of *QRS* complex and *T* wave of the electrocardiogram, and high voltage and slow frequency (2–4 c/s) of electroencephalogram waves were characteristic of responses in the abnormal goat (Table 1) Similar pathophysiological changes have been observed following the administration of massive doses of mixed neutrons and γ-radiation of the goat[2]

Gross necropsy disclosed normal conditions in the thymus, heart, liver, thyroid, parathyroids, spleen, trachea, genito-urinary and alimentary tracts The alveolar membrane and alveoli of the diaphragmatic lobe of the right lung were filled with fluid and cells from a pneumonic condition Dissection of the forelimbs disclosed an underdeveloped left scapula, humerus, radius and ulna, the latter having no articular surface at the carpal extremity, and two fragmentary phalanges Total overall length of forelimbs (eliminating ulna) were left, 19 8 cm, right, 40 4 cm

Lack of critical irradiation data in the goat plus the single abnormal individual in a total of 4 animals born to dams irradiated on the 30th day of pregnancy make difficult the evaluation of results obtained The possibility that these defects were not radiation induced cannot be ruled out, but each of the defects reported have been observed in rodents following acute prenatal irradiation. The results of numerous investigations[3–10] demonstrate that critical periods for induction of almost all irradiation-induced abnormalities in the rodent are short and specific In the rat for example, at 300 r acute X-irradiation, short upper jaw occurs from irradiation on day 10, anophthalmia and microphthalmia on day 11, cleft palate on days 12 to 14 and forefoot reduction on day 13, to cite a few examples of separate stages in which there is little or no overlap of critical periods[5] The animal cited in this report had numerous observed defects following a single 3 h period of irradiation totalling 300 r , the same as for the rats The effective period of irradiation disturbance is reported to be lengthened by raising the dose[4] As the dose in this case was quite large, having produced death in three of 18 pregnant females irradiated at various stages of pregnancy, and is larger than the developing rodent will usually tolerate, it may have contributed to the multiple anomalies The level chosen, however, produced low maternal and fœtal mortality in irradiated swine and cattle[11,12]

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C A O'BRIEN
E W HUPP
J W AUSTIN
M SZABUNIEWICZ
A M SORENSEN, JUN

Departments of Animal Husbandry,
Veterinary Physiology and Pharmacology
and Radiation Biology Laboratory,
A and M College of Texas,
College Station,
Texas

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BIOLOGY

Hydroxyproline as an Inhibitor of Auxin-induced Cell Elongation

AUXIN-INDUCED cell elongation can be inhibited by a variety of substances[1] Among these inhibitors are the amino-acid antagonists canavanine[2], β-2-thienylalanine[3] and ethionine[4,5] This has been taken as evidence that protein synthesis is necessary for cell elongation[6]. Recently, Steward *et al* [7] have presented evidence that hydroxy-L-proline can also be an amino-acid antagonist The growth of carrot callus explants could be blocked by this compound, and the inhibition could be completely reversed with L-proline Since growth in this tissue is largely due to cell division, an investigation has been undertaken into the effects of hydroxyproline on the elongation of *Avena* coleoptile sections in order to assess the effect of this compound on auxin-induced cell elongation

The following experimental procedure was used in this investigation 5-mm sections of 4-day-old *Avena* seedlings were obtained as detailed elsewhere[5] Lots of 10 sections were placed in 25 × 150 mm test-tubes containing 5 ml basal medium (0 0025 M potassium maleate at pH 4 8 + 2 per cent sucrose) When desired the solutions also contained indolyl-3-acetic acid (5 p p m) and hydroxy-L-proline at the desired concentration The tubes were then incubated on a 'Rollerdrum' in the dark After the desired length of time, the tubes were removed and the sections were measured with a microscope fitted with an eyepiece micrometer Each treatment was run in duplicate, each experiment was repeated at least three times

The effect of hydroxyproline on the elongation of *Avena* coleoptile sections during 24 h is shown in Table 1 Auxin-induced elongation was inhibited 50 per cent by 4×10^{-5} M hydroxyproline and almost 100 per cent by 10^{-3} M hydroxyproline The effect of 10^{-3} M hydroxyproline could be almost completely reversed by 10^{-3} M L-proline L-Proline itself has no effect on elongation These results indicate that hydroxy-L-proline is acting as an antagonist of proline metabolism in this system

Table 1 Effect of hydroxyproline on auxin-induced cell elongation All sections incubated 24 h in basal medium + 5 p p m indolyl-3-acetic acid and with L-hydroxyproline or L-proline at the concentration indicated

Concentration (M) L-Hydroxyproline	 L-Proline	% Elongation (24 h)
0	0	100
10^{-5}	0	99
10^{-4}	0	36
10^{-3}	0	20
10^{-3}	10^{-5}	38
10^{-3}	10^{-4}	90
10^{-3}	10^{-3}	97
Control (no auxin)		19

The time curve for the hydroxyproline inhibition is shown in Fig 1 It can be seen that hydroxyproline has no effect on cell elongation during the first 2–3 h Thereafter elongation is severely inhibited This lag in inhibition could be ascribed to two causes First, it might be a measure of the length of time needed for hydroxyproline to exert its inhibition Alternatively, the effect of this inhibition may only manifest itself after a certain amount of auxin-induced elongation has occurred Evidence to favour this latter possibility was obtained in the following manner Sections were placed in the basal medium containing 10^{-3} M hydroxyproline, auxin was added to groups of sections after 0, 6, 9, or 21 h, and the resulting elongation curves were determined It can be seen from Fig 2 that irrespective of the length of the hydroxyproline pretreatment, auxin elicited the same general response In each case sections showed rapid elongation for about 2 h Thereafter the elongation was inhibited such that the final rate of elongation was comparable to that of the non-auxin-treated control sections It is apparent that the ability of these sections to respond to auxin persists for at least 24 h after addition of hydroxyproline The magnitude of the elongation elicited decreased with increasing pretreatment with hydroxyproline However, this decrease is more likely due to the stiffening of the sections which occurs during periods of low elongation than to any specific effect of the hydroxyproline itself[8]

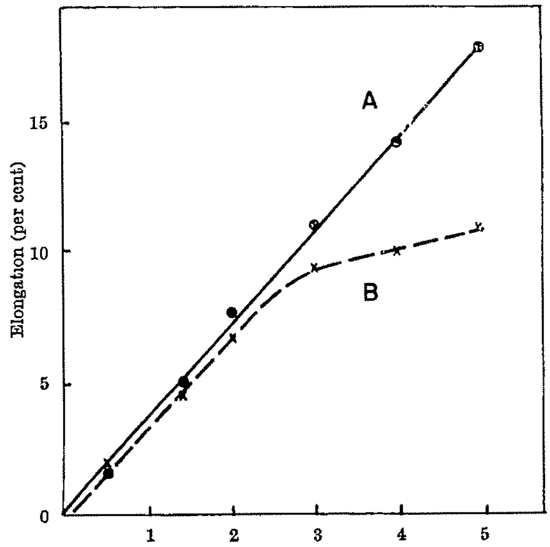

Fig 1 Time course of hydroxyproline inhibition Sections incubated in basal medium + 5 p p m indolyl-3-acetic acid and either with (*B*) or without (*A*) 10^{-3} M L-hydroxyproline

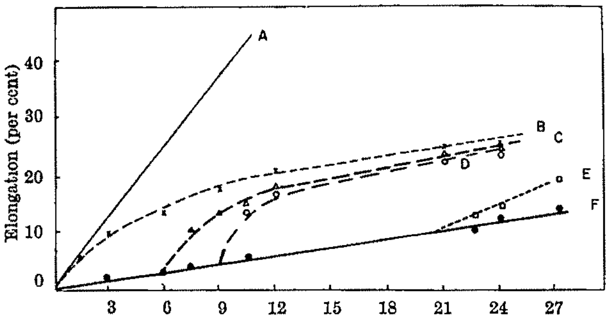

Fig 2 Effect of hydroxyproline pretreatment on auxin response Sections incubated in basal medium + 5 p p m indolyl-3-acetic acid (*A*) or + 10^{-3} M hydroxyproline (*B*–*F*) Indolyl-3-acetic acid (5 p p m) added after 0 h (*B*), 6 h (*C*), 9 h (*D*), 21 h (*E*) or not at all (*F*)

The manner in which hydroxyproline inhibits cell elongation is unknown, but there is ample reason to believe that it must interfere in some way with protein synthesis Other amino-acid antagonists act by either blocking protein synthesis[9] or by causing the formation of aberrant, inactive proteins[10] Furthermore, Steward *et al* [7] have cited evidence that hydroxyproline can prevent the incorporation of proline into proteins

The role of protein synthesis in auxin-induced cell elongation is still uncertain The fact that auxin is still able to induce cell elongation even after sections have been pretreated for 21 h with hydroxyproline suggests that protein synthesis is not required for the initiation of auxin-action Instead, the present results seem to favour the following picture Some product of protein synthesis is necessary for the actual process of rapid cell elongation This product becomes consumed during the cell elongation In normal circumstances it is being replenished by protein synthesis, but in the presence of hydroxyproline no new product is being formed Under these conditions elongation is limited by the amount of this substance In these sections the pool of this substance is sufficient to allow about 15 per cent elongation to occur This product of protein synthesis is stable for periods of up to 24 h This suggests that the substance is a protein itself rather than some secondary product such as ATP. This essential protein may be some enzyme which controls cell wall loosening and which is destroyed in the course of its action. Or it may be some structural cell wall protein, such as that reported by Lamport[11], which must be incorporated into the wall in order that continued elongation may occur. The effect of hydroxyproline on protein synthesis and the existence and nature of the essential product of protein synthesis are now under investigation.

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ROBERT CLELAND

Department of Botany,
University of California,
Berkeley 4.

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Relationship of Relative Leaf Growth Rate to Net Assimilation Rate and its Relevance to the Physiological Analysis of Plant Yield

IN the growing plant, where the photosynthetic tissues make the major contribution to dry-matter production, the absolute growth rate at any time is the product of the rate of increase in weight per unit of leaf (net assimilation rate[1], N.A.R.) and the amount of leaf present. The latter is usually expressed in terms of leaf area, which is the product of leaf weight and the area-to-weight ratio.

The relative rate of increase in leaf weight (relative leaf growth rate—$R.L_W.G.R.$ expressed on a weight basis) may be increased either by an increase in N.A.R. or by an increase in the proportion of the products of assimilation which are devoted towards leaf growth. This does not seem to be widely appreciated, and the proportion of the products of assimilation going towards leaf growth is not generally calculated in growth analysis. This variable can be expressed as the ratio of $R.L_W.G.R.$ to N.A.R. and is most simply calculated for a time-interval t_1-----t_2 as $(L_{t_2} - L_{t_1})/(W_{t_2} - W_{t_1})$, where L refers to leaf weight and W refers to total weight; it is hereafter referred to as the leaf-to-total growth ratio L.T.G.R.

If plant growth is considered as a compound interest phenomenon[2] and the leaf area to leaf weight ratio to be constant, plant weight at any time would depend on the initial productive capital (leaf weight), the rate of interest on this capital (N.A.R. calculated on a leaf weight basis), the proportion of interest re-invested in productive capital (L.T.G.R.) and the period of time involved.

Leaf area/leaf weight is not, however, constant, and since area is probably a more useful measure of the amount of photosynthetic tissue than is weight, plant growth over its entire period must be considered to depend on: (*a*) the initial leaf area; (*b*) N.A.R. calculated on the basis of leaf area; (*c*) L.T.G.R.; (*d*) relationship of leaf area to leaf weight; (*e*) the length of the growing period.

It is clear, therefore, that the yield of crop plants can depend in large measure on the way in which the products of assimilation are allocated, that is, on the L.T.G.R. Thus the supply of nitrogenous fertilizer to cotton plants increases the relative leaf growth rate but not the net assimilation rate[3]; therefore, as Heath[4] pointed out, it increases the proportion of assimilate going to leaf, raising the ratio of leaf weight to total weight and consequently the relative growth rate. This treatment results in a large increase in leaf weight, in total weight, and in economic yield (seed cotton). It is, however, under conditions where the net assimilation rate and the relative leaf growth rate are varying in the same direction that it is of most value to calculate the L.T.G.R. in order to determine how much of the change in relative leaf growth rate and therefore in ultimate leafiness is in fact solely due to change in N.A.R.

The concept of L.T.G.R. is being used in a detailed investigation of the physiological basis of treatment effects on and seasonal variation in the yield of cotton which will be reported in detail elsewhere. This variable has also been calculated by Rees[5] working with oil palm.

JOHN E. JACKSON*

Research Division,
Ministry of Agriculture,
Wad Medani,
Sudan.

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* At present: Smuts Visiting Fellow, School of Agriculture, Cambridge.

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Stomatal Distribution in Relation to Xeromorphy in Aquatic Plants

IT is well known that xeromorphy is of physiological importance[1] to the aquatic plants which exhibit it and certain tissues become altered in relation to environment. The modifications in internal leaf structure include a higher stomatal frequency. Limited enlargements of epidermal cells result in the increase in number of stomata per unit area. Though these are the features of a xerophyte, experimental evidences to show the stomatal distribution in relation to xeromorphy exhibited by an aquatic plant are meagre. In an attempt to elucidate the problem of xeromorphy in aquatic plants the wild species of *Ipomoea carnea* Jacq growing in different ecological habitats, that is, aquatic and xerophytic, was selected. The aquatic plants were selected from those growing at a 3–5 ft. depth of fresh-water in ponds and ditches and the xerophytic plants from those growing in the sandy soils of the plains. Though these plants belong to the same species they are not alike but have become adapted through generations of growth in the particular habitat. We consider that a comparison between the same species which has grown naturally under different ecological habitats might better contribute towards our understanding of the nature of xeromorphy than inducing xeromorphy under artificial conditions. The species considered here is a straggling shrub with milky juice and ovate-cordate leaves with large convolvulaceous pink flowers. The plant grows abundantly both in dry and marshy places besides its aquatic habit in various parts of India.

The healthy branches were selected from the plants growing under different habitats, leaves serially numbered from top to bottom and the stomatal counts were made after the nitric acid method as already described[2]. The area of the leaf was measured with a planimeter. Absolute stomatal number was determined by multiplying the average number of stomata per square mm of leaf area with the square mm area of the leaf. The results are expressed as percentages of that of the aquatic plant in Table 1 with respect to the absolute stomatal number and stomatal distribution per unit area.

The results indicate that when xeromorphy is exhibited by an aquatic plant, the absolute stomatal number increases 3–5-fold. There is a decrease in the ratio of absolute stomatal number between the upper to the lower surface of the leaf, as the leaf matures. A gradual shifting

Table 1. VALUE EXPRESSED AS PERCENTAGES OF THE RESPECTIVE LEAVES OF AQUATIC PLANTS

Leaf No.	Upper	Lower	Ratio upper/lower
(1) Absolute stomatal No.			
1	—	248	—
2	341	346	99
3	387	544	71
4	285	426	67
5	353	403	87
(2) No. of stomata per square mm leaf area			
1	—	148	—
2	138	145	95
3	99	141	70
4	99	120	82
5	106	115	92

in the formation of stomata to the lower leaf surface is a feature associated with xeromorphy. No significant change is observed on the upper leaf surface so far as stomatal distribution per unit area is concerned.

Reduced leaf lamina is a feature of aquatic plants, and in the species reported here the xeromorphic plants of the same aquatic species had four times the leaf area. Though leaf area was increased four times during xeromorphy, stomatal distribution per unit area was not changed on the upper leaf surface. However, there was a small but consistent increase on the lower leaf surface of the upper leaves, the increase being 41–48 per cent with respect to the same leaves of the aquatic plant.

Upper leaves, farther removed from water supply and exposed to high light intensity, are supposed to be more xeromorphic than the lower leaves of the same plant, and the results obtained in the present investigation suggest that xeromorphy when exhibited by an aquatic plant results in: (*a*) an increase in the absolute stomatal number; (*b*) increased stomatal distribution per unit area on the lower surfaces of leaves.

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P. K. MOHANTY
D. MISHRA *

Department of Botany,
G.M. College, Utkal University,
Sambalpur, India.

* Present address: Department of Botany, University of Manitoba, Winnipeg, Canada.

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Occurrence of Indole Compounds containing Phosphorus in Etiolated Pea Seedlings

IN the course of paper chromatographic identification of compounds containing phosphorus-32 in different parts of plants the presence of phosphorus compounds with an indole skeleton seemed probable. To prove this, 10-day-old etiolated pea seedlings were kept for 24 h in the dark, in Knop's solution containing phosphorus-32 as KH_2PO_4 (the activity was 5 µc./ml.), and an alcoholic extract was chromatographed; on the start points of the one- and two-dimensional chromatograms an extract corresponding to 36 g fresh material was dropped. Developing was carried out first in isopropanol/ammonia/water, 10 : 1 : 1, and secondly in butanol/acetic acid/water, 4 : 1 : 5 solvent. The chromatograms were sprayed with Salkowski and Ehrlich reagent and exposed on X-ray film for 8 days. On the chromatograms prepared from the extract of epicotyls, five (Fig. 1), and on those prepared from the extract of roots, six, indole-positive spots appeared (Fig. 2): in both cases two of them proved to be radioactive.

In the case of the epicotyl, one of the two radioactive spots was found to correspond to the location and colour reaction of tryptophan, while in the case of the root to those of indolyl-3-acetic acid (IAA). The second radioactive spot gave in both cases a brick-red colour reaction with Ehrlich reagent.

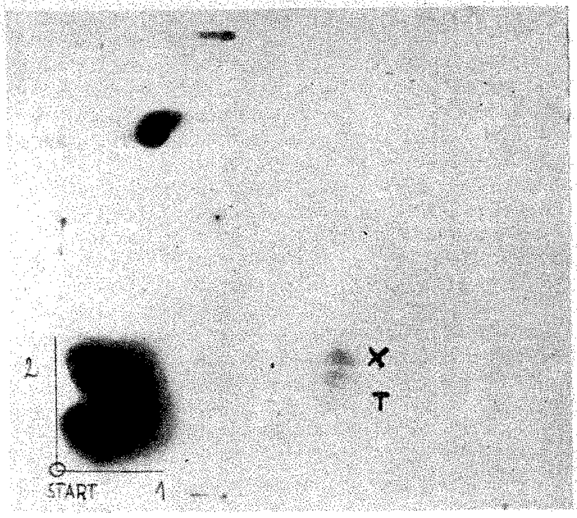

Fig. 1. Radiogram of compounds containing phosphorus-32 soluble in alcohol in the epicotyl of the etiolated pea. *T*, Tryptophan; *X*, unknown indole compound

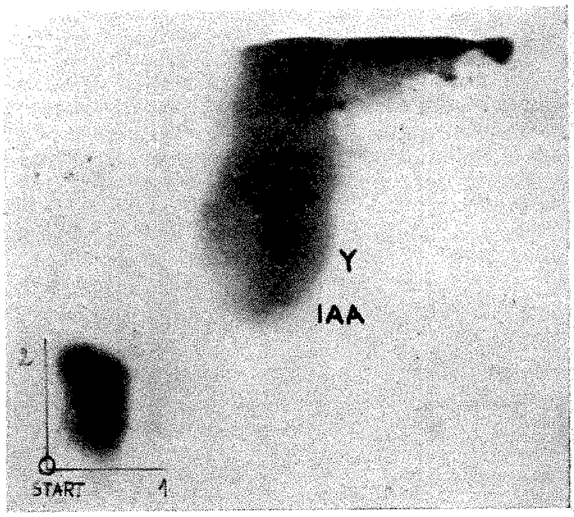

Fig. 2. Radiogram of compounds containing phosphorus-32 soluble in alcohol in the root of the pea. *IAA*, Indolyl-3-acetic acid; *Y*, unknown indole compound

Investigations are now being carried out to elucidate the structure and physiological importance of these compounds.

It may be assumed that the compounds involved are partly intermediary products of the biogenesis of IAA and the indole skeleton respectively[1,2] and, on the other hand, one of them is a compound of IAA formed with phosphoric acid playing a part in the growth regulator system in the plants. Our results, that an IAA derivative containing phosphorus is present in growing plants, is in accordance with the assumption of Rhodes and Ashworth[3] whereby the primary effect of IAA is based on the formation of energy-rich phosphate bonds.

ELIZABETH KÖVES

Institute of Plant Physiology,

F. SIROKMÁN

Central Isotopic Laboratory,
University of Szeged,
Hungary.

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Cytoplasmic Dimorphism within Pollen Grains

MATURE pollen grains contain a vegetative nucleus and either a generative nucleus or two sperm nuclei (derived by mitotic division of the generative nucleus). It has been generally accepted that the generative nucleus has an associated cytoplasm containing basic cell organelles[1,2] and is separated from the vegetative cell cytoplasm by plasma membranes. While Venema and Koopmans[3] have recently reported that a phase-contrast study of germinating pollen yielded no evidence for the 'bicellular'[4] nature of pollen grains, confirmation has been achieved by Safijovska[2] utilizing light microscopy, and in a definitive manner in the electron microscopic examination reported by Bopp-Hassenkamp[5]. In addition to demonstrating the presence of a generative cell, the investigation by Bopp-Hassenkamp indicated that vegetative and generative cell cytoplasms differ in relative organelle numbers, and to a degree in organelle morphologies as seen in profile. Safijovska has observed that generative cell cytoplasm is longer-lived than vegetative cell cytoplasm in both aged pollen tubes and following pollen tube rupture. A comparative investigation of the fine structure of pollen cytoplasm now being conducted by me has made possible the observation of generative cells within pollen grains of a number of plant species.

Mature pollen of *Quercus virginiana*, *Aquilegia* sp., *Ranunculus macranthus*, *Prosopis glandulosa*, *Tradescantia paludosa*, *Parkinsonia aculeata*, *Hippeastrum belladonna*, and *Ambrosia psilostachya*, have been examined in the electron microscope. In addition, developmental stages of *Parkinsonia aculeata* and germinated pollen of *P. aculeata* and *Ranunculus macranthus* have been observed.

The fixative for electron microscopy was predominantly $KMnO_4$, though $Sr(MnO_4)_2$, $NaMnO_4$, and OsO_4 have also been used. Epoxy resins have been used as the embedding matrix and observations following diamond knife sectioning were made in Radio Corporation of America electron microscope models *EMU-3-D* and *EMU-3-F*.

In every species examined, the generative nucleus has had an associated cytoplasm separated from the vegetative cytoplasm by paired plasma membranes (Fig. 1). In *Ambrosia*, which is tri-nucleate at pollen maturity, sperm cells exist with obvious plasma membranes, and sperms in pollen tubes of *R. macranthus* also exist as cells. Between the paired plasma membranes is an area of variable width (in fixed grains) which is devoid of hyaloplasm but which does have a number of minute vesicles. No complete intercellular connexions have been observed and the mobility and highly plastic nature of the generative cell argues against the presence of such connexions.

No evidence of a cell wall has been observed, though cell walls are obvious in electron micrographs of multicellular mature cycad pollen. The pleomorphic nature of the generative cell is best observed during pollen tube growth, but it can also be seen in fixed specimens when the generative cell had progressed into the pollen tube prior to fixation, or when 'frozen' in the process of flowing out a ruptured pollen grain.

In mature grains of every species studied, the cytoplasm of the generative cell differs markedly from that of the vegetative cell (in *Parkinsonia aculeata*, the differentiation of the two cytoplasms out of a uniform common cytoplasm begins immediately after the establishment of the two cells following the first microspore mitosis). Every cell organelle and the hyaloplasm, or ground substance, is found to differ in fixed specimens. Mitochondria are fewer in number per unit area, have smaller dimensions in profile and have fewer cristæ ; distribution of mitochondria is generally random. Plastids were identified in a majority of the species and are most clearly seen in *Hippeastrum belladonna*. In general, generative cell plastids are difficult to identify because of their smaller dimensions, paucity of internal membranes, and because they do not contain stored starch; in *Ranunculus macranthus*, and others in which amyloplasts occur in the vegetative cell, starch is definitely absent from mature generative cells. Generative cell plastids appear to be rare and the average section does not contain a plastid profile.

Golgi structures are a normal element of the generative cell ; but they, too, are fewer in number, have fewer cisternæ, and do not have nearly the number of associated vesicles that the vegetative cell Golgi structures do. Free vesicles, abundant in vegetative cell cytoplasm, are infrequent in the generative cell. During pollen tube growth, Golgi structures in the vegetative cell produce numerous large vesicles while the Golgi structures in the generative cell remain inactive. The large vesicles produced by the Golgi of the vegetative cell are associated with pollen tube wall synthesis[6].

The endoplasmic reticulum in the generative cell is significantly reduced in amount. Especially noteworthy is the reduced membrane spacing in generative cells, while the endoplasmic reticulum of vegetative cells has a wide membrane spacing. Differences in hyaloplasm densities between the two pollen cells occur in each species, but the appearance is not consistent, being either of greater or lesser density in the generative cell. Inclusions often identified in the literature as lipid or protein bodies are common in vegetative cells ; they have only been observed in generative cells of *Hippeastrum*. Not uncommonly, the vegetative nucleus is polymorphic and appears to be degenerative. Venema and Koopmans[3], among others, have reported degeneration of the vegetative nuclei of some species prior to or during pollen germination.

These observations of cytoplasmic fine structure are consistent with the limited results of Bopp-Hassenkamp and in general detail with earlier optical studies. The taxonomic distribution of the species studied so far permits one to state a tentative generalization, namely, that the generative and sperm cells have a cytoplasm distinct from that of pollen vegetative cells and that the appearance of generative cell cytoplasm is one of reduced metabolic potential. Fischberg and Blackler[7] have suggested that, in animal development, germ plasm serves to protect germ nuclei from developmental modifications. It is possible that generative and sperm cell cytoplasm functions in like manner. Safijovska's observation on generative cell longevity and the fact that during pollen germination generative cell cytoplasm does not show the increased activity that vegetative cell cytoplasm exhibits suggests a decreased environmental sensitivity.

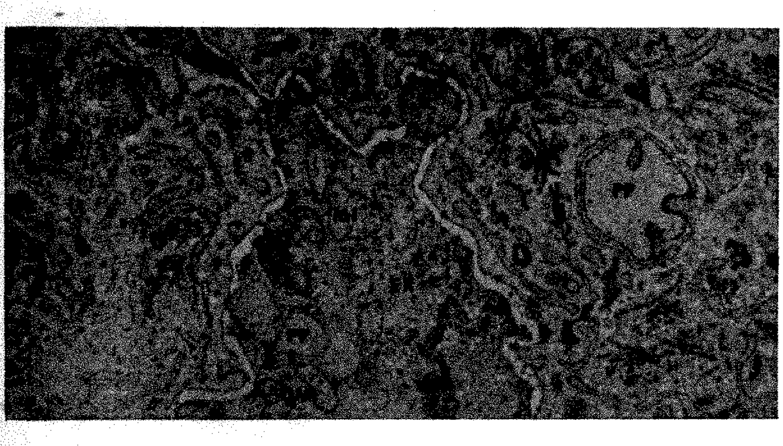

Fig. 1. Generative cell (centre of figure) in mature pollen grain of *Hippeastrum belladonna*. *MI*, mitochondrion; *ER*, endoplasmic reticulum; *GA*, Golgi apparatus; *PP*, proplastid; *CM*, cell membrane; *I*, inclusion. (Permanganate fixed. × *c*. 10,200)

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Donald A. Larson

Department of Botany and Plant Research Institute,
University of Texas, Austin 12.

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Effects of 2-Chloroethyltrimethylammonium Chloride and 2,4-Dichlorobenzyltributyl Phosphonium Chloride on Growth and Transpiration of Slash Pine

The growth of many plants, including several tree species, can be retarded by CCC (2-chloroethyltrimethylammonium chloride) and 'Phosfon-D' (2,4-dichlorobenzyltributyl phosphonium chloride)[1–5]. In addition, CCC applied as a soil drench has led to increased flowering in some plants[6–8].

A small lath-house experiment, designed to test the effects of these compounds on 1-year-old potted slash pine (*Pinus elliottii* var. *elliottii*) seedlings, was installed at Olustee, Florida, in February 1962. Treatments consisted of: (1) CCC soil drench at rates of 0, 5, 10, 20 and 40 g/ft.^{3} potting soil; (2) 'Phosfon-D' soil drench at rates of 0, 0·4, 0·8, 1·6, and 3·2 g of technical material per cubic foot; (3) CCC foliar spray at concentrations of 0, 2,000, 4,000, 8,000 and 16,000 p.p.m.; and (4) CCC foliar spray at the above concentrations plus 0·15 per cent wetting agent (Fasco 'Spreadhesit'). Soil drench applications were made in February, April, May, and July 1962. Foliar sprays were applied with a hand-operated 'flit gun' twice weekly, from February to September 1962. The experiment was set up in a randomized block design, replicated six times. Height measurements to the nearest 0·1 cm were taken at 2-week intervals throughout the growing season.

'Phosfon-D' concentrations were apparently too low to affect height growth of the seedlings, although it is possible that slash pine does not respond to this compound. Since 'Phosfon-D' is reported to be highly persistent in the soil, concentrations of 1·2, 2·4, 4·8 and 9·6 g per cubic foot should have existed in the soil by July. Cathey[2] has observed that approximately 25 times as much CCC as 'Phosfon-D' is required to retard the height growth of chrysanthemums. Nevertheless, complete lack of toxicity or other symptoms at the highest level of 'Phosfon-D' indicates that the dosages were too low.

CCC applied as a foliar spray, either with or without a wetting agent, significantly depressed seedling height growth. However, extensive damage to foliage (needles stunted and chlorotic) and developing shoots resulted at the two highest concentrations (8,000 and 16,000 p.p.m.), to the extent that this form of application is considered impractical.

The treatment showing most promise was the application of CCC as a soil drench (Table 1). Growth of trees receiving 40 g/CCC/ft.^{3} soil became significantly less than check tree growth by the 10th week of treatment, levelling off at approximately 35 per cent of check tree growth from the 20th week on. Treatment effects were significant at the 0·005 level, with seedling height growth decreasing linearly with the logarithm of concentration. There was no apparent damage to the foliage.

Table 1. Effect of CCC Soil Drench on Total Seedling Height Growth

Concentration (g/ft.^{3} soil)	Average height growth (cm)	Percentage of check tree growth
0	28·8	100·0
5	25·9	89·8
10	29·0	100·7
20	18·6	64·6
40	18·6	64·6

Table 2. Effects of CCC Soil Drench on Seedling Transpiration (72-h Weight Loss in g)

Concentration (g/ft.^{3} soil)	Average 72-h weight-loss (g)	Percentage of check tree loss
0	409·2	100·0
5	235·8	57·6
10	177·5	43·4
20	90·8	22·2
40	85·8	21·0

It was noted early in the course of the experiment that soil in pots receiving CCC as a soil drench remained moist when other pots were relatively dry. On June 4, 1962, the potting soil was saturated with tapwater and allowed to drain overnight. On June 5, all pots were enclosed in polyethylene bags and weighed. Subsequent weighings were made on June 6, 7 and 8. Analysis of the data (total 72-h weight-loss) revealed a reduction, significant at the 0·005 level, in transpiration of seedlings receiving CCC either as a soil drench or foliar spray (Table 2). Response, as in the case of height growth, was linearly related to the logarithm of concentration.

As in the case of height growth, reduced transpiration of seedlings receiving CCC as a crown spray was difficult to assess because of foliar damage. The response to soil application is more clear-cut, since foliar damage is entirely absent. Height of the tree was considered as a possible contributing factor, but only a slight correlation ($r = -0{\cdot}055$) was found between total height of the check seedlings at the time of initial weighing and 72-h weight-loss.

After soil application of CCC at the two highest levels (20 and 40 g/ft.^{3} soil), needles on treated trees were noticeably shorter than check tree needles. Fascicles from three different vertical positions on the current season's leader growth were clipped off and measured. Comparison of the needle-lengths of check trees with those from trees treated with the two high-level soil applications of CCC showed a significant difference ($t = 5{\cdot}132$, $P < 0{\cdot}001$). However, that needle length *per se* cannot account for the observed reduction in transpiration of treated seedlings is evidenced by the fact that the correlation between average needle length of check trees and 72-h weight-loss is not significant ($r = 0{\cdot}146$). One can only conclude that: (1) the observed reduction in transpiration is not due solely to reduced length of needle or size of tree; (2) the reduction is not due to blocking of water movement into the tree stem, since wilting does not occur. Possible factors are a reduction in stomatal opening due to the treatment, either directly or via reduced photosynthetic rate, reduced water utilization in the treated trees, and retarded root development.

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William C. Asher

Southeastern Forest
Experiment Station,
Forest Service,
U.S. Department of Agriculture,
Asheville,
North Carolina.

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Diffusion Chambers for the Collection of Helminth Antigens

In comparing the immunological responsiveness, in relation to a particular nematode, of old-established hosts on one hand and newly established hosts on the other[1], it was necessary to obtain an antigen which could be presumed to have been immunologically active over evolutionary time, that is, a natural product of normal infection. It was therefore essential to collect the excretions and secretions of worms living under conditions resembling as nearly as possible the natural environment, in this instance the liver and abdominal cavity of rats.

Recourse was made to diffusion chambers for this purpose, the principle involved being to transfer third-stage larvæ from the original host to the diffusion chamber, which was then inserted into the abdominal cavity of a recipient host. Larvæ removed from the donor 5 weeks–6 months after experimental infection were observed to remain alive in the capsules for at least a week.

The technique involved removal of 10–15 worms from the abdominal cavity under aseptic conditions and their insertion into the capsule, which was then inserted into the peritoneal cavity, which had been opened by midline incision. The recipient rats were given intramuscular benethamine penicillin (10,000 I.U.) and 0·03 g streptomycin sulphate after the insertion of the capsule, which was left *in situ* for 7–10 days, then removed and opened. The contents of the capsule were washed out with about 1 c.c. of sterile distilled water and, after separation of the worms, the fluid was centrifuged and used as antigen.

The diffusion chambers were made from three types of membrane, each with a different pore size. The first type (*DC*1) was made from size 8/32 Visking dialysis tubing (Visking Co., Chicago). About 18 cm of the tubing was cut and sterilized by placing in 1 per cent 'Cetavlon–Hibitane' solution (Imperial Chemical Industries, Ltd.) for 48 h before use. One end was tied off and the worms were inserted into the open end with about 3 c.c. of sterile physiological saline. The other end was then tied off and both ends carefully trimmed, so as to leave as little rough edge as possible.

The other capsules (*DC*2 and *DC*3) were made from membranes prepared by 'Membranfilter', Göttingen, Germany. Two 50-mm membranes were used to form each capsule. The two sizes used were 'Membranfilter Gruppe 5' (*DC*2) and 'Ultrafein Filter' (*DC*3). These capsules were prepared by means of a carpenter's 'G' clamp and two stainless cylindrical moulds 29 mm diam. and 19 mm thick (Fig. 1). On one of the flat sides of each mould was a depression 25 mm diam. and 9 mm depth. One 50-mm circular sheet of membrane was placed over the mould with the depression uppermost. The worms in sterile physiological saline were placed on the membrane, which was pushed into the depression on the mould. Another membrane was placed over the worms and the second mould, with depression downwards, was placed over this membrane. The two moulds were placed in the clamp and tightly opposed. The opposing membrane edges, protruding outside the moulds, were then joined with acetone, trimmed, and after a few seconds for drying, they were removed from between the moulds by loosening the clamp. After washing in sterile distilled water, the chamber was now ready for insertion.

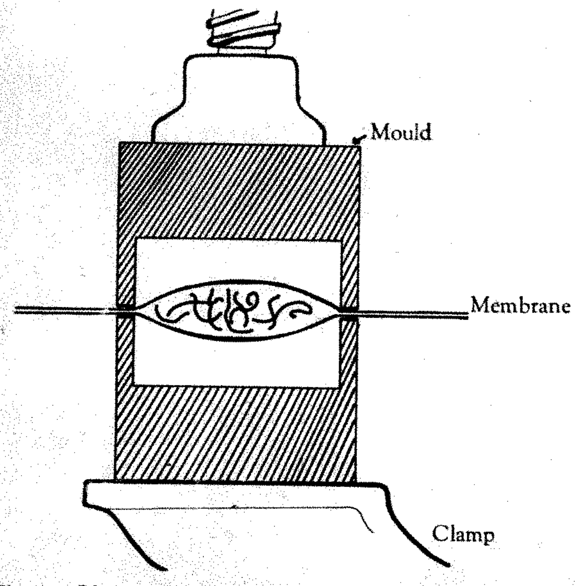

Fig. 1. Diagram showing preparation of capsules *DC*2 and *DC*3

The fluid obtained from the different chambers (*DC*1, *DC*2 and *DC*3) was tested by the complement fixation reaction and by gel-diffusion against sera from infected rabbits and rats, as well as against normal serum, and against the serum of rats after harbouring the capsules. The fluid from each disk gave positive complement fixation, and strong Ouchterlony gel diffusion lines with infected rabbit sera. With infected rat sera no complement fixation occurred, the serum showing anticomplementary activities, but gel-diffusion lines were strongly developed. No reaction occurred with normal rabbit and rat sera, or with the sera of rats harbouring the capsules.

The contents of the three types of capsule varied in reactivity with infected rabbit serum, that obtained from capsule *DC*1 showing a higher complement fixation titre than that from capsules *DC*2 and *DC*3.

J. F. A. Sprent
R. S. Hogarth Scott
H. Timourian

Department of Parasitology,
University of Queensland,
St. Lucia, Brisbane.

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ENTOMOLOGY

Histology and Possible Functions of Neurosecretory Cells in the Ventral Ganglia of *Schistocerca gregaria* Forsk.

Although neurosecretory cells were shown to exist in the ventral ganglia of insects as early as 1940[1], students of neurosecretion in the group have concentrated on the brain and rather neglected the ventral nerve-cord. This neglect prompted me in 1959 to begin studying the ventral ganglia of *Schistocerca gregaria* (Orthoptera : Acrididae) in some detail, and though, since starting the work, three papers on the histology of the ventral ganglia of other insects have appeared[2–4], a summary of the rather different conditions in *Schistocerca* seems desirable.

All four main types of neurosecretory cells found in insects and often denoted, for example by Highnam[5], as *A*, *B*, *C* and *D* cells have been shown to occur in the ventral ganglia of *S. gregaria* (Fig. 1). The *B* cells are not phloxinophil, however, and therefore not identical with the *B* cells of the pars intercerebralis of the brain[5]. The *B*, *C* and *D* cells occur in all the ventral ganglia, but their number and position vary from one insect to another. The *A* cells are constant in number and location, and occur in all ventral ganglia except the last abdominal one. Neurosecretory cells are absent from the frontal, hypocerebral and ingluvial ganglia of the stomatogastric nervous system.

The *A* cells may be divided into three sub-types : *A*1, *A*2 and *A*3 cells. The *A*1 cells are peculiar in undergoing great changes in volume during sexual maturation of the female, while the *A*2 cells are unique in that their secretory material appears in Azan-stained sections as large droplets which are coloured brilliant red with azocarmine. Both are thus easily distinguished from the *A*3 cells which show neither of these characteristics. *A*1 cells occur in all the ventral ganglia except the last ; there are 2 in each ganglion except for the third (compound) thoracic ganglion, which has 6 arranged in three pairs, apparently derived from the three primitive abdominal ganglia which have fused with the metathoracic ganglion to form the compound structure. *A*2 cells are found in the third thoracic and first four abdominal ganglia ; there are 4 cells in each abdominal ganglion and this pattern is repeated three times in the posterior end of the third thoracic ganglion. The *A*3 cells, of which there are 2, lie close together one on each side of the mid-ventral line of the sub-œsophageal ganglion. The *B* cells fall into two sub-types : *B*2 cells stain with aniline blue in Azan preparations, while *B*1 cells do not. The *A* cells undergo secretory changes correlated with maturation and oviposition in the female ; no such changes have been demonstrated for the *B*, *C* and *D* cells. On histological grounds, the *C* and *D* cells appear to be different stages in the secretory cycle of a single functional type of cell.

Axonal transport of neurosecretory material was studied by ligaturing experiments and by nerve section. Neurosecretory material passes backwards and forwards along the double ventral nerve cord, outwards along at least some of the nerves originating from the ganglia, as well as along the axonal tracts from the neurosecretory cells of the pars intercerebralis to the corpora cardiaca. No such transport has been demonstrated along the circum-œsophageal connectives. Neurosecretory cells whose axons have been cut contain after five days less neurosecretory material than those with intact axons. Evidence from ligation of the axonal routes of neurosecretory cells suggests that, in the ventral ganglia, cells packed with neurosecretory material may be actively secreting and those with little such material are quiescent. The same conclusion on secretory dynamics seems to be supported by preliminary autoradiographic investigations on the *A*3 cells of the sub-œsophageal ganglion, using ^{35}S-DL-cystine.

Students of reproductive physiology have in the past correlated the process of egg maturation with the neurosecretory material derived from the *A* cells of the brain[6,7] and the hormones secreted by the corpora allata[8,9]. In the work recorded here it was found that extirpation of the last abdominal ganglion in newly emerged *Schistocerca* prevents egg development ; that implantation of the ganglion into a locust the ganglion of which had previously been extirpated results in egg development if the implantation is done after about 2–3 weeks following the extirpation, and if the donor was about the same age as the recipient ; and that the complete nervous isolation of the ganglion by section of the ventral connectives and the peripheral nerves did not prevent egg maturation. The desert locust normally matures after about 3 or 4 weeks of adult life, and the first egg pod is not normally laid before the age of 4 weeks[10]. But when either the last abdominal ganglion or the corpus cardiacum of a mature female is implanted into a newly emerged female, the latter begins to copulate after about a week and lays the first egg pod on the 10th day. Similar results are obtained when both the ganglion and the gland from a mature female are implanted together into a newly emerged female ; that is, maturation is not accelerated further by a double implant. Controls consisting of locusts with cuticular incisions matured in 3 or 4 weeks, as did normal locusts. These findings support the view that a hormonal factor, in the provision of which the last abdominal ganglion is concerned, helps to control egg maturation, though the source of such a hormone does not seem to lie in any of the neurosecretory cells found in this last ganglion by the above histological study.

Examination of the neurosecretory system of locusts which had been flown for periods of 4–58 h on a simple motorized flight roundabout showed that there had been a heavy discharge of neurosecretory material from the third thoracic and all the abdominal ganglia, and no discharge in the sub-œsophageal and first two thoracic ganglia. This discharge is therefore not likely to be due to 'stress'[11] since under such conditions one would imagine that the neurosecretory cells of the anterior ganglia would also discharge their contents. Furthermore, the distribution of the *A*2 cells in the posterior ganglia would seem to suggest that it is these cells which discharge during flight.

A series of simple dehydration experiments tend to suggest that the discharge of neurosecretory material from the *A*2 cells of the ventral ganglia is also correlated with water loss. Forty newly emerged females were separated into four groups of 10 each. One group was reared on bran alone, a second without food or water (that is, starved), a third on water alone, and a fourth on both food and water. Examination of the neurosecretory system after 14 days showed that there was a heavy discharge of material from the third thoracic and abdom-

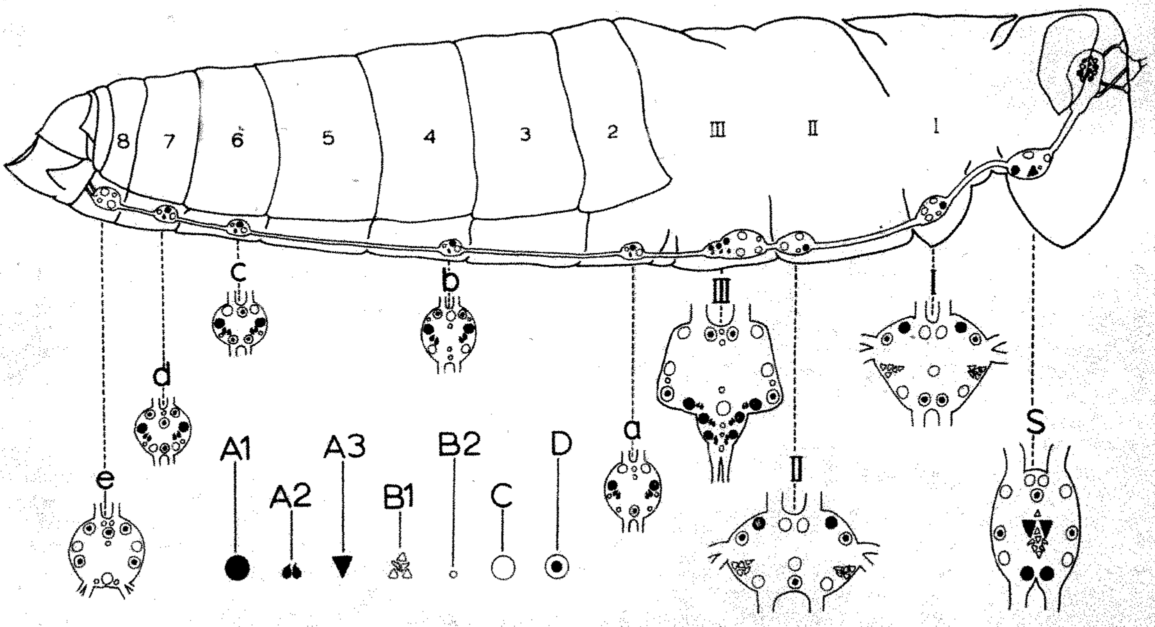

Fig. 1. Distribution of neurosecretory cells in the ventral ganglia of *Schistocerca gregaria* Forsk. *S*, sub-œsophageal ganglion ; I, II, III, thoracic ganglia ; *a* – *e*, abdominal ganglia

inal ganglia of locusts reared without water, similar to the results obtained in the flight experiments, whereas no discharge occurred in locusts with access to water. Neurosecretory discharge under these conditions has no parallel in the literature, and it is tentatively suggested that the *A*2 neurosecretory cells of the abdominal ganglia secrete an antidiuretic hormone[12] which is released under conditions in which water conservation might be necessary.

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F. Delphin*

Department of Zoology,
Imperial College of Science and Technology,
London, S.W.7.

* Present address: Department of Biology, University of Mandalay, Burma.

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Transmission by Filariasis in the Philippine Islands by *Anopheles minimus flavirostris* Ludlow

The highest endemicity of filariasis in the Philippine Islands occurs in the extensive abaca-growing areas of the Bicol Peninsula, Samar, Leyte and Mindanao[1]. Here the principal vector is the plant-cavity breeding *Aedes* (*Finlaya*) *poicilius* (Theobald), which is able to exist in dense populations because the abundant rainfall keeps the leaf axils of the abaca trees filled with water[1]. In our survey of 1954–55 (ref. 2), we showed a general correlation between the presence of abaca and filarial endemicity, but there were two notable exceptions. One of these was in the northern mountain province of Luzon. Through the co-operation of the Malaria Eradication Unit stationed at Tabuk, we received a sample of night blood films which showed a 29 per cent microfilaræmia-rate.

At that time we were unable to inspect the area from which the smears were reported to have been taken. The purpose of the work recorded here was to confirm the presence of filariasis among the indigenous, non-immigrant population, and to ascertain the environmental factors which would permit the disease to exist in this improbable area.

Our study area includes the barrio of Calaccad, some 15 miles south of Tabuk; from which it is accessible only by foot trails. The Kalinga tribespeople live in isolated, single family units, or in groups of 2 to about 6 families. These single houses, or groups of houses, are half a mile or more apart. Most of the dwellings are situated near small streams, which are heavily wooded with bamboo and deciduous trees. In Calaccad the forests are being depleted rapidly by dry season burning for rice cultivation. A few large trees remain in the relict forest patches.

Our night blood film survey during the past dry season showed an overall microfilaræmia-rate of 10·7 per cent in this diffuse population at Calaccad. There was a tendency for positive cases to be grouped, so that in several of the tiny settlements or 'sitios', of from 8 or 9 to 25 people examined, we obtained infection-rates up to 32 per cent.

The houses are built several feet above the ground on posts; the walls are made of split bamboo or sawali, and the roofs are thatched with cogon-grass. Almost all have been sprayed with DDT for the past 10 years. We have been unable to capture more than an occasional mosquito inside the houses. In order to obtain large numbers for dissection, we have made use of a large screened trap baited with a carabao. This trap was set up in the vicinity of a house in which 4 of the 7 inhabitants are infected with periodic *Wuchereria bancrofti*. Although most mosquitoes attracted to a carabao-baited trap are zoophilic, it seemed to us reasonable to assume that some of the insects which had previously fed on one of the human cases would be seeking their later meals from a carabao. It must also be understood that a large proportion of the mosquitoes caught in such a trap would be taking blood for the first time. Therefore, one could not anticipate a high infection-rate. The collections were made early in the morning, and the dissections were completed during the following day or two.

Apart from *Aedes vexans*, the only persistent man-biting species which also occurred regularly in the trap was *Anopheles minimus flavirostris*. Of 207 *flavirostris* examined so far, 4 have been found infected with filarial worms. In one there was a single second-stage larva in the thoracic muscles. Two of the specimens each contained 3 mature third-stage infective larvæ. In one the larvæ were in the thorax, and in the other they were in the head. In the fourth specimen, there were 2 sausage-stage larvæ in the thorax and a highly active motile infective larva in the proboscis. All the mature larvæ agreed in morphology with published descriptions of *Wuchereria bancrofti*. Also they were identical with *W. bancrofti* larvæ we have observed in mosquitoes in our previous investigations. We are offering these findings as evidence that in this area, where small foothill streams furnish an ideal environment for *A. minimus flavirostris*, this species is a natural vector of *Wuchereria bancrofti*.

This investigation was conducted under the auspices of the Commission on Parasitic Diseases of the Armed Forces Epidemiological Board, and was supported in part by the Surgeon General, Department of the U.S. Army.

Lloyd E. Rozeboom

Laboratories of Medical Entomology,
Department of Pathobiology,
School of Hygiene and Public Health,
Johns Hopkins University.

Benjamin D. Cabrera

Department of Parasitology,
Institute of Hygiene,
University of the Philippines.

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CYTOLOGY

A Cytochemical Method for Electron Microscopy

Methods for specifically staining tissue constituents for electron microscopy are at present inadequate for the identification of important molecular species in the cell. The range of application of the electron microscope in biology is thereby severely limited.

It occurred to us that more specific stains might be prepared by complexing heavy metal atoms with enzymes of high substrate specificity. It was hypothesized that, even though the activity of the enzyme might be impaired by the presence of the metal atoms, substrate-bonding specificity might be retained sufficiently to bring about localization of heavy atoms at the substrate locus in the cell.

This communication describes preliminary experiments which tend to bear out this hypothesis.

The substrate and enzyme selected for the first experiments were DNA and DNase. The DNase was treated by a procedure believed likely to result in the formation of a mercuric–DNase complex. A solution of the enzyme (Sigma Biochemical Co., Grade *DN-C*) at a concentration of 1 mg/ml. in deionized water was mixed with nine volumes of a 0·005 M solution of mercuric chloride in 0·05 M pH 7·4 glycylglycine buffer at 5° C. The mixture was dialysed against cold glycylglycine buffer for three days to remove the non-enzyme-bound mercuric ion. The activity of the treated enzyme after dialysis was determined to be a small fraction of the original activity.

Although it seemed unlikely that after fixation, dehydration and embedding, a cellular substrate such as DNA would be in sufficiently native condition to interact with enzyme, it was decided to make a staining trial under these conditions because of the technical advantages to be realized in the event of it working. The dialysed mercuric enzyme solution was therefore used directly as a stain on thin sections of tissues embedded in 'Araldite' resin. The sections, adherent to 500-mesh copper specimen grids, were immersed in the mercuric-enzyme solution at room temperature for 5 min, rinsed vigorously with distilled water, and examined in the electron microscope.

Various cells of neural origin were used as test specimens. These included : human meningioma cells (in tissue culture for 37 days prior to fixation) ; re-aggregates of 7-day chick neural retinal cells; neurones from the earthworm nerve cord; and neurones and associated glial cells from hamster spinal sympathetic ganglia.

The test specimens had been fixed by several different procedures. Isotonic 0·6 per cent permanganate fixation at physiological pH had been used for most tissues ; some permanganate-fixed tissues had been intensified after sectioning with a potassium hydroxide-complexed potassium plumbate solution (our method, not yet published) ; the embryonal neural retinal re-aggregates had been fixed in an isotonic buffered osmic acid solution. All specimens had been dehydrated in graded acetone solutions and embedded by transfer from 100 per cent acetone into the final embedding mixture at 55° C. No influence of the method of tissue preparation was apparent in results obtained with the enzymatic staining procedure.

All staining trials were positive in that, after immersion in the mercuric–DNase solution, the nuclei of the cells, and only the nuclei, showed areas of increased electron density which were unlike any figures seen in sections not thus treated. The shapes of the stained regions of the nuclei were in all cases more or less fibrillar. In certain kinds of cells including embryonal neurones, Schwann cells, and meningioma cells, the darkened regions were relatively condensed and had high contrast. In neurones of the sympathetic ganglia, the contrast of the stained regions was relatively low, but the resolution of strands was comparatively good. Suggestions of a banded structure were not infrequently present in the fibrils.

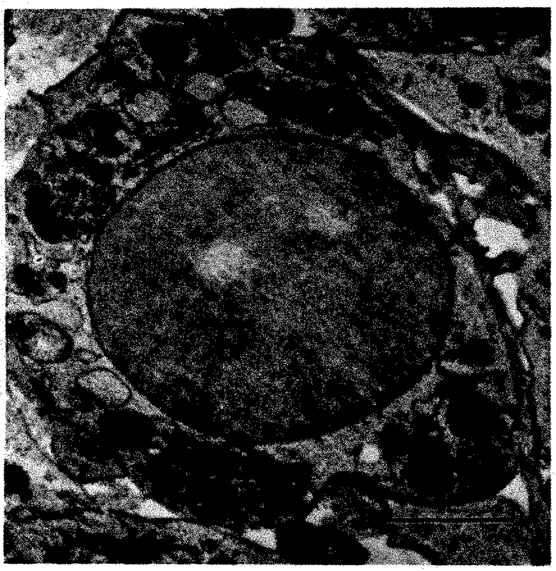

Fig. 1. Electron photomicrograph of cell from tissue culture of human meningioma. Permanganate fixed, 'Araldite' embedded. Scale = 2μ

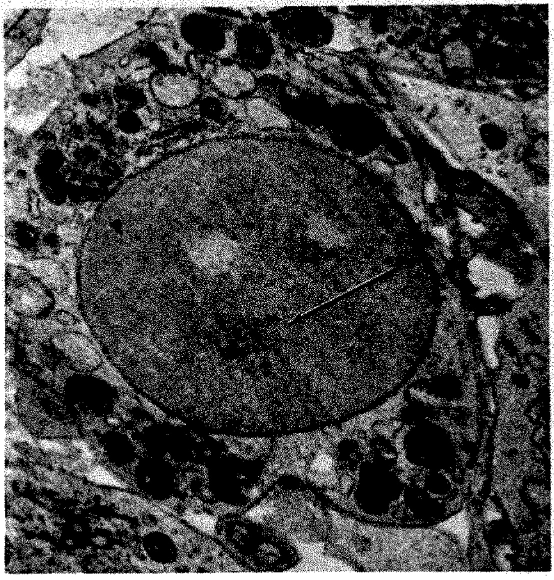

Fig. 2. Same area as in Fig. 1 after exposure of section to mercuric–DNAase solution

To demonstrate the change which results from immersion in the mercuric–DNase solution, a selected nucleus from a human meningioma culture was photographed before and after exposure to the staining solution. Fig. 1 shows this nucleus prior to staining. Fig. 2 is the post-staining picture. The arrow in Fig. 2 indicates the region of density increase. Note that the mercuric-DNase stain took effect on this specimen despite a lengthy exposure to the microscope beam prior to staining. The two light regions in this nucleus are areas of reduced electron density surrounding nucleoli not seen in this section. The dense masses in the cytoplasm, regularly seen in electron micrographs of this tumour culture, are believed to be an abnormal lipoidal product.

In other micrographs, the stranded material the density of which was increased by exposure to the complex was seen to terminate on the nuclear membrane in frequent instances. When a number of such strands were present parallel to the plane of sectioning, the nucleus had the unexpected appearance of a giant mitochondrion, the strands resembling, though not in detail, the arrangement of mitochondrial cristæ.

It is likely that the modified enzyme did not penetrate the epoxide-embedding material, but rather bound only to DNA at or near the surface of the section. A better result might be expected if the technique were used in conjunction with a more permeable or wettable embedding medium, or from the use of enzymes of smaller molecular dimensions than DNase. The dielectric properties of the embedding material might also be found to affect the bonding between the enzyme–metal complex and the fixed substrate. Surface charge patterns induced in the resin by the presence of the substrate might be effective in attracting the enzyme to the surface of the section. Experiments with various embedding materials are in progress.

Should our present work of testing the generality of this method show it to be applicable to other enzyme–substrate combinations, a widely useful tool will be available for electron microscope cytochemistry. Our present work includes efforts at extension of this technique to include localization of intracellular RNA and neurotransmitter substances, as well as application of metal-complexed DNase to metaphase chromosomes. The investigations also include the use of metal-complexed enzymes as vital stains prior to fixation and thin sectioning of tissues. Further work is in progress to characterize the presumed mercuric–DNase complex in detail.

In view of the possibility that enzyme-substrate interactions can occur between an embedded substrate and an aqueous enzyme solution, it would seem reasonable to attempt the complementary experiment of attempting to locate intracellular enzyme by applying a solution of metal-complexed substrate to tissue sections.

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Richard Roppel
Carl Melton

Battelle Memorial Institute,
505 King Avenue,
Columbus 1, Ohio.

GENETICS

Karyotype of *Theligonum cynocrambe* L.

The family Theligonaceae has one genus *Theligonum* L. (= *Cynocrambe* Tourn.) in which four species have been reported: *T. japonicum* Okubo and Makino, *T. macranthum* Franch., *T. cynocrambe* L. and *Cynocrambe formosana* Ohwi. Two of these species have been cytologically examined previously by other workers. Schneider[1] reported that *T. cynocrambe*, a Mediterranean species and an annual, had a somatic chromosome number of $2n = 20$ and a meiotic count of $n = 10$. In contrast to this, Sugiura[2] found that *T. japonicum*, a perennial from Japan, had a meiotic count of $n = 11$. He did not report on the somatic chromosomes of this species.

In 1961 seed of *T. cynocrambe* was collected from the Piana degli Albanesi district of Sicily about ten miles south of Palermo by Dr. C. D. Cook. Plants were grown from this seed, the chromosome complement was examined, and a voucher specimen deposited at the British Museum Herbarium.

Root tips were pre-treated for 2 h in 0·05 per cent colchicine solution and fixed in 3:1 alcohol/acetic. They were then hydrolysed for 10 min in hydrochloric acid at 60° C, stained for 1 h in Feulgen, mounted in aceto-orcein to counterstain, teased and squashed. The chromosome number of $2n = 20$ was observed in all cells. This agrees with the count made by Schneider in 1914.

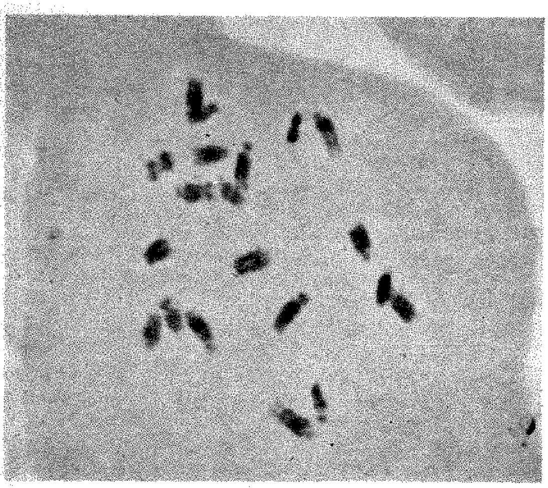

Fig. 1. Somatic metaphase plate from a root tip of *Theligonum cynocrambe*, $2n = 20$ (× 2,000)

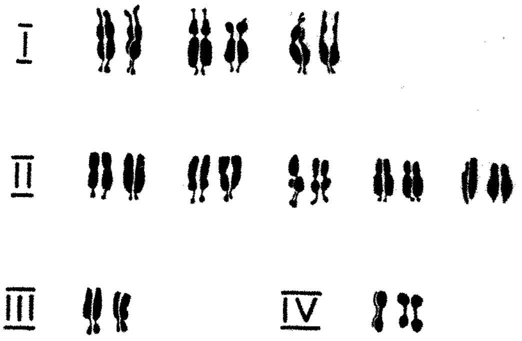

Fig. 2. Karyotype analysis of *Theligonum cynocrambe* (× 3,000)

The degree of chromosome contraction, due to the colchicine pre-treatment, varied slightly from cell to cell, but this method allowed karyotype analysis to be carried out with accuracy. Four groups of chromosomes can be distinguished (Figs. 1 and 2). (I) Three pairs of large acrocentric chromosomes ranging from 2·6 to 2·8μ in length; (II) five pairs of small acrocentric chromosomes ranging from 1·7 to 2·0μ in length; (III) one pair of acrocentric chromosomes with more prominent short arms, the short arm 0·6μ and the long arm ranging from 1·3 to 1·4μ in length; (IV) one pair of metacentric chromosomes, 1·7μ in length.

Chromosome morphology was seen best in those cells where the pre-treatment had produced the least contraction of the chromosomes but had clarified the position of the centromere and secondary constriction regions. The members of group I have two secondary constrictions and in those of group II there is evidence of a single secondary constriction. Drawings from root-tip cells by Schneider[1] do not show any of these details.

Similar karyotype analysis of the other species of this genus will be of interest in view of the different basic chromosome numbers reported. These will be done as material becomes available.

N. M. Gregson

Department of Genetics,
University of Liverpool.

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A Red Eye Colour Mutation in *Culex pipiens* after X-irradiation

Four 1–2-day-old males of *Culex pipiens* were irradiated with a dose of 4,000 r. The mutant 'red eye' (*r*) was isolated from F_3 cultures of two of the irradiated males (♂ II and ♂ IV). From male II, there were three red-eyed females which arose out of a single F_2 brother–sister mating; from male IV, 80 red-eyed females and one red-eyed male from 14 F_2 brother–sister matings. According to the experimental procedure, this means that at least one sperm from male II and at least fourteen sperms from male IV carried the mutation *r*. Thus, the same mutation was recovered in parallel from two irradiated males. The clustered appearance of the mutation in male IV was presumably caused by the occurrence of the mutation in a spermatogonial cell.

While the normal eyes of the larvæ, pupæ, and adults appear pigmented dark brown to black, the mutation *r* causes a red eye colour in place of black. In the late embryo of the normal animals, one recognizes two brownish red eye spots, which in the larvæ darken to brown or black. The eyes of the advanced larvæ, pupæ, and adults appear completely black. The embryonic eye spots of the mutants, on the other hand, are faded orange in colour. The orange hue becomes more intense and turns to red in the fourth larval instar. The eyes of young adults are light red. In several days old animals, the red colour begins to darken; in older animals the eyes are deep dark red. The nature of the red colour substance has not been clarified as yet.

Crossings of the various *r*-isolations with one another gave red-eyed F_1 progeny in every case. Accordingly, the mutation is the same in all red-eyed animals. *r* is inherited as a recessive, shows full penetrance and no variability of expression.

Table 1. DATA ON INHERITANCE OF THE MUTATION *r*: NORMAL ♀♀ CROSSED WITH RED-EYED ♂♂

Generation	♀♀ Normal	♀♀ Red-eye	♂♂ Normal	♂♂ Red-eye
F_1	68	—	98	—
F_2	378	—	199	165

Among the red-eyed animals there were 83 females and only one male. This indicates that the factor for red eyes is sex-linked. Sex in *Culex* is determined by a single gene[1]. The male is heterogametic and the female homogametic, with dominance of the male factor *M* over *m*. Crosses with normal-eyed animals confirm the linkage of the mutation to sex (Table 1). In the F_2 the red eye colour reappeared in the males only. In this experiment, the linkage with sex was complete; in other cases, however, as a result of crossing over between the sex factor and the factor *r*, a small percentage of red-eyed animals appeared among the females. The only male in the radiation experiment must have originated also through crossing over.

A spontaneous white-eyed mutation is also available in this laboratory. Since white is also sex-linked, it was suggested that white and red were multiple alleles. However, a fully normal F_1 progeny was obtained from a cross with white-eyed females and red-eyed males. Thus, the two factors are not multiple alleles.

A. WILD

Institut für Genetik,
Johannes Gutenberg-Universität,
Mainz, Germany.

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Autoradiographic Studies of *X*-chromosome Duplication in an *XO*/*X*-isochromosome *X* Mosaic Human Female

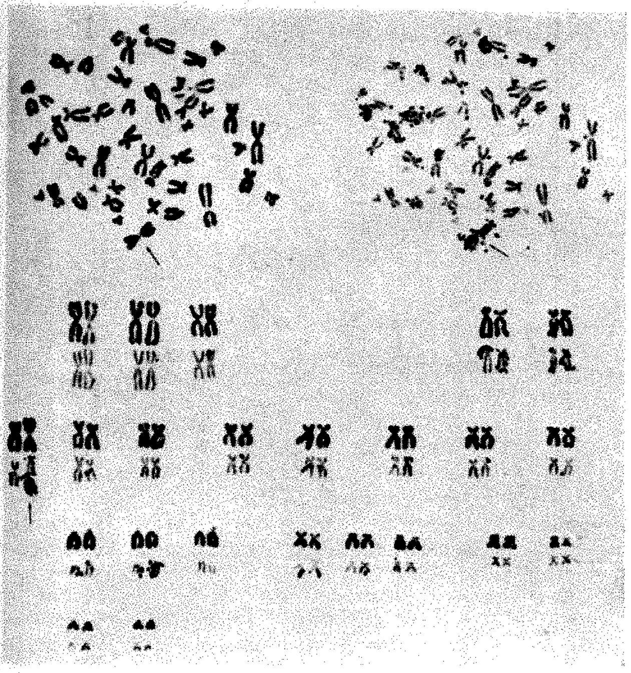
ACCORDING to Lyon's hypothesis[1,2], one of the two *X*-chromosomes in each *XX* cell of the normal human female and perhaps in all mammalian females is genetically inactivated at a certain stage of embryogenesis. This inactive *X* chromosome becomes condensed or heteropycnotic and forms the sex chromatin. Inactivation is presumed to occur at random. The heteropycnotic *X* can thus be either maternal or paternal in origin. Ohno and Cattanach[3] have recently provided direct cytological evidence of this in the mouse. In the skin of animals with a variegated phenotype due to a translocation of the wild-type allele of a coat colour gene to the *X* chromosome (X^t), the wild-type patches of skin were populated by cells containing a condensed inactive chromosome (presumably the normal *X*). Mutant-type patches from the same animal contained cells in which a visibly larger chromosome, presumably the X^t, was heteropycnotic.

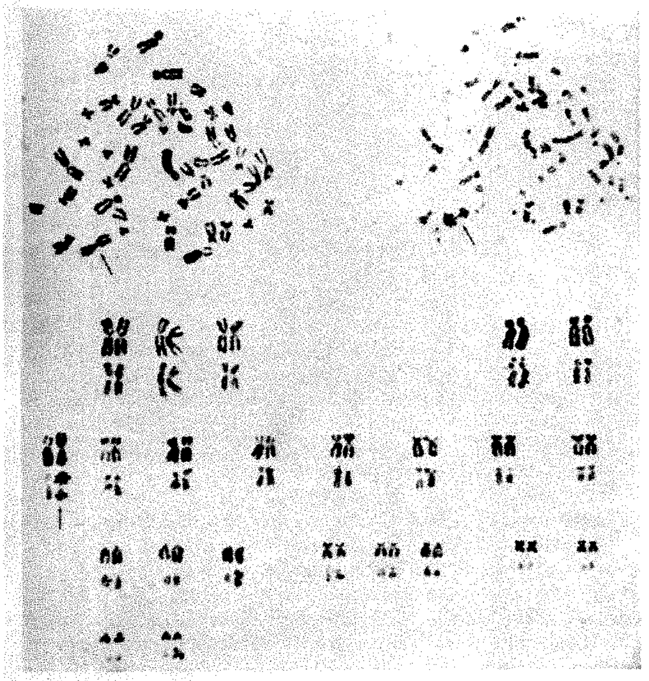
Random inactivation of maternally or paternally derived *X* chromosomes may not always occur. In human females with one normal *X* and one structurally abnormal *X* chromosome, the sex chromatin is consistently abnormal in size. It conforms to the size of the abnormal *X* chromosome which can be either larger or smaller than normal[4]. If the sex chromatin can be formed only by inactivated, heteropycnotic *X* chromosomes, then the structurally abnormal *X* chromosome in cells from these heterozygous females undergoes preferential inactivation. We have demonstrated the consistent inactivation of an isochromosome-*X* in human leucocytes heterozygous for this structurally abnormal *X* chromosome by means of an autoradiographic examination of chromosome duplication (DNA replication)[5].

For this investigation reported here, peripheral blood containing leucocytes with this abnormal complement was obtained from a 40-year-old woman. She had primary amenorrhœa and sexual infantilism as well as many other anomalies of the type frequently seen in Turner's syndrome. Approximately 20 per cent of the chromatin positive nuclei from the oral mucosa contained two sex chromatin masses. Both the sex chromatin masses in the epithelial cells and the drumsticks in the neutrophils were of two sizes. They were either normal or enlarged[6]. However, there were only two clearly evident cell lines in cultured leucocytes. One cell line had 45 chromosomes with an *XO* sex chromosome complement. The other cell line had 46 chromosomes with an *X*-isochromosome-*X* sex chromosome complement. Since the isochromosome-*X* was larger than the other *X* chromosome in the latter cell line and could be clearly identified, we examined the replication patterns of these two *X* chromosomes in a series of cells in order to find out whether the isochromosome *X* always had the late-replication pattern characteristic of heteropycnotic, inactive *X* chromosomes[7-13]. Leucocyte cultures were prepared[14]. Tritiated thymidine and colchicine were added to a final concentration of 1 μc./ml. (specific activity 0·36 c./mM) and 10^{-6} M respectively. Five hours or nine hours later, metaphase cells were collected directly from the isotope-containing medium. The procedure is described more fully elsewhere[11]. The metaphase figures were photographed before stripping film (Kodak '*AR*-10') or emulsion (Kodak '*NTB*-3') was applied, and re-photographed at the plane of the silver grains overlying the labelled metaphase figures after exposure and development of the photographic emulsion. Under these conditions, the more heavily labelled chromosomes were those which terminated replication late in the synthetic period of interphase.

The isochromosome *X* was the most heavily labelled chromosome in the complement in 12 labelled cells illustrating the end of synthesis of DNA which were examined and karyotyped (Fig. 1). The normal-sized *X* chromosome always terminated DNA replication earlier than the late-replicating isochromosome *X*. This may indicate that the abnormal *X* chromosome was always inactivated in preference to the structurally normal *X* chromosome in these leucocytes. On the other hand, if inactivation of *X* chromosomes occurs very early in embryonic life, the isochromosomes-*X* might be inactivated in a given individual by chance alone in every one of the few cells from which all the leucocytes afterwards arise. Other females heterozygous for a structurally abnormal *X* chromosome have now been examined[15-17]. In cultured leucocytes from these individuals, the isochromosome-*X* was consistently the late-replicating *X* chromosome. A much stronger case can therefore be made for preferential or non-random inactivation of the abnormal *X* chromosome. It is still possible that inactivation of *X* chromosomes occurs at random, and that cells in which the normal *X* chromosome is inactive are inviable.

The results obtained in the work recorded here do not provide any simple explanation for the complex sex chromatin pattern seen in this patient, who is both chromatin-two positive and has sex chromatin bodies of two different sizes. Since there appears to be a consistent relationship between the maximum number and size of sex chromatin masses and the number and size of *X* chromosomes[4], this patient should either be chromatin positive, with every sex chromatin mass enlarged, or there should be at least two more cell lines in this patient than we have been able to demonstrate by culturing leucocytes. A cell line with two normal *X* chromosomes would account for the presence of sex chromatin masses of normal size.





Figs. 1 (above) and 2 (below). Mitotic figures from a leucocyte culture which contained tritiated thymidine for its final 5 h. Each mitotic figure has been photographed before, as well as after, the application of photographic emulsion. Karyotypes have been prepared from the matched photographs. The isochromosome *X* (arrow) is consistently more heavily labelled (late-replicating) than any other chromosome. The isochromosome is also frequently at the periphery of the metaphase figure, as shown here

A cell line with three *X* chromosomes, two being isochromosomes, would account for the presence of cells with two large sex chromatin masses. One possible *XX* sex chromosome complement has been observed among the 42 karyotypes of labelled metaphase figures from cultured leucocytes which have been examined. No metaphase figure with three *X* chromosomes has been observed. If such a cell line exists in this individual, it may be present only in some tissues. An autoradiographic examination of dividing cells from cultures of another tissue such as skin or mucosa, in which the sex chromatin pattern and the karyotype with its replication pattern can be directly compared might resolve the apparent discrepancy between these characteristics.

An examination of the labelling patterns of the autosomes in this *X*-isochromosome *X* complement is in progress. The labelling patterns of the autosomes will be compared with the labelling patterns of the autosomes in *XX*, *XXX*, and *XXXXY* genotypes. In the *XXX* and *XXXXY* complements, the autosomes have a characteristic pattern of DNA replication, which is indistinguishable from that seen in *XX* complements[11,13].

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Orlando J. Miller
Barid B. Mukherjee
Saul Bader
Arthur C. Christakos

Department of Obstetrics and Gynecology,
College of Physicians and Surgeons,
Columbia University,
New York, 32.

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VIROLOGY

Pigs and Rift Valley Fever

One of the earliest papers[1] on Rift Valley fever included pigs in the list of refractory species because Daubney, Hudson and Garnham[2] had failed to elicit a clinical response in two pigs they had injected. Later, however, Weiss[3] directed attention to the circumstantial field evidence that sows had aborted on farms in South Africa where sheep had died. Nevertheless, when Easterday, Murphy and Bennett[4] injected three young pigs intraperitoneally with doses of $10^{2\cdot2}$ and $10^{3\cdot2}$ mouse LD_{50} of Rift Valley fever virus there was no clinical response, no viræmia and no development of antibody. Preliminary findings, herein recorded, suggest that the innate resistance of pigs is not absolute, the dose of virus being critical.

Six immature Large White pigs, weighing 34–45 kg, were split into two groups. The pigs in the first group were injected intravenously with 1·0 ml. of a dilution of infected sheep serum estimated to contain 10^2–10^3 hamster LD_{50} of the van Wyk strain of Rift Valley fever virus. Those in the second group were injected similarly with 10^5–10^6 hamster LD_{50} virus. Simultaneously the infected sheep serum was titrated in hamsters.

The pigs were bled at intervals of two days until the 14th day after inoculation and each serum was injected intraperitoneally into four hamsters. The hamsters were observed for 14 days, after which the survivors were challenged with Rift Valley fever virus. Finally, on the 21st day after inoculation, the serum of each pig was checked for the presence of Rift Valley fever antibodies by neutralization tests in hamsters.

The pigs remained healthy. Neither virus nor antibody was detected in the pigs given the lower dose, but virus was recovered on the 2nd and 4th days after inoculation from one pig given the higher dose. Antibodies were detected in the sera of all three pigs given the higher dose.

G. R. Scott*

East African Veterinary Research Organization,
Muguga,
Kenya.

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[*] Present address: Royal (Dick) School of Veterinary Studies, Easter Bush, Roslin, Midlothian, Scotland.

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Shortening by Actinomycin *D* of Latent Period of Multiplication of Sendai Virus

It has been shown earlier that actinomycin *D* inhibits the multiplication of influenza viruses without any influence on Newcastle disease virus and Sendai virus multiplication[1,2]. It was found that the dynamics of Sendai virus reproduction in actinomycin-treated cultures of chicken fibroblasts and human amniotic cells were changed after the latent period of multiplication was shortened by 2 h (Fig. 1). To clarify this problem an autoradiographic investigation was carried out. Cultures of human amniotic cells infected with Sendai virus (1,000 ID_{50} per cell) were held at 35° C in Medium 199 or in this medium with 0·5 γ/ml. of actinomycin *D*. 30 min, 1, 2, 3 and 5 h after onset of incubation samples of cultures were exposed to 1 μc/ml. of tritiated uridine for 30 min, washed in BBS containing excess of unlabelled uridine, fixed in Carnoy solution, covered with emulsion type *M* and exposed for 1 month. Developed autographs were stained by the Unna method.

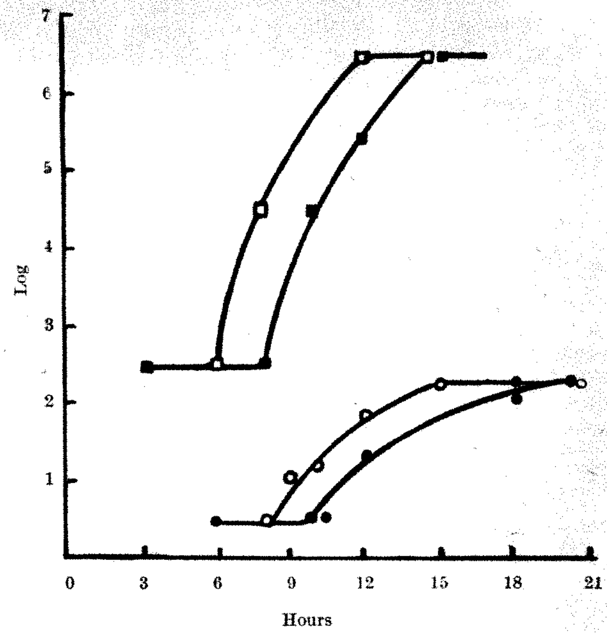

Fig. 1. Influence of actinomycin *D* on the dynamics of Sendai virus reproduction in human amniotic cells
●, HA titre in control cells; ○, HA titre in actinomycin-treated cells; ■, ID_{50} titre for chicken embryos in control cells; □, ID_{50} titre in actinomycin-treated cells

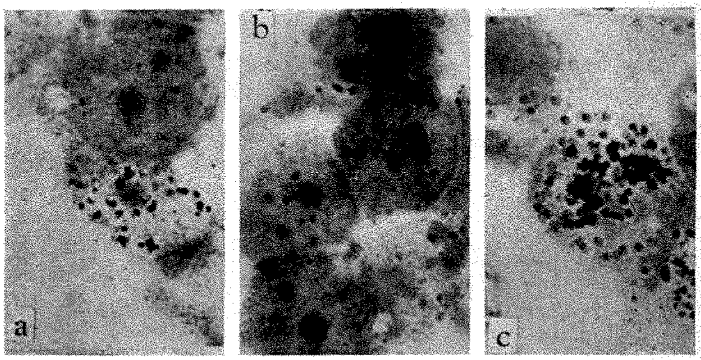

Fig. 2. Human amniotic cells: autoradiographs (× 900)
a, Control cells; *b*, actinomycin-treated cells; *c*, actinomycin-treated cells 2 h after Sendai virus infection

Four sets of experiments were performed: (*a*) control cultures; (*b*) cultures treated with actinomycin *D*; (*c*) infected cultures; (*d*) infected cultures treated with actinomycin *D*. All incorporated radioactivity in the control cells was localized in the cell nuclei, mainly in the internucleolar area; in some cells grains were found both in the internucleolar area and in the nucleoli (Table 1 and Fig. 2). Actinomycin *D* inhibited completely incorporation of tritiated uridine into the internucleolar area and partially into the nucleoli. No differences were observed in grain localization between control cells and infected cells for the first hour after infection; but 2 h after infection and later there was no labelling of internucleolar area. Heavy labelling of nucleoli was observed 3 h after infection (Fig. 2) and disappeared within 5 h. In the infected cells treated with actinomycin *D* no labelling of the internucleolar area was seen; an increased incorporation of tritiated uridine into nucleoli was observed 1 h after infection, became more intense at 2 h and disappeared 3 h after infection. The number of grains in nucleoli of infected cells was considerably greater than that in nucleoli of the control cells.

The results obtained show that actinomycin *D* inhibits the synthesis of RNA in internucleolar area and partially in nucleoli. Differences between our results and those of Perry[3,4] seem to be explained either by different properties of cultures used or by different experimental conditions.

Table 1. Percentage of Human Amniotic Cells labelled with Tritiated Uridine

Intervals after infection	Experimental							
	Control cells		Actinomycin-treated cells		Infected cells		Actinomycin-treated infected cells	
	N	*n*	*N*	*n*	*N*	*n*	*N*	*n*
30 min	70 ± 3·6	13 ± 2·8	0	9 ± 1·6	65 ± 2·6	11 ± 2·4	0	2 ± 1·1
1 h	52 ± 4·0	5 ± 1·7	0	4 ± 1·6	74 ± 3·6	4 ± 1·6	0	18 ± 2·0
2 h	49 ± 4·0	13 ± 2·6	0	6 ± 1·4	0	2 ± 1·1	0	44 ± 4·0
3 h	65 ± 2·6	9 ± 1·6	0	0	0	34 ± 2·6	0	3 ± 1·3
5 h	61 ± 4·0	5 ± 1·7	0	± 1·3	0	9 ± 1·6	0	6 ± 1·4

N, nucleus excluding nucleoli; *n*, nucleolus

Action of Sendai virus on the cells was similar to that of actinomycin *D*; but virus-induced inhibition of nuclear RNA synthesis was observed only 2 h after infection. The synthesis of viral RNA occurred 3 h after infection and was localized in nucleoli where RNA of the Sendai virus inoculum penetrates[5,6]. An earlier synthesis of viral RNA and shortening of the latent period of multiplication by 2 h in actinomycin-treated cultures may be explained by inhibition of messenger RNA of a cell; as a result, enzymatic systems start to work earlier according to a genetic programme of virus.

A. G. Bukrinskaya
V. M. Zhdanov

Institute of Virology,
Academy of Medical Sciences, U.S.S.R.,
Moscow.

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Nicotinamide Adenine Dinucleotide and Nicotinamide Adenine Dinucleotide Phosphate Concentrations in Leaves of *Nicotiana glutinosa* infected by Tobacco Mosaic Virus

When leaves of *Nicotiana glutinosa* are inoculated with tobacco mosaic virus (TMV) the formation of local necrotic lesions is accompanied by an increase in the rate of respiration[1,2]. This increase is related to the development of the lesions and to the total number of lesions produced. Although tissue extracts of the lesions show increased polyphenoloxidase activity[3,4] evidence is lacking to show that polyphenoloxidase is concerned in the respiration of the intact virus-infected cell or that the increased repiration is related to increased polyphenoloxidase activity. Two possible functions for polyphenoloxidase in the metabolism of the virus-infected cell can be suggested. It may function as a terminal oxidase, and the increased respiration is the result of the increased polyphenoloxidase activity. However, this is most unlikely as the respiration rate in plant tissues is not usually limited by the activity of the terminal oxidases. A second possibility is that increased polyphenoloxidase activity is concerned with the oxidation of polyphenolic compounds to oxidized polyphenols. If this made any significant contribution to the respiration of the virus-infected tissue an increase in the respiratory quotient would be expected, but this was shown not to be so by Yamaguchi and Hirai[2] who found in *N. glutinosa* leaves infected by TMV the respiratory quotient was unchanged.

In looking for an explanation of the increased respiration rate of *N. glutinosa* leaves showing local lesions formed as a result of TMV-infection, nicotinamide-adenine dinucleotide (NAD), reduced nicotinamide-adenine dinucleotide ($NADH_2$), nicotinamide-adenine dinucleotide phosphate (NADP) and reduced nicotinamide-adenine dinucleotide phosphate ($NADPH_2$) concentrations in the leaves were determined.

Leaves of *Nicotiana glutinosa* were inoculated with TMV and five days after the appearance of local lesions healthy and infected leaves were assayed for NAD, $NADH_2$, NADP and $NADPH_2$. An acid medium of 5 ml. of 0·1 M *tris*-hydrochloric acid buffer *p*H 8·2, 1 ml. N hydrochloric acid and 2 ml. water was used to extract NAD and NADP. $NADH_2$ and $NADPH_2$ were extracted by an alkaline medium, 5 ml. *tris*-hydrochloric acid buffer, 1 ml. N sodium hydroxide and 2 ml. of water. The extraction medium was contained in a Potter–Elvehjem homogenizer immersed in a bath of boiling water. The leaf was cut into pieces of convenient size and rapidly dropped into the homogenizer, heated for 30 sec, homogenized for 90 sec, and then rapidly cooled by immersion in a freezing mixture of dry-ice and ethanol. The extracts were thawed and centrifuged at 20,000*g* for 15 min in a refrigerated centrifuge. The supernatants were decanted; the *p*H of the alkaline-extract adjusted to 8·0 and that of the acid-extract to 7·5 by dropwise addition of acid or alkali in the cold. The nicotinamide-adenine dinucleotide content of the extracts was determined immediately by the colorimetric method of Slater and Sawyer[5]. NAD, $NADH_2$, NADP, and $NADPH_2$ (Sigma purest grade) were tested for purity by the usual spectrophotometric methods[6] and used to construct calibration curves for the method of Slater and Sawyer and to determine the recovery of dinucleotides added to the extracting medium. The recovery was more than 90 per cent for all four dinucleotides.

Table 1. NICOTINAMIDE-ADENINE DINUCLEOTIDE AND NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE CONCENTRATION OF *Nicotiana glutinosa* LEAVES INOCULATED WITH TMV

Nucleotide	μg/g fresh weight	
	Healthy	TMV-infected
NAD	5·5	4·1
$NADH_2$	0·8	1·7
NADP	3·0	1·0
$NADPH_2$	0·2	0·7
Total	9·5	7·5

The results given in Table 1 show an increase in the level of $NADH_2$ and $NADPH_2$ in the leaves showing TMV-induced lesions. As these results represent a complete leaf it may be that the increase in reduced nicotinamide-adenine dinucleotides is even greater in the region of the local lesion. It is not possible to cut out and assay a number of individual local lesions because the tissue contains an active $NADH_2$ oxidase which rapidly oxidizes $NADH_2$ in tissue homogenates. The decrease in the $NAD/NADH_2$ ratio in the tissue from 7 to 1 for healthy leaves to 2·4 to 1 for virus-infected leaves and a decrease from 15 to 1 for healthy to 1·4 to 1 in the virus-infected leaves for the $NADP/NADPH_2$ ratio show an accumulation of reduced nicotinamide-adenine dinucleotides in the virus-infected leaf. It has been shown by Solymosy and Farkas[7] that the enzymes of the pentose phosphate shunt are activated in the periphery of the local lesion. This may account for the increase in $NADPH_2$ in the virus-infected tissue. However, an increased respiration rate in the tissue requires an increase in electron transfer from $NADH_2$ to oxygen. It may be that virus multiplication in the lesion results in protein synthesis leading to an increased utilization of adenosine triphosphate and concomitant oxygen uptake. Even so it would appear that the electron-transfer chain is unable to reoxidize reduced nicotinamide-adenine dinucleotides as rapidly as they are formed in the virus-induced local lesion.

D. W. Sunderland
M. J. Merrett

Department of Biological Sciences,
Institute of Technology,
Bradford. 7.

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MISCELLANEOUS

Equipment for Facilitating Movement on an Intertidal Mud-flat

It is probable that the lack of literature relating to intertidal mud ecology as compared with other intertidal habitats is due, in part, to the restrictions placed on movement by the softness of the substratum. Yonge[1] reported that at Stolford, Bridgewater Bay, the shrimp fishermen, whose nets were secured on soft banks of mud, used a type of sledge or 'mud-horse', which they pushed in front of them. The sledge served the double purpose of preventing them from sinking deeply into the mud and of carrying back the catch. Barnett (personal communication) used mud shoes of his own design to aid movement.

Ecological investigations on littoral muds often necessitate travelling for distances of several hundred yards, and then remaining in one position for periods of several minutes while samples are taken. The problem of performing these tasks has been overcome by designing mud skis.

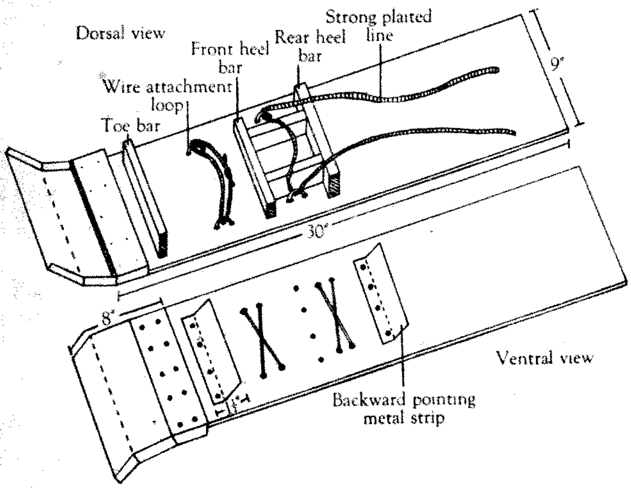

Fig. 1. Diagrammatic sketch of the mud skis

The main body of each ski is of ¼-in. thick 3-ply wood, 30 in. long and 9 in. wide, to which are attached a toe, front heel, and a rear heel bar (Fig. 1). These wooden bars prevent fore and aft movement of the foot relative to the ski, bars on either side of the heel eliminating sideways movement. The upturned forward end of the ski is fashioned from 18 gauge galvanized iron, as are the backward pointing strips on the ventral surface. For extra strength the galvanized attachments and wooden bars are fixed to the 3-ply board with ¾ in. brass screws. The ski is fastened to the foot in two places by means of strong plaited line threaded through attachment loops made from ⅛-in. diameter galvanized wire.

Forward movement is effected by using one ski as a pivot, sliding the other ski forward relative to it, then using this ski as a pivot. The backwardly directed metal strips on the underside of the pivot ski prevent slipping.

This method of progression allows a sledge laden with sampling equipment to be drawn along by hand.

J. D. GEORGE*

Department of Zoology,
University of Southampton.

* Present address: Institute of Oceanography, Dalhousie University, Halifax, Nova Scotia.

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Impurities in Commercially Prepared Tritiated Folic Acid

RADIOISOTOPICALLY labelled compounds are being used with increasing frequency in both basic science and clinical investigation. Most laboratories are dependent on commercial sources for these products, and although the specifications attesting to the purity of a compound are usually accurate, exceptions do occur.

We have recently had occasion to use commercially available tritiated folic acid specified by the manufacturer to be 100 per cent pure by dilution analysis and chromatography. As a check on the purity a sample of the tritiated folic acid was subjected to paper chromatography in subdued light, using 0·2 M phosphate buffer *p*H 7·0. Fig. 1 is a photograph taken under ultra-violet illumination of the chromatograms of the tritiated folic acid as well as unpurified and purified stable folic acid. Strip *A* is the tritiated folic acid with accompanying radioscanogram. Radioactivity is clearly evident at several positions corresponding to two slow-moving fluorescent areas, a middle absorbent area representing folic acid, a second faster-moving fluorescent area, and a less-clearly visible absorbent spot near the end of the strip. Strip *B* is unpurified grade *C* folic acid. Similar slow-moving fluorescent areas are evident, but the fluorescence just ahead of the folic acid absorbent spot is moving slower than that seen in the tritiated folic acid strip. However, this impurity on repeat chromatography sometimes spreads from the folate absorbent area, and, therefore, probably represents the same impurity. Strip *C* is the same commercial folic acid purified in our laboratory by the method of Sakami and Knowles[1]. Practically all but a slight trace of the faster-moving fluorescent impurity has been removed. We did not analyse these impurities, but from the observations of Belcher *et al.*[2] it would appear that the faster-moving fluorescent area seen in strip *A* represents free tritium and/or para-aminobenzylglutamates. The slower-moving fluorescent areas are probably pteridine fractions. The fluorescent areas at the end of the strips are due to accumulation of phosphate salts at the solvent front.

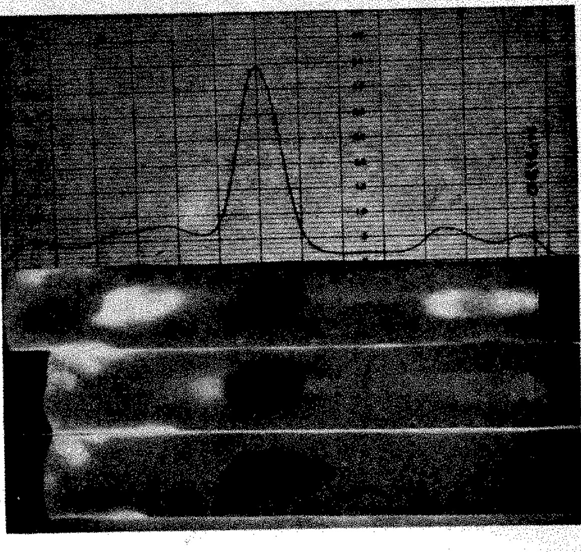

Fig. 1

Planimetric analysis of the tritiated folic acid radioscanogram revealed that only 50 per cent of the radioactivity corresponded to the folic acid absorbent area. The slow- and fast-fluorescent areas had approximately 15 and 35 per cent of the radioactivity, respectively.

The pure folate absorbent area was easily eluted from the paper with 0·2 M phosphate buffer, *p*H 7, and, when rechromatographed under similar conditions, moved as a single radioactive peak corresponding in mobility to pure folic acid.

This work was supported by a New York City Health Research Council grant *U*–1190.

SHELDON P. ROTHENBERG
RUBEN GARCIA

Blood Research Laboratory,
Department of Medicine,
New York Medical College, New York.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 2

University of London (at the Royal Veterinary College, Royal College Street, London, N W 1), at 4 p m —Prof John Francis (University of Queensland) "Veterinary Research and Education in Australia" *

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Mr F Horner "Atmospheric Radio Noise as a Problem in Radio Communications"

British Institution of Radio Engineers, Computer Group (joint meeting with the Computer Group of the Institution of Electrical Engineers, at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Symposium on "Analogue Circuit Techniques Using Transistors"

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m — Discussion on "Some Experience on Projects in Courses for Diplomas in Technology"

Royal Society of Arts (at John Adam Street, Adelphi, London, W C 2), at 6 p m —Mr Hulme Chadwick "Colour and Community" (Last of three Cantor Lectures on "Colour in Modern Life")

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S W 1), at 6 30 p m —Mr H R Hearn "Technical Developments in the Aerosol Field"

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 8 15 p m —Mr Norman G Dyhrenfurth "America on Everest".

Tuesday, December 3

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1 15 p m —Dr P J Ucko "The Significance of Prehistoric Human Figurines" *

University of London (at Imperial College, London, S W 7), at 1 30 p m —Mr D E Broadbent "Decision and Action" (Last of five lectures on "The Basis of Experimental Psychology")*

Institution of Civil Engineers (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr J C Dickinson and Mr R T Gerrard "Cameron Highlands Hydroelectric Scheme"

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Mr R M Ware and Mr D J McNeil "Iron Disilicide as a Thermoelectric Generator Material", Dr K Crystal "Silicon Solar Cells for Ground Level Use"

University of London (at Imperial College, London, S W 7), at 5 30 p m —Prof A V Bradshaw "The Third Phase in Metallurgy" (Inaugural Lecture)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m — Dr F T Perkins "Virus Vaccines—Living or Dead ?" (Fourteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Society for Analytical Chemistry, Atomic Absorption Spectroscopy Discussion Panel of the Physical Methods Group (at the Chemical Society, Burlington House, Piccadilly, London, W 1), at 6 30 p m —First Annual General Meeting, followed by a Discussion opened by Dr W Slavin (U S A)

Wednesday, December 4

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 2 p m —Prof A Latner "Isoenzymes" *

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 2 30 p m and 5 30 p m —Colloquium on "Connections in Electronic Circuits"

Royal Society of Arts (at John Adam Street, Adelphi, London, W C 2), at 2 30 p m —Dr B A Southgate "The Prevention of Water Pollution—the Present Position"

Colour Group (Great Britain) (in the new Physics Department, Imperial College, London, S W 7), at 3 p m —Mr J Charlton "The Use of Colour in Eighteenth Century Interiors" 4 p m —Continued Discussion of the C I E Conference Reports with an Introduction by Dr R W G Hunt.

Institute of Petroleum (at 61 New Cavendish Street, London, W 1), at 5 30 p m —Dr T F Gaskell "The Study of Oceanography and Its Bearing on Exploration for Oil"

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Electrical Aspects of the Offices, Shops and Railway Premises Bill" opened by Mr J R Freeth

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 6 p m —Symposium on "New Developments in Television Cameras and Zoom Lenses"

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Mr R S Silver "An Approach to a New Theory of Surface Condensers"

Plastics Institute (at the Wellcome Building, Euston Road, London, N W 1), at 6 30 p m —Mr J M J Estevez "Some Thoughts on the Weathering and Ageing of Plastics"

Society for Analytical Chemistry (at the Chemical Society, Burlington House, Piccadilly, London, W 1), at 7 p.m —Meeting on "Differential Thermal Analysis"

Thursday, December 5

Nutrition Society (joint meeting with the British Grassland Society, at the Federation of British Industries, 21 Tothill Street, London, S W 1)—Symposium on "The Utilization of Grass by Ruminants"

Royal Society (at Burlington House, Piccadilly, London, W 1), at 4 30 p m —Mr N W Pirie, F R S "The Size of Small Organisms" (The Leeuwenhoek Lecture)

Sir Thomas Middleton Memorial Trust (at the Wellcome Building, 183 Euston Road, London, N W 1), at 5 p m —Dr J B Brooksby "The Impact of Research on the Control of Foot-and-Mouth Disease" (Ninth Middleton Memorial Lecture)

Institution of Civil Engineers (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Informal Discussion on "Supersonic Concorde Aircraft—Their Effect on Airport Layout" introduced by Mr A C Johnson

Royal Institution, Library Circle (at 21 Albemarle Street, London, W 1), at 5 30 p m —Dr F N L Poynter "On Collecting Books for a Library of the History of Medicine"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m — Prof L Saunders "Interfacial Films and Cell Membranes" (Fifteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Institute of Refrigeration (at the National College for Heating, Ventilation, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S E 1), at 6 p m —Mr V S Meadows "Fluidised Bed Techniques in Refrigeration"

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S W 1) at 6 p m —Discussion on "Should Hydraulic Equipment be Cheaper ?"

Society of Chemical Industry, London Section (joint meeting with the Industrial Water and Effluents Group, at the Royal Institution, Albemarle Street, London, W 1), at 6 30 p m —Dr J S Carter "The Alkali Inspectorate Look Back"

Royal Institute of Chemistry, London Section (joint meeting with the Northampton College of Advanced Technology Chemical Society, at the Northampton College of Advanced Technology, St John Street, London, E C 1), at 7 p m —Prof G J Hills "The Structure of Water and Aqueous Solutions"

Friday, December 6

Association of Applied Biologists (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S W 7), at 11 a m — Discussion on "Host Specific Replanting Problems in Fruit Trees" introduced by Dr R S Pitcher and Dr J E Crosse 12 noon—Discussion on "Viruses in the Cultivated Mushroom" introduced by Mr M Hollings, Miss Doreen G Gandy, Dr F T Last and Dr Olwen M Stone 2 30 p m —Dr L Broadbent "Winds of Change in Applied Biology" (Barnes Memorial Lecture)

Royal Institution (at 21 Albemarle Street, London, W 1), at 9 p m — Dr L S B Leakey "The Search for Man's Ancestors and Cousins in East Africa"

Saturday, December 7

London County Council (at the Horniman Museum, London Road Forest Hill, London, S E 23), at 3 30 p m —Dr G H S Bushnell "Pre-Columbian Mexican Art" *

Monday, December 9

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Dr I Hacking "On the Foundations of Statistics"

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Measurements at Very Low Frequencies with particular reference to Noise" opened by Dr J L Douce Mr H Sutcliffe and Prof J C West

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Dr F Fallside and Mr M R Patel "Effect of a Back E M F Non-linearity on Responses of a Second-Order Position Control System", Dr F Fallside and J O C Ezeilo "Application of the Second Method of Lyapunov to the Stability of Certain Position Control Systems Containing a Back-E M F Non-linearity"

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 6 p m —"The Pitcairn People" (colour films).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturer/Lecturer (well qualified in one of the human biological sciences) in Ergonomics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref 36/G (December 6)

Lecturer (with industrial experience in the field of corrosion and surface technology, and preferably an honours degree or equivalent qualifications) in the Department of Metallurgy—The Secretary, Battersea College of Technology, Battersea Park Road, London, S W 11 (December 6)

Senior Research Assistant (with a Ph D in physics or equivalent research experience) in the Department of Physics, to work on the optical and electrical properties of some magnetic oxide semi-conductors, this will include the Raman effect, using an infra-red gas laser—The Registrar, The University Sheffield (December 7)

Tutor in Philosophy—The Principal, St Anne's College, Oxford (December 7)

Assistant Librarian (honours graduate with library qualifications)—The Secretary, The Queen's University, Belfast, Northern Ireland (December 10)

Assistant Lecturer or Demonstrator (with medical qualifications registrable in the United Kingdom) in Pathology—The Registrar, The University, Manchester 13 (December 14)

Chair of Statistics—The Registrar, The University, Sheffield (December 14)

Senior Research Associate (plant physiologist or physical scientist interested in plant physiological problems) in the School of Biological Sciences, to work under the general direction of Prof J Dainty on a programme of ionic and water relations of plant cells and tissues—The Registrar, University of East Anglia, Earlham Hall, Norwich, Nor 05G (December 16)

Senior Lecturer or Lecturer in Ophthalmology—The Registrar, The University, Sheffield (December 18)

Senior Lecturer or Lecturer in Geography at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 20)

Senior Lecturer or Lecturer in Pharmacology at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 20)

Senior Lecturer (with a degree in chemistry or biochemistry and extensive experience in clinical chemistry) in the Department of Clinical Chemistry—The Secretary, The University, Edinburgh (December 21)

Assistant Lecturer in the Department of Zoology, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 22)

Deputy Chief Scientific Officer (Virologist) to take charge of the Virology Section of the Microbiological Research Establishment, Porton Down, Wilts, and to undertake research in the field of animal viruses, particularly those groups which attack man, and to supervise the work of a group of scientific staff working in allied fields—The Secretary, Civil Service Commission, 23 Savile Row, London, W 1, quoting Ref S/5790/63 (December 31)

Lecturer (qualified in physics and an interest in the application of physical techniques to biology and particularly macromolecules or electron microscopy) in the Department of Botany—The Secretary, University College, Gower Street, London, W C 1 (December 31)

Lecturer (with a special interest in gas dynamics) in the Department of Mechanical Engineering—The Secretary, University College, Gower Street, London, W C 1 (December 31)

Senior Lecturer in Experimental Physics, and a Lecturer and an Assistant Lecturer in Experimental or Theoretical Physics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton (December 31)

Reader or Senior Lecturer in Chemistry, and a Lecturer and an Assistant Lecturer in Chemistry—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton (December 31)

Marguerite A Keasbey Official Fellow and Lecturer in Theoretical Physics—The Warden, New College, Oxford (January 1)

Chair of Applied Electromagnetism, with particular reference to plasma engineering—The Registrar, The University, Liverpool, quoting Ref No CV/462 (January 11)

Chair of Public Health and Social Medicine—The Secretary, The University, Edinburgh (January 15)

Senior Lecturer or Lecturer in Entomology at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Commonwealth Universities (Branch Office) Marlborough House, Pall Mall, London, S W 1 (South Africa and London, January 15)

Chair of Mathematical Statistics at Monash University—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1, or The Registrar, Monash, University, P O Box 92, Clayton, Victoria, Australia (Australia and London, January 31)

University Lecturer in Biochemistry—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford (January 31)

Assistant or Assistant Lecturer (with special interests in inorganic chemistry) in the Department of Chemistry—The Professor, Department of Chemistry, University College, Upper Merrion Street, Dublin 2, Republic of Ireland

Lecturer in Mathematics, and an Assistant Lecturer in Zoology at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1

Lecturer (with good academic qualifications and preferably experience of teaching, research or industry) in Mathematics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire quoting Ref 35/G

Physicist (with experience of electronics), to work in the application of computers to experiments in the field of nuclear structure—Nuclear Physics Laboratory, The University, 21 Banbury Road, Oxford

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Admiralty, Hydrographic Department Admiralty Marine Science Publication No 4 Bathymetric, Magnetic and Gravity Investigations, H M S *Owen*, 1961–1962 Part 2 Profile Pp 2+35 profiles+track chart and profile index (H D 539) (London Admiralty, Hydrographic Department, 1963) [109

University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1961–1962 Pp xvi+46 (Cmnd 2135) (London H M Stationery Office, 1963) 6*s* net [189

Hospital Engineering Research Unit A Study of the Performance of the Air Conditioning Plants at Three Operating Theatre Suites Pp 32 (Glasgow Hospital Engineering Research Unit, 3 Lilybank Gardens, 1963) 5*s* [189

The State of Our Schools a Report of the Findings of the National Survey of Schools, 1962 Part 3 An Analysis of the Findings Relating to Grammar Schools, Comprehensive Schools and Special Schools Pp 19 (London National Union of Teachers, 1963) 2*s* 6*d* [189

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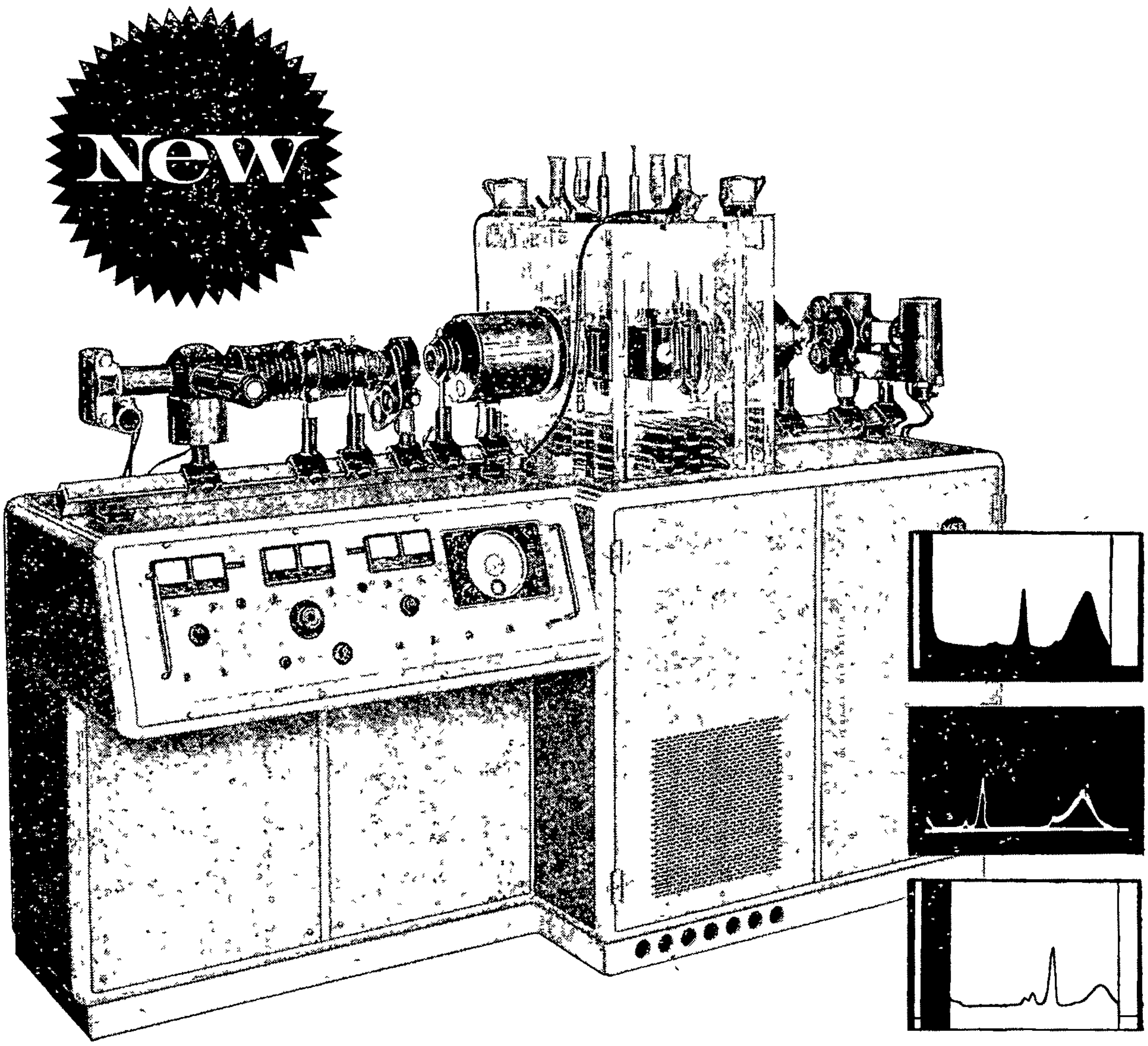

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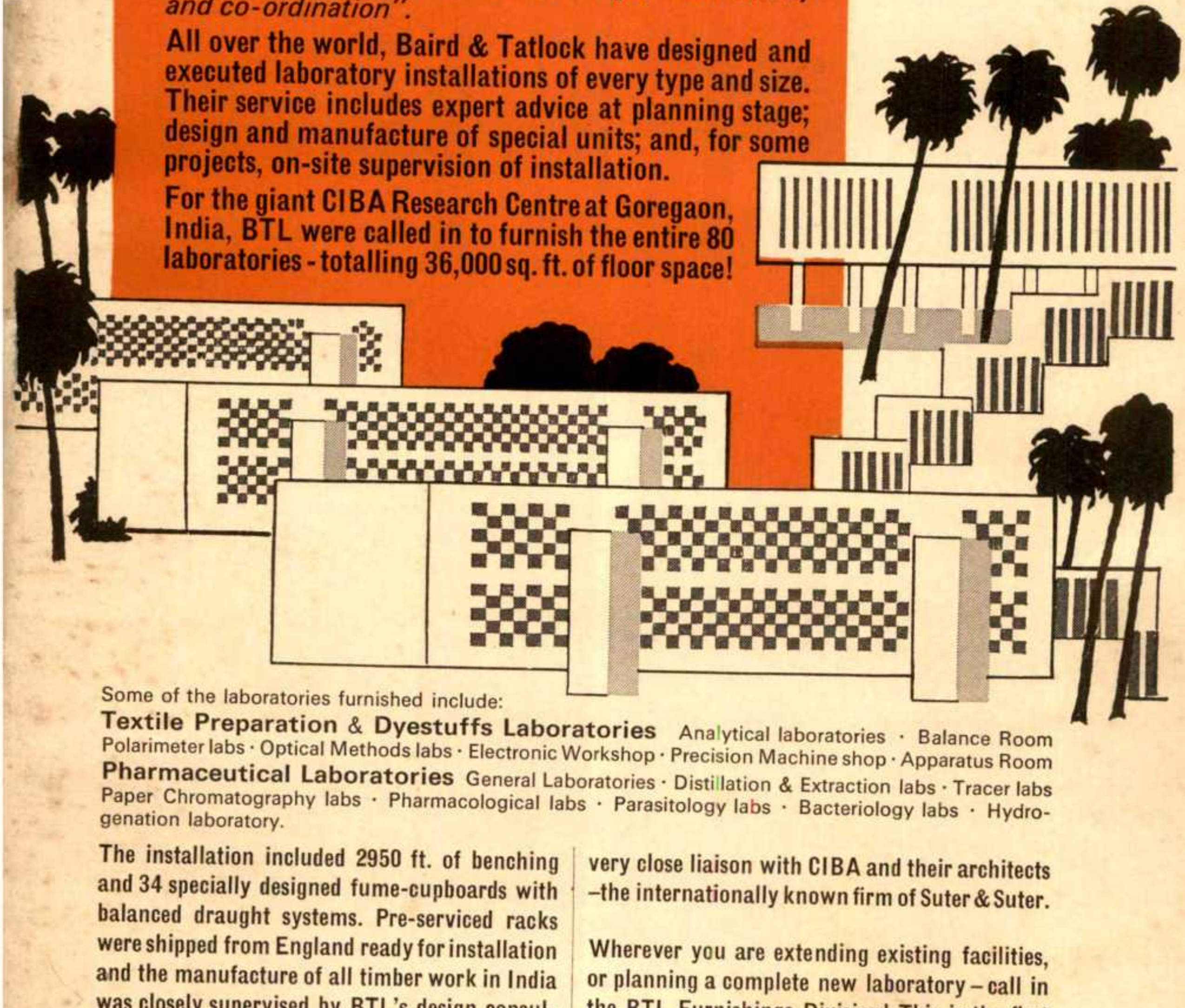

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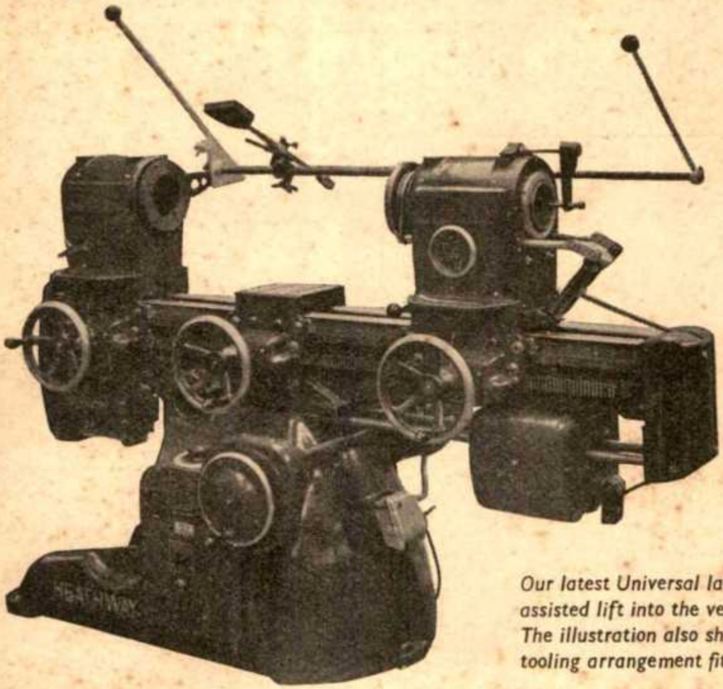

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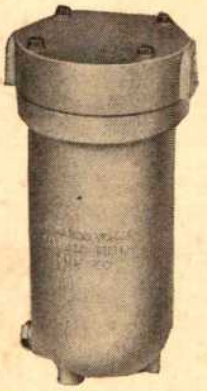

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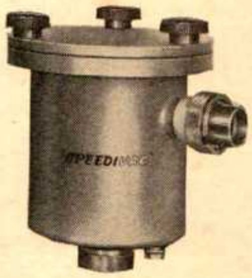

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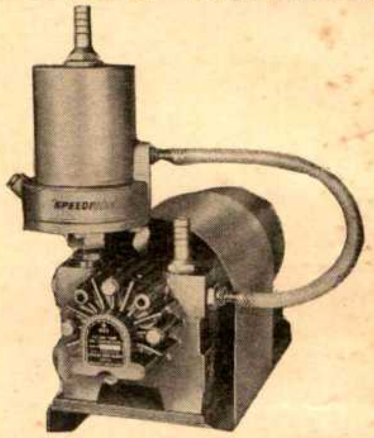

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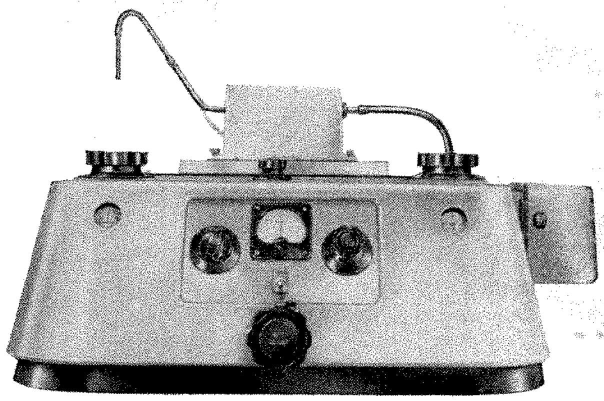

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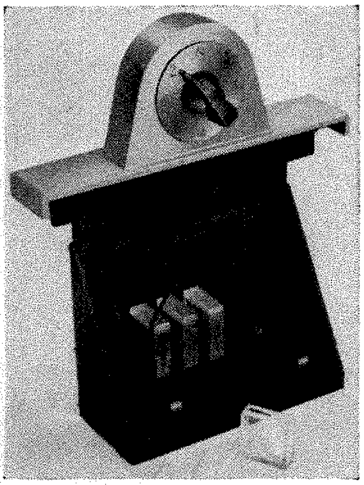

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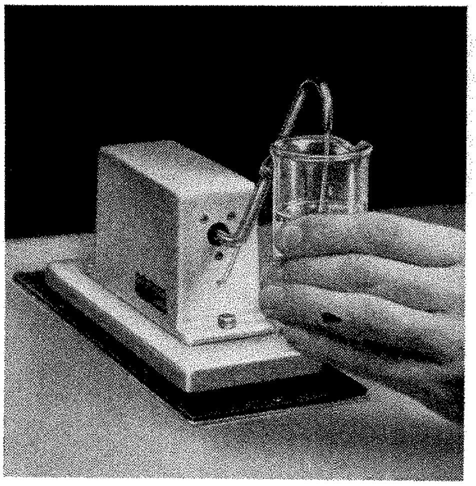

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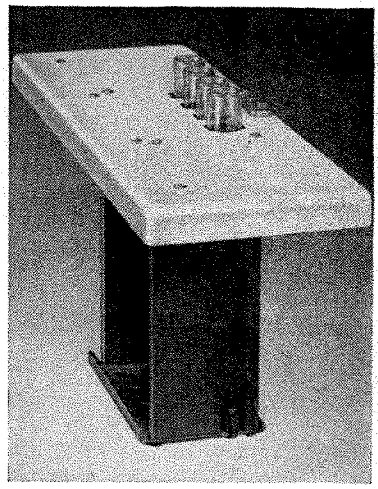

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A BLUEPRINT FOR HIGHER EDUCATION

THE Report of the Committee on Higher Education, appointed with Lord Robbins as chairman in February 1961, is both a major educational document in itself and also something of a social landmark*. The terms of reference of the Committee were to review the pattern of full-time higher education in Great Britain and, in the light of national needs and resources, to advise on what principles the long-term development of higher education should be based, including whether any changes in that pattern or any new types of institution were desirable, and whether any modifications should be made in the present arrangements for planning and co-ordinating the development of the various types of institution. It brings to an end a period of uncertainty and delay, removing the last excuses for procrastination and setting the full weight of searching enquiry behind its recommendation that Britain should and can plan to admit 17 per cent of the age-group to higher education in place of the present 15 per cent, of whom some 8.5 per cent are full-time. It disposes authoritatively of the idea of there being any risk within the next twenty years that the growth in the proportion of young people with qualifications and aptitudes suitable for entry to higher education will be restrained by a shortage of potential ability. Fears that expansion will lead to a lowering of the average ability of students in higher education have proved unfounded.

Nor is this inconsistent with the view widely entertained that there are already, in any event, some students at the universities ill-qualified by aptitude or character to profit by a university education. This may be due in part to the differentiation between institutions which the Robbins Committee deplores: distinctions based on adventitious grounds, whether historical or social, are, it observes, wholly alien to the spirit that should pervade higher education. The Committee wishes to see the removal of any designations or limitations that cause differentiation between institutions performing similar functions, while fully recognizing the need for a variety of institutions with differing functions. Student wastage and incompatibility can, however, equally be due to imperfections in the selection process, and it should be noted that the Committee specifically recommends research by an independent body into the extent to which aptitude tests might supplement other features of the selection process.

The Committee's proposals for the expansion of higher education from 216,000 full-time places in 1962–63 to about 390,000 in 1973–74 and some 560,000 (on present estimates) in 1980–81, of whom 350,000 will be in university institutions, are not based on narrow calculations of the demand for manpower. They are based on the conviction that there is a broad connexion between the size of the stock of trained manpower in a community and its level of productivity per head. In modern societies the skills and versatilities required are increasingly those conferred by higher education, but while the Report takes it as axiomatic that courses of higher education should be available for all those who are qualified by ability and attainment to pursue them and who wish to do so, it lends no support to the idea of a 'right' to higher education. It points out unequivocally that all those engaged in higher education, both teachers and taught, are privileged persons and the freedom they enjoy carries great responsibility. Public opinion will not support the cost of higher education unless teachers are actuated by a high sense of professional obligation and students are actuated by a corresponding sense of the obligation to work.

In advocating a programme of expansion which is estimated to cost £742 million in 1980–81 in place of £206 million in 1962–63, that was wisely and timely put. No less cognate, however, is the insistence of the Committee that higher education not only requires expansion but it also requires a system—a feature that has hitherto been lacking. To secure the smooth functioning of the proposed system, however, the Report also proposes some changes in the administrative arrangements, including ministerial responsibility. It is on this last point that there is one dissentient from the measures proposed to secure the necessary co-ordination: Mr. H. C. Shearman, who would have a single Minister for Education rather than a Minister of Arts and Science, responsible for the University Grants Committee, the Research Councils and like institutions, with other institutions of higher education under the Minister of Education or, in Scotland, the Secretary of State, as proposed by his colleagues.

These and the rest of the Committee's 178 recommendations, however, should be considered first in the light of the aims of higher education set forth alongside the principles which guided the Committee in framing its recommendations as well as conducting its enquiries, which are set forth in the second chapter of the Report. First, then, while assuming that needs of the present and still more of the future demand that there should be a system of higher education, replacing the largely uncoordinated activities and initiative of the past, the Robbins Committee does not suggest that all the activities concerned should be planned and controlled. It attaches great value to the freedom of individuals and institutions in any academic system, and the chapter on academic freedom and its scope is forthright and unequivocal: the principle of the University Grants Committee is not merely endorsed, but regarded as an essential ingredient in any administrative structure for higher education. The Committee considers that central decisions should be coherent and take account of the interests of all sectors of higher education, and that decentralized initiative should be inspired by common principles.

Next, in any properly balanced system at least four essential objectives are visualized. Instruction in skills suitable to play a part in the general division of labour is put first, because this vocational aspect is sometimes ignored or undervalued, and Britain's progress depends to a much greater extent on skills demanding special training. With this the Robbins Committee postulates that what is taught should be so taught as to promote the general powers of the mind. The aim should be to produce not more specialists, but cultivated men and women. Nor can higher education be dissociated from the advancement of learning. The search for truth is an essential function of institutions of higher education, and the process of education, the Committee adds, is itself most vital when it partakes of the nature of discovery.

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* *Committee on Higher Education. Higher Education.* Report of the Committee appointed by the Prime Minister under the Chairmanship of Lord Robbins. Pp. xvi+335. (Cmnd. 2154.) (London: H.M.S.O., 1963.) 15s. net.

Elsewhere in the Report, the Committee discusses some of the controversial issues concerning the balance between teaching and research and the distribution of research between the institutions of higher education and other bodies. The answers given to those questions have a close bearing on the actual structure of Government organization and Ministerial responsibility. Fourth among its aims, however, the Committee places the transmission of a common culture and of common standards of citizenship. It believes that it is a proper function of higher education to provide in partnership with the family that background of culture and social habit on which a healthy society depends.

Coming now to the guiding principles of the Report, that of availability of courses to all who are qualified and wish to pursue them has already been noted. Next, the Committee places the principle of equal academic awards for equal performance, rejecting the retention of formal differences in terminology that do not reflect real differences in attainment. On the status of institutions, it insists that distinction should be based on difference in functions and in emphasis or acknowledged excellence: there should be no freezing of institutions into established hierarchies, but excellence should be recognized and encouraged wherever it exists and whenever it appears. Great importance is attached to opportunities for the transfer of a student from one institution to another when appropriate, and the organization of higher education must allow opportunity for free development of institutions, for experiment, for the safeguarding of standards and the production of as much high excellence as possible.

While the Robbins Committee was concerned primarily with the long-term development of higher education, it recognized that during the years 1965–68 Britain would be faced with an emergency arising out of the very large numbers of boys and girls born just after the Second World War who will then reach the age of higher education. If this situation is not met adequately the long-term plans may well be frustrated. The present plans for the universities are designed to allow for 150,000 places by 1966–67 and 170,000 in 1973–74. For the colleges of advanced technology, 15,000 places are planned in 1966–67, rising to 21,000 by a date depending on capital investment after 1963–64, and for training colleges in England and Wales authorized plans allow for 80,000 students in general and specialist colleges in 1970–71. It is now apparent that in 1966–67 there will be a shortage of 19,000 places in universities and 25,000 in 1967–68; for higher education as a whole the total shortage is estimated as 20,000 and 25,000, respectively.

To meet this shortage, the Robbins Committee considers that the present target figure of 168,000 places for the universities, including the colleges of advanced technology, in 1966–67 must be increased by some 10 per cent. It recommends to this end a substantial increase in the existing capital building programmes of the universities for 1964 and succeeding years. More accommodation, for teaching and especially for residence, is a prime essential, and the Committee insists on the need for firm assurances to the universities that sufficient funds will be available forthwith both to meet the short-term problem and for the long-term developments which the Committee recommends, if confidence in the Government is to be restored. It recognizes, too, that there will be difficulty in finding sufficient qualified teachers to meet all the needs of the emergency period, and whatever means of improvisation are adopted, including the provision of more technical and secretarial assistance, the extra burden on teachers is likely to be considerable.

It is all the more important, accordingly, that it should be clearly understood that any inroads on present standards are transitory and will be redressed as soon as possible and that adequate recurrent grants are available for all the necessary measures. It is not the least merit of the Robbins Committee that it faces the full implications of its proposals generally or that it leaves no doubt as to the deplorable effect of Government vacillation and changes in university policy in the past. Further, while it does not go into details of possible methods of improvisation for this emergency period, it does point out that some of the changes in organization recommended as part of its long-term proposals could assist in meeting the immediate need. This is a further and weighty reason for an early decision on some of the Committee's recommendations in regard to administration and also on the importance of speedy action on some of the less controversial measures proposed.

Further thought may well be desirable as to whether a Minister of Arts and Science should be appointed as co-ordinating Minister rather than one of the other alternatives. However, there would seem to be no reason why inter-ministerial and inter-departmental consultations to concert measures to meet the crisis should not be put in hand forthwith, or the Ministries concerned, the University Grants Committee, the Committee of Vice-Chancellors and Principals and representatives of other relevant associations brought together for appropriate consultation on the proposals of the Report. Nor does there seem to be any *prima facie* reason for delay in establishing and bringing into action the Consultative Council, consisting of representatives of educational and other interests, the creation of which is recommended by the Committee to consider major questions covering the whole field of higher education as well as its relations with the schools. The Statement issued from 10 Downing Street, on October 24, indicates indeed that the Government appreciates the urgency of the situation, but it gives no hint of an early decision on changes in organization, although these will not be allowed to delay the work on expansion which is now being carried out.

The hard core of the Committee's proposals for long-term expansion of higher education is to be found in the chapter entitled "The Future Pattern of Higher Education". It is visualized that by 1980–81 some 346,000 places will be provided in universities when some 60 per cent of students enter full-time higher education and go to a university instead of the present 55 per cent. This will involve expansion of many of the multi-faculty universities to accommodate 8,000–10,000 students, but even so there will be a need for some 50,000 places over and above what could be provided from existing institutions. A further 30,000 places will be provided by the foundation of six new universities in addition to those now in formation, one or perhaps two of which would be in Scotland and one a Special Institution for Scientific and Technological Education and Research, development of which the Committee recommends as a matter of special urgency. The remaining 20,000 places could be met by giving university status to some of the regional colleges, central institutions and colleges of technology

The colleges of advanced technology, which elsewhere in the Report it is recommended should become technological universities with full university status, are included in the calculation of the expansion of existing universities, and it should be noted that, besides the special institution already mentioned, the Committee recommends development of a similar institution from a selected college of advanced technology Institutions like the Imperial College of Science and Technology, the Manchester College of Science and Technology and the new University of Strathclyde, will have strong postgraduate elements, and together these five technological institutions should fill a gap that has been most noticeable in Britain and facilitate the major effort to expand technological research which is also recommended in the Report As regards the colleges of education, while the Committee proposes that the numbers entering some colleges in England and Wales should rise to 40,000 by 1974, it does not propose a rise in entry after that date the colleges will then be supplying 111,000 students, rising to 131,000 in the middle and later years of the decade For colleges of education in Scotland the Committee proposes expansion to 15,000 students by 1980–81, and the numbers of places in colleges of further education is assumed to rise from its present 31,000 to some 65,000

The question of places is, of course, only one aspect, and the Robbins Committee then considers the location of universities, the areas of study, professional education and the education of adults generally It urges the claims of the large cities and centres of population in determining the location of the new universities, and envisages that the present 51 per cent of students taking science and technology will increase further, though it would view with some concern any continuing reduction in the proportion of students taking arts subjects A growing proportion of such students has been taking social studies, and any decline or weakening in the study of the humanities would impoverish Britain's intellectual and spiritual life The implications of a more intimate contact between professional bodies and institutions of higher education are perhaps passed over too lightly, probably because the Committee's terms of reference relate to full-time higher education However, it is fully aware of the importance of the activities of the extra-mural departments of the universities, of the Workers' Education Association and local education authorities in adult education and that it is desirable that the universities should co-operate in developments opened up by the television services and other media of communication

Of vital importance in expansion is the quality of the staff, and the Report examines in some detail the problems which staffing the new and expanded institutions will present It deprecates any attempt to draw a sharp line between university teachers and the teachers in other institutions of higher education while research is a primary function of universities, this does not mean that it is only in universities that fresh work is needed and original work is done On the contrary, both in further education and the colleges of education, the Committee expects the student/staff ratio to recognize that teachers need time for reflexion and personal study Moreover, the Robbins Committee is concerned about the deterioration in the qualifications of graduates teaching in the upper forms of schools This itself can affect higher education adversely, but the deterioration can only be halted if the proportion of good honours graduates who become school teachers is, in the long term, higher than at present

Nevertheless, while the Committee anticipates in higher education in the immediate future a serious aggravation of the strains and stresses of the past two decades, it does not consider that the expansion of higher education of itself need cause a deterioration in the staffing of schools It emphasizes rather that higher education itself can only attract recruits of the necessary quality if it offers appropriate conditions of service and economic inducements In this connexion the Committee directs attention to the importance of adequate prospects of promotion, recommending a review of the structure of university staffing as a whole, and of freer movement of staff between higher education, Government and other research establishments and industry, and to the serious danger of a shortage of technicians, if not of secretarial assistance On teaching and research, pertinent observations are made in the Report Apart from recommending a major effort to encourage an increase in technological as well as scientific research (including an increase from 20 to 30 per cent by 1980 of the proportion of graduates proceeding to postgraduate work) and closer links between university institutions and Government research establishments and industry, it considers that in making appointments and in promotions, the diversity of gifts is not sufficiently honoured and published work counts far too much in comparison with other kinds of excellence The Committee recommends specifically the abolition of distinctions in salary scale between reader and senior lecturer and that in promotions to the latter grade particular attention should be paid to distinction as a teacher The full weight of the Committee's authority is put behind further attention to teaching methods and to curriculum research and development in the schools as well as in institutions of higher education The need for further educational research in the universities and into the many aspects of higher education is clearly recognized without any specific comment on the proposal for an educational research council

It will be clear that in recommending that the colleges of advanced technology should in general be designated as technological universities, with power to award both first and higher degrees, and that responsibility for their finance should be transferred to the University Grants Committee, the Robbins Committee concedes most of their claims and what was generally recognized as reasonable by most people Friction is more likely over the proposal to rename the teachers' training colleges of education, more particularly in their relations with the university departments, and there are numerous points of administrative detail to be determined even when the formal organization recommended by the Committee has been established This in itself, as already indicated, is a matter of some complexity on which the Committee was divided and will be discussed more fully later in the light of the recommendations of the Trend Committee It should suffice here to indicate the broad outline of the arrangements proposed up to the level of a Minister for Arts and Science, to whom the University Grants Committee and the Research Councils should be responsible

It is visualized that governing bodies for autonomous institutes, which normally contain a majority of lay members, and for institutions of higher learning, which are administered by local authorities, should be properly constituted with suitable academic representation and lay membership According to the Report, the Committee of Vice-Chancellors and Principals should be reconstituted, each university being represented on the Committee by its chief administrator and by a member of the academic staff elected by the senate and this Committee should appoint a standing committee of 15–20 members, and standing or *ad hoc* committees as required Likewise, the University Grants Committee should be responsible for advising the Government on the needs of all autonomous institutions of higher learning in Great Britain and for distributing grants and should set up standing committees to deal with areas of interest and *ad hoc* committees to deal with current topics The present practice in regard to accountability should be maintained, but improvements are needed in the present system for allocating recurrent grants, and an independent body should be set up to advise the Government on salaries of academic staff in autonomous institutions of higher learning

Leaving this matter of ministerial responsibility and that of administration generally with the observation that the Robbins Committee is as aware of the importance of reducing the burden on Vice-Chancellors as it is of that of associating non-professional staff with internal self-government and particularly of keeping staff at all levels informed of the reasons for policy decisions, there are two related matters which call for note Like Sir Burke Trend and his colleagues* the Robbins Committee is concerned that the finance of universities and university research should come from more than one public source and this is one of the main reasons why it recommends an upward revision of university fees so that they provide at least 20 per cent of current expenditure in place of the present 11 per cent. The effect on public expenditure would be mainly a change in the channels of distribution, but there would be another important consequence the subsidy to overseas students could be handled on more rational lines

Unlike Sir Burke Trend and his colleagues, the Robbins Committee shows itself well aware of the vital importance of the situation overseas and its bearing on Britain's future It found no firm basis for estimating the future demand for places for overseas students but was convinced that even in the short-term emergency Britain must continue to find places for such students The Committee's long-term forecast assumes that the present proportion of places will continue with an increase in numbers from about 20,000 in 1962–63 to about 50,000 in 1980–81 It is pointed out, however, that with fees at their present level and under present conditions this represents a substantial subsidy over which the nation has no control and for which it receives no credit By calculating the fees for overseas students on a uniform basis which does not vary from one institution to another, but is no higher than for other students and creating at the same time, as part of Britain's contribution to educational assistance overseas, a fund for helping any students requiring such assistance, a more rational procedure would be established and one which would also make clear the full magnitude of the British contribution

While the Government accepts that changes in organization will be necessary and has undertaken to make a full review of higher education and student finance in the light of the considerations put forward in the Report, its attitude on this and many other particular points is not defined in the Statement which has already been issued That Statement indicates that the Government has accepted the basic assumption of the Report that courses of higher education should be available for all those who are qualified and wish to pursue them and that it has adopted the Committee's calculations for 1967–68 and 1973–74 as its objective Recurrent and capital grants for university institutions concerned are being adjusted to this new objective of 197,000 full-time students in university institutions in 1967–68 and 328,000 in all higher education, the University Grants Committee and the education departments are being asked to discuss the best methods for securing this expansion This expansion, moreover, is to be the first step in formulating a ten-year programme to give 390,000 places in full-time higher education by 1973–74, 217,000 will be in institutions of university status The preliminary action to confer university status on the colleges of advanced technology and certain Scottish institutions is also being taken, the University Grants Committee is being asked for early reports on the location of a new university in Scotland and on the further development of the special institutions for scientific and technological education and research recommended in the Report This all adds up to a programme with which considerable and valuable progress could be made while clear decisions are being reached on difficult problems of organization None the less, the value of such work and the sincerity of the Government's intentions will only be attested by the swiftness with which those decisions are reached and the firmness with which its financial and other support is maintained independent of fluctuating circumstances, whether economic or political

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* *Committee of Enquiry into the Organization of Civil Science* Pp 59 (Cmnd 2171) (London H M S O, 1963) 4s net

SEEKING AND SHAPING

By Sir ROBERT WATSON-WATT, C.B, F.R.S

THE distinguished author of the remarkable and important volume, *Forschen und Gestalten Reden und Aufsatze, 1930–1962**, is almost as well known in London as in Dusseldorf, Bonn and Berlin As Staats Sekretar of Land Nordrhein Westfalen, he has been responsible for a major portion of the renaissance of the most concentrated and most important industrial area in Germany, the land between the Rhine and the Ruhr In another field, in which I first met him in the mid-'twenties, he was an energetic pioneer of 'the electronic age' In this field, too, he has led, in co-operation with the late General Wolfgang Martini, the vigorous Ausschuss fur Funkortung (now the Deutsche Gesellschaft fur Ortung und Navigation, E V), in international conferences on radio-aids to travel by air, sea and land

Brandt's weighty volume earns the adjective 'formidable' in the abstract and the concrete alike Its two-thirds of a thousand pages and some three-pounds weight are autobiographic in a very special sense, which may fairly be described as 'autobiography by exhibited sample' It presents the text of discourses bearing on his major activities from 1930, when he was president of the German Republican Students' Union, to January 1962, when he presided over the Section of Culture and Science in the Assembly of the Atlantic Community in Paris. It is thus, by implication, a sampling review and self-analysis of his personal contributions to the technological progress, in peace and in war, of his native land. The range of specialized activities represented in this collection of writings and addresses is covered in four sections, dealing respectively with radio and radar techniques (120 pp., 1953–62), transport policy and techniques (273 pp., 1949–59), rationalization (63 pp., 1952–56), research and science (175 pp., 1953–62).

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* *Forschen und Gestalten Reden und Aufsätze, 1930–1962* Von Leo Brandt Pp xii+669 (Koln und Opladen, Westdeutscher Verlag, 1962)

These main sections are supplemented by two fragments of technical history, each of 9 pp., from "The General Weekly of the Jews in Germany", 1960, by a postscript of 13 pp., and an index of more than 250 personal names appearing in the text.

Surviving workers from the 'twenties and 'thirties will, in turn, become nostalgically biographic as they read Leo Brandt's account, written in 1934, of his screened cage and its measuring equipment for investigation of radio-receiver performance. A much wider population of initiators, designers, producers, users and victims will find his summary of "German Radio Technique, 1934" absorbing, and (in widely different degrees) satisfying, surprising and/or illuminating.

As Entwicklungsgruppenleiter fur Funkmessgerate des Generalbevollmachtigen fur die technischen Nachrichtenmittel, and as Vorsitzender der Arbeitsgemeinschaft "Rotterdam"—this for the post-British introduction of centimetric wave-length equipment—he gives in 21 pp. a compact account of the technical effort in Germany which was directed mainly to 9-cm and 3-cm wave-lengths.

This review is followed by a brief account, dated 1949, of the development in the preceding 15 years of commercial radio receivers, by a 28-pp. retrospective review of German radar technique, a 34-pp. survey of the state of German centimetre-wave technique at the end of the Second World War and, appropriately, 13 pp. on the German station for radioastronomy and for basic research on radar. The whole of Section 1 of the book is essential, though necessarily inadequate, to this general history of centimetric radio in general and of radar in particular. It is to be hoped, in particular, that the papers of the late General Wolfgang Martini, general commanding the communication troops of the Luftwaffe, can be made available to the student of radio and radar devices in naval, military and air warfare.

Section 2, of this book on 'seeking' and 'doing', is devoted, in its 273 pp., to policy and techniques in the field of transport, beginning with a 1949 article on the reconstruction of the very important Rheinbahn, which serves the great and crowded industrial area between the Rhine and the Ruhr. Its principal city, Dusseldorf, is the focus of a population of six million or more, within a radius of some 30 miles. The articles in Section 2 are illustrated by copious photographs, graphs and tables which give a clear, compact and impressive account of the restoration and expansion of public transport facilities, with text and tables illuminating the requirements in material as well as the costing and financing of the system in Nordrhein-Westfalen.

While railway transport was the most urgent and demanding section of the whole, road transport was of no less importance in the total picture: indeed, the theoretical maximum carrying capacity of road transport was estimated at 16 per cent greater than that of rail—70 milliard ton-km against 60 in the year 1949. To these major services there must be added that of the inland waterways, and these are considered in a chapter dealing with the whole post-war problem of the nation's inland water-transport network up to 1950. The influence of the jet aircraft in international air transport, especially in Europe, is discussed in the last chapter of Section 2, based on a lecture in Genoa, in October 1959.

Section 3 deals with rationalization, in five discourses dating from 1952 to 1956, four of these having appeared in the records of the Working-group for Rationalization of the Land Nordrhein-Westfalen. They deal, in turn, with problems in rationalization, the influence of post-war occurrences on German productivity, the road to the exhibition "All Shall Live Better", Dusseldorf, 1953, rationalization as a state-political problem, 1953, the road to the Research Institute for Rationalization, 1956—dealing with the opening of the Research Institute for Rationalization at Rhine-Westfalen's Technische Hochschule, Aachen.

Section 4, "Research and Science", the second longest in the volume (175 pp.), comprises seven addresses and four articles dated between 1953 and 1962. The titles are, in all cases, self-explanatory: man and technology, the need for German research, atomic energy as an economic source of power for Germany; report on the renaissance of German aviation research (1955), the furtherance of science in the atomic age (1956), science in need (1957), research, the gate to the future (1958), the joint atomic energy research installation of the Land Nordrhein-Westfalen in Julich (1958), phases in the technical development of our time (1958); the group for atomic research in common with the Land Nordrhein Westfalen (1960), the strengthening of Atlantic Co-operation in the area of science (1962).

This catalogue of activities is in itself sufficient to reveal the astonishing range of the public interests to which Leo Brandt has devoted his gifts in research, technology, economics, political science, organization, management, public institutions, and in their application to the service of the State and the nation. But the supplements to the texts, tables, diagrams, maps, illustrations, and—perhaps most illuminating of all—the frontispiece showing the author at work, under the drive of a multitude of needs and the vision of a multitude of facilities, are the clearest pictures of the man who was successively dedicated to the advance, to the defence, to the renaissance and to the world-service now demanded of his country in the fields of Forschen und Gestalten, of "Seeking and Shaping". In serving his State and his country he is serving well the world at large.

Section 5 of the book contains two specially individual chapters, the first "On the Share of Jewish Personalities in the Development of the German Electrical Industry"; the second, "To the Memory of Leo Lowenstein". The former is, as every student of modern science knows, an impressive gallery of giants. Here are Heinrich Hertz, Albert Einstein, Max Born, James Frank, Lise Meitner, Georg Schlesinger. In the technological "Who was Who" are such pioneers as Emil Rathenau, Carl Furstenberg, Walter Rathenau, Felix Deutsch, Reinhold Rudenberg, Julius Laufer, Alfred Berliner, Paul Heinrich Perls, Rudolf Goldschmidt, Emil Mayer, Graf Arco, Otto Bohm, Erich Zepler, (Prof.) Korn, and so on.

One Leo Lowenstein has a special place in this list. Born in 1879, he studied chemistry in the Technische Hochschule in 1908, then in Gottingen and Berlin under the great Nernst. He became an assistant to Geheimrat Nernst, and was succeeded in that position by one Frederick Alexander Lindemann, later to be the first Baron Cherwell.

Lowenstein, as a reserve officer in a Bavarian telegraph battalion in October 1913, introduced sound-ranging as a military technique. In the relatively static phases of the First World War, his equipment became a remote precursor of radar, and when Lindemann and I came together at the Royal Aircraft Establishment, in 1915, Lindemann talked to me interestedly about sound-ranging. Lowenstein went on to achieve radio communications on 20-cm wave-lengths, using 3-m diameter parabolic antennæ, over a distance of 40 km. On August 12

1929, he was authorized to initiate experimental work on guided rockets. The lassitude which delayed many promising military inventions was overcome by the young Wernher von Braun, who was engaged by General Dornberger—although no one appears to have thought of telling him about Lowenstein's priority.

The Fates have their own sense of the amusing and the dramatic in their weaving of the webs of human destiny. The Lowenstein project, for guided rockets, the younger and later Wernher von Braun project, and the close association between Winston Churchill and Frederick A. Lindemann, brought the thoughts of two successive ex-assistants to Prof. Nernst in Berlin and also those of another brilliant ex-student from the Nernst 'stable', Henry T. Tizard, into the webs of initiative and of scepticism, and to the diverse weighing machines of optimism and pessimism on the fate of nations.

If I may be allowed a closing excursion beyond the ambit of the important papers of Leo Brandt, I would like to direct attention to the close association just mentioned. A treasured reward for the writing of *The Pulse of Radar* came in the form of a letter from a distinguished American lawyer who made history through a week-end tennis match. Having at his country home, not far from Banbury Cross, a guest named Winston Spencer Churchill he invited the University of Oxford tennis enthusiasts to send a team to play a week-end match against members of his house party. Thus did the frolicsome Fates bring Winston Churchill and 'The Prof', F. A. Lindemann, to their first meeting and their intimate friendship.

'PSYCHOGENETIC STRESS' AND AUTOINTOXICATION IN THE HONEY BEE

By ROY J. PENCE, ROBERT D. CHAMBERS and MANUEL S. VIRAY

Department of Entomology, University of California, Los Angeles

THE theory of the 'general-adaptation syndrome'[1] has engendered preliminary investigation to determine the effects of stress as it occurs in the honey bee, *Apis mellifera* Linn., on the assumption that social insects of the higher orders are more susceptible to symptoms of stress than insects of a more primitive nature. Results of these investigations have shown: (1) a similarity of physiological decline of a honey bee reacting to forced confinement, and a honey bee reacting to a stress created by externally applied chemicals; (2) the variance of activity of a honey bee abdomen, after it has been excised (disconnected from the co-ordinating centre), in comparison with that of an intact bee held in confinement; (3) the comparative reactivity of both systems, excised and intact, following exposure to chemically applied stress.

The fundamental question in this instance is: Why does a foraging honey bee returning to the hive laden with nectar or pollen succumb within a period of 1.5–2 h after capture and confinement, while an excised abdomen from an identical bee continues to remain active for as long as 48 h? On the surface it would appear that mortality of the intact bee could be attributed to over-exertion during its attempts to escape. However, it must be borne in mind that the honey bee is capable of many miles of tiring flight yet eventually succeeds in returning to the colony. The bee's 'objectivity', measured in habitat-maintaining behaviour or homing instinct, will usually prevail. Why then does the same insect surrender so quickly when denied the exercise of this instinct? It is felt that the limited period of tolerance to confinement is not the result of mere exhaustion. If energy expenditure alone is not accountable for the bee's 'resignation', leading to prostration and subsequent death, then there must be other factors instrumental in instigating this rapid decline.

It would appear that a syndrome is involved in association with stress—perhaps an autointoxicating, triggering mechanism which becomes involuntarily activated during the processes of stress[2]. When a foraging honey bee is confined to a glass jar placed near an open window, it makes hyperactive attempts to escape in the direction of natural light. If escape is denied the bee quickly dies. The time required for death is of primary interest, and it is on this period that attention is centred.

There obviously exists in the foraging honey bee some navigational instrumentation which ensures its return to the colony. Von Frisch[3,4] propounds the theory of internal clocks and the dependence of these mechanisms, in part, on the Sun. Considering the period of time that a honey bee can survive confinement, removed from all familiar objects of environment, it is found that this period is inversely related to the amount of natural light available to it[5]. Experiments also show that the bee's tolerance for confinement is much greater in the absence of natural light, and there is some indication that total darkness is less excitable than substituted artificial light. We are uncertain at this point what effect the various properties of light have on the related nervous and hormonal functions, and exactly how they are interrelated. Observing that a confined honey bee is more strongly attracted to ultra-violet than to natural light and, proportionately, the bee's period of endurance of confinement becomes significantly shorter, it appears that light in the ultra-violet spectrum is of importance[6,7].

In addition to light there are indications of other visual or sensory factors affecting the honey bee's behaviour and tolerance of confinement. The mere presence of small amounts of honeycomb exerts a striking placebo effect on a confined bee. Comb that has been coated to mask its characteristic odour, while retaining its original form, will also exert a placatory effect on an otherwise excitable honey bee. Activity is then lessened, with longer life resulting. There appears to be, however, a fairly specific amount of comb necessary to pacify a confined bee for a day or more. Comb which is coated to decrease the likelihood of chemoreception offers less pacification, while amounts smaller than the bee itself offer none. Light augments stress while a placebo decreases it. Blinding the bee, or placing it in a dark enclosure, will increase its tolerance to forced confinement. Elimination of the antennæ will, however, impose an opposite effect, for without these chemoreceptors the bee succumbs to confinement more readily. Behavioural studies of confined honey bees from which the antennæ have been severed have led to the belief that resignation occurs long before any factor of energy expenditure takes place. Subsequent examinations of respiration have substantially contributed toward this theory[8].

The area within which a foraging honey bee is confined has a definite relationship to its measure of endurance. A bee confined in a transparent enclosure of several square feet will succumb within an hour while, in a quart jar, life will be extended for from 1–2 h. Within an area of an inch or two, the bee remains seemingly co-ordinated from 1.5 to approximately 3 h; in a tightly fitted, transparent gelatin capsule for some 20 h. Freedom of motion

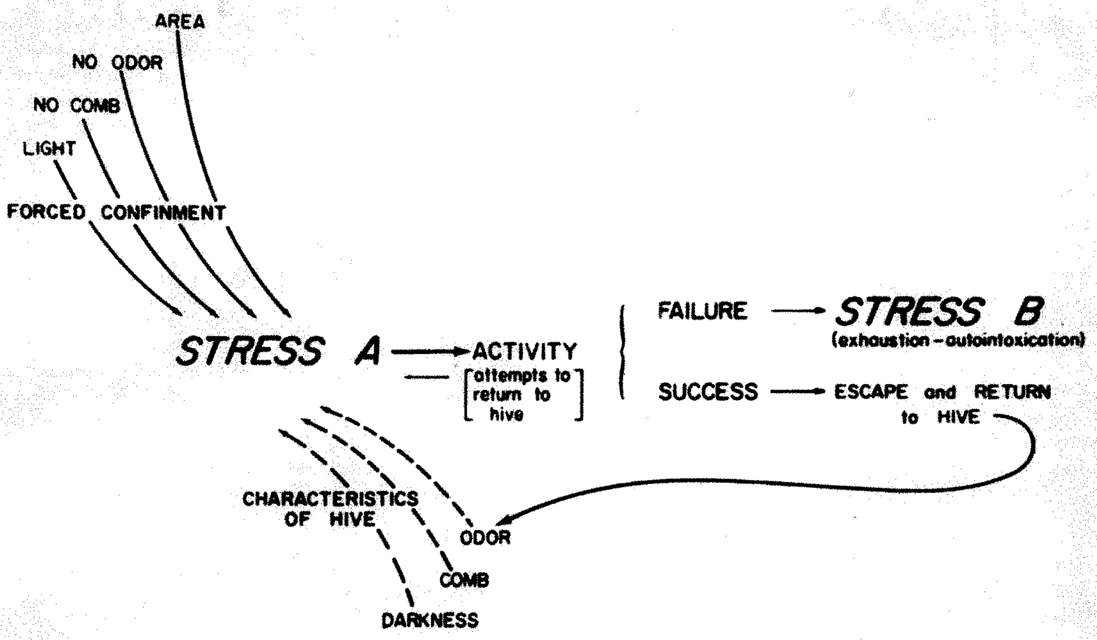

Fig. 1. Scheme of factors contributing to two 'types' of stress in the foraging honey bee
Forced confinement in natural light, in the absence of certain other environmental factors, suppresses exercise of habitat-maintaining behaviour. Continued suppression leads to beginning symptoms of stress *A*, manifested in hyperactive attempts to escape. Failure to regain natural balance results in stress *B*: death by exhaustion and autointoxication. Normality may be regained only if freedom is attained prior to debilitating effects of stress *A*

appears to be related to the bee's degree of 'frustration'. If a bee is given room to take wing toward its destination and then suddenly encounters an invisible barrier, its 'objectivity' is more abruptly thwarted. Conversely, constricted confinement will suppress or even eliminate objectivity.

Several factors of environment have an apparent effect on the highly organized, foraging honey bee in confinement. Light has been found to increase activity while the placebo effect of honeycomb reduces it. Honey bees in collective confinement have comparable objectivity, yet behave independently in their efforts to return to the colony.

Light and area affect activity which, when excessive, is deleterious and constitutes stress. Stress is the result of activity and energy expenditure and initiates activity. Thus stress, if not relieved, contributes to autotoxicity which, in turn, leads to mortality if no adaptation to the circumstances imposing stress is forthcoming.

The problem becomes one of defining the nature of a stress imposed on a foraging honey bee under confinement by separately examining similar symptoms of prostration primarily brought on by energy expenditure. To differentiate between the two, it was reasoned that first a stress would have to be induced by some externally applied chemical means. In this manner the energy expenditure resulting from chemical irritation could be compared with stress of a 'psychogenetic' nature induced by confinement alone.

The chemical selected for this investigation was menadione (vitamin K_3) which, though relatively non-toxic when applied topically at low concentrations and in minimal amounts, has an irritating effect which causes the bee to react with such violence that it eventually perishes due to exhaustion associated with chemically induced stress.

The results of an application of 0·001 ml. of 0·25 per cent menadione to the venter of a honey bee abdomen are comparable with stress symptoms of an untreated, confined bee. The time required for prostration is approximately the same, with both bees demonstrating comparable overall degrees of total activity. The important difference is that the untreated bee will be activated toward light in energetic attempts to escape, while the treated bee ignores light and expends comparable reflexive energy in attempting to rid itself of the chemical irritant.

Present evaluations clearly indicate that a topically applied irritant serves as a distracting agent which appears to supplant the original homing instinct. Thus, a stress induced by external sub-lethal chemical means becomes a substitute for a stress resulting from interference of the insect's natural behavioural pattern. This has been further substantiated through observations that irritated bees under confined conditions expend more energy yet live longer than those that are untreated. In other words, a confined bee more quickly falls prey to 'autogenetic stress' fostered through denial of its objectivity associated with the homing instinct. Measurements of losses in weight of both treated and untreated bees following death show losses to be greater in the treated than in the untreated bees; however, losses in weight alone only suggest energy expenditure.

The Warburg respirometric method of evaluation has proved particularly suitable for experiments in honey bee respiration. This technique enables one to evaluate respiration in terms of environmental, applied, and inherent factors which contribute either to decline or pacification of an isolated subject[8]. Considering the total oxygen consumption as the most reliable index of energy expenditure, it has now been confirmed that bees reacting to a chemically applied stress expend more energy yet live longer than untreated bees which react to confinement. Conversely, it has also been learned that untreated, excised abdomens expend more energy and live longer than treated abdomens.

Table 1. RELATION OF OXYGEN CONSUMPTION TO MORTALITY FOR: (1) INTACT HONEY BEES UNDER CONFINEMENT, IRRITATED AND NON-IRRITATED; (2) IRRITATED AND NON-IRRITATED SEVERED ABDOMEN*

Condition of bees	Oxygen consumption (μl.)	Period for 100% mortality (h)†
Severed abdomen irritated	52	36
Severed abdomen non-irritated	56	40
Intact bees irritated	144·5	10
Intact bees non-irritated	122·2	8

* Irritated with menadione 0·001 ml. of 0·25 per cent.
† Sixteen insects per test, three replicas of each test.

Respiration investigations have presented evidence that there exists in the foraging honey bee some potential of fatal autointoxication which becomes manifest when it is denied the exercise of normal habitat-maintaining behaviour. Results of these investigations have produced evidence that the period a bee can endure forced confinement is not based on expenditure of energy alone. The relationship of light to 'psychogenetic stress' has been evaluated by the simple expedience of altering light and its properties over bees confined within respirometric flasks. Quantitative analyses of the insects' threshold tolerances have thus been recorded, and take into consideration sensory mechanisms which moderate differences between normal and abnormal behaviour.

It is felt that more consideration might be given to insecticides which are known to trigger early stress symptoms, with subsequent autotoxicity, to promote the desired total lethal effect. One cannot help but question the part played by the insecticide *per se* when the important factor of 'physiological' stress remains undetermined. Therefore one is led to speculate on the possibility of using lower dosages of hazardous insecticides, with allowances for associated stress to contribute its role in mortality.

The still undetermined chemistry of toxic substances generated during the processes of stress should be considered[2]. It is suspected that these substances are released into the hæmolymph as evidenced by the dramatic effects this fluid has on normal tissue. When minute amounts of hæmolymph are transferred from the body of a 'stressed' honey bee on to the exposed organ of sting from a normal bee, a muscular hyperactivity occurs which is followed by abrupt and final cessation of any further movement. A freshly extracted, intact sting, when carefully removed and placed on a clean surface, continues to pulsate for some 20 min and becomes an excellent medium with which to test the pharmacodynamics of certain chemicals[9]. Body fluids removed from a 'stressed' bee 24 h after death are even more toxic. They will take immediate effect on an exposed sting and after injection into a healthy bee will cause death. It appears that a form of 'septicæmia' slowly materializes within the body of a bee which has succumbed to stress which is not detected in bees that have perished from other causes.

These preliminary observations have resulted in two new conclusions: (1) The honey bee, already recognized as a highly specialized insect, is still basically a creature of instinct. Apart from a limited number of reactions which give some evidence of adaptation, association, memory and learning, the bee responds in a fixed way to a given change in its environment[10]. We have known for some time that a factor of morale exists in the colony and we now have evidence that a honey bee is also subject to symptoms of 'neuroses' which become manifest in stress. (2) The processes of stress give rise to autotoxicity, which proves fatal to the bee, and the toxic substances, especially when aged and transferred to normal bees, are equally fatal.

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FORMATION OF TRUE THREE-DIMENSIONAL CRYSTALS OF THE TOBACCO MOSAIC VIRUS PROTEIN

By R. MacLEOD, G. J. HILLS and Dr. ROY MARKHAM, F.R.S.

Agricultural Research Council, Virus Research Unit, Huntingdon Road, Cambridge

THE protein components of the tobacco mosaic virus and its relatives are rather remarkable substances. Although they can vary considerably in their amino-acid compositions, they can all assume a configuration that is ideally adapted to shield the sensitive and important ribonucleic acid part of the virus from its external environment, by forming a left-handed[1] tube having $16\frac{1}{3}$ peptide chains per turn[2] which not only encloses the nucleic acid thread as an inclusion compound but also fits it in such a way that there is a stoichiometric relationship between the primary phosphate groupings and the protein, presumably involving ionic compounds with the arginine guanidino groups[3]. The protein is also able to form this type of tube in the absence of the nucleic acid, either under natural conditions, when it is referred to as 'X' protein[4], or when the nucleic acid has been removed artificially by the use of dilute alkali or by acetic acid, when it is usually referred to as the 'A' protein[5,6]. However, in these last instances the restraints imposed by the inclusion of the nucleic acid thread are not present, and the protein molecules are then also able to form tubes composed of disks stacked

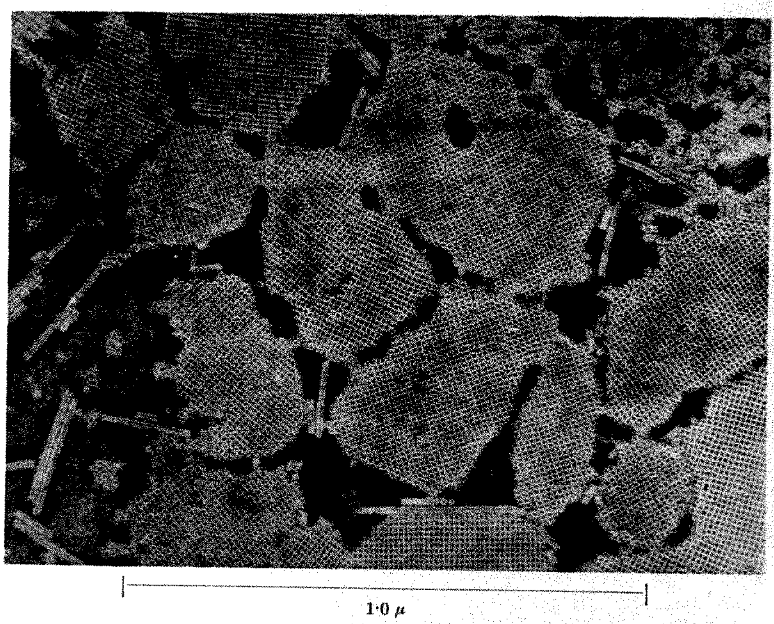

Fig. 1. Low-magnification electron micrograph of tobacco mosaic virus protein crystal. Note the angles on the crystal edges, the rods composed of stacked pairs of disks and individual flat disks

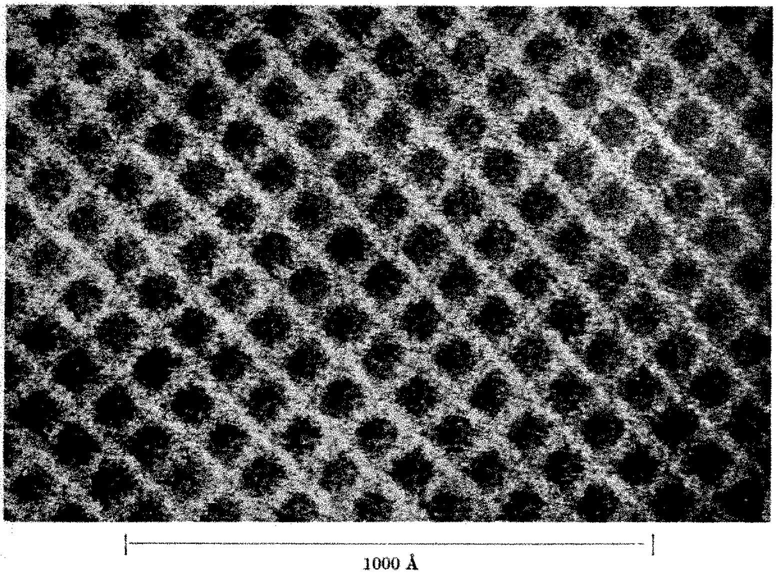

Fig. 2. High-magnification electron micrograph of the square mesh structure seen on the surface of the virus protein crystals

on each other in pairs and having 16 peptide chains per disk arranged radially[7]. This type of structure so closely resembles the helical tube that it is only quite recently that it has been recognized. However, its structure is readily noticed once one is aware of its existence, and we have noted that this type of tube is a not uncommon constituent of tobacco mosaic virus preparations.

Re-polymerized tobacco mosaic virus protein, whether in the form of a helix or a stack of disks, differs from the intact virus in that it has little tendency to form rods having a uniform length, and some rods may be as short as one pair of disks, which would appear to be the smallest stable structure of this type. Quite recently we have come across an entirely new way in which these disks can arrange themselves, namely, as true three-dimensional crystals. It seems not unlikely that when larger crystals of this type are grown, they may be used to determine the precise configuration of the peptide chains constituting the sub-units of the virus, using X-ray diffraction techniques, because such crystals should have advantages over the gels of virus that have been used so far.

The virus preparation used in this work was derived from the 'type' strain from Berkeley, California, and was adjusted to pH 10·3 with sodium hydroxide and left at 4° C for 24 h. The solution was then dialysed exhaustively against distilled water. On examination under the electron microscope, using potassium phosphotungstate as a negative stain[8,9], a number of flat plates were seen having a striking rectangular network rather like a mesh screen (Figs. 1 and 2). At first sight it was difficult to interpret these pictures because the size of the mesh was considerably smaller than the diameter of the virus rods, and the apparent width of the elements composing the mesh was not consistent with any of the dimensions with which we were familiar.

It transpired that these structures were artefacts of a new type. The appearance of negatively stained objects is very often so appealing to the eye that one tends to overlook the fact that the final image is not formed by those regions in the object where matter is present but, on the contrary, by those places where there is no matter to prevent the ingress of the negative stain, and that these are by no means complementary situations, particularly when one is examining a crystal lattice which may well have several planes in which the voids are distributed differently at different levels. In such cases any transparent, electron-transmitting, areas merely represent the projection on the plane of the image of those vertical regions where the stain is more or less excluded, and such a projection need not necessarily resemble in its major details the structures under examination, though, of course, there is a definite relationship between them.

This state of affairs is shown diagrammatically in Fig. 3. This figure shows the situation obtaining when transparent disks are arranged in a square lattice, with the interstices filled with an opaque stain. One then sees an array of four-cusped, squarish, stained regions arranged with the same spacing as are the disks, but displaced with respect to them. If a second layer of disks is then superimposed with their centres on the centres of the spaces between the disks in the first layer, two layers of cusped areas are superimposed out of step and the only regions not obscured by stain are the places where the disks in the two layers overlap. It will be seen that one then obtains an approximation to a square mesh with an edge period of $1/\sqrt{2}$ times the diameter of a single disk at 45° to the array of disks, which incidentally are now arranged in two successive layers of a body-centred tetragonal lattice as viewed normal to the (001) face. Further layers of disks can be

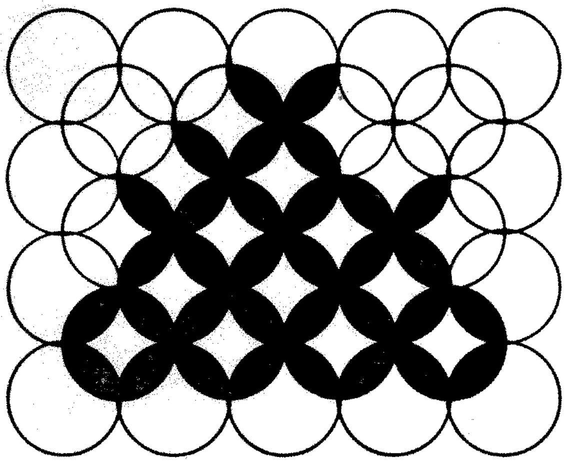

Fig. 3. Diagram showing how the square mesh of Fig. 2 is formed by the overlapping of virus protein disks arranged in a body-centred tetragonal lattice. In this figure the areas which overlap are mostly shaded in black, but some are left unshaded to show how they originate

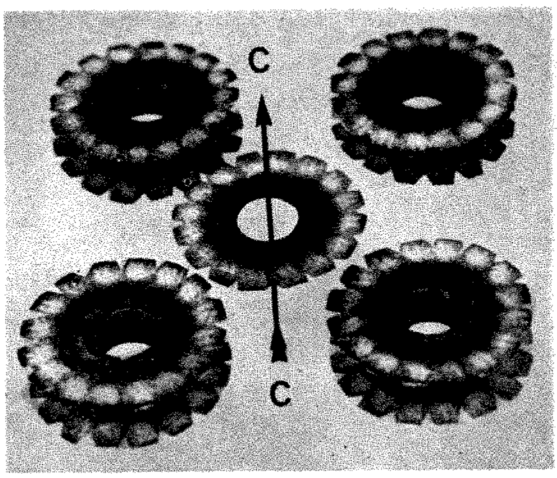

Fig. 4. Diagrammatic exploded view of the unit cell of the tobacco mosaic virus protein. The individual disks in this figure are copies of the radially averaged electron micrograph in reference No. 7

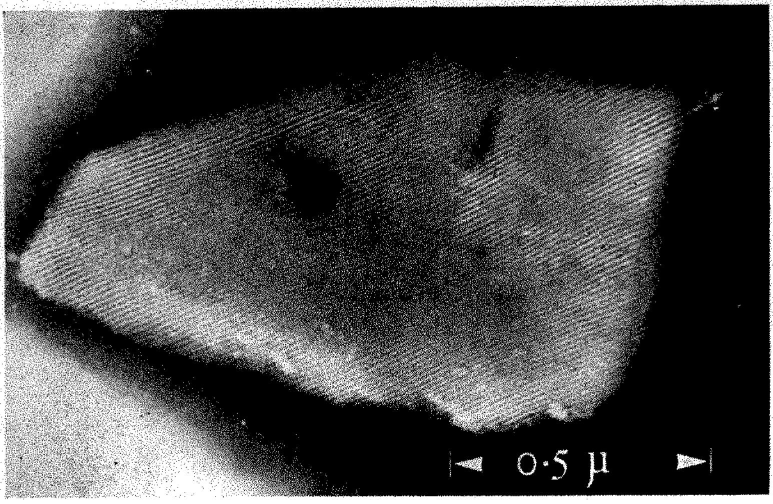

Fig. 5. Medium-sized crystal showing parallel lines on its surface

superimposed indefinitely using the same rules without altering the appearance of the image in any way, and it will be noted that if the disks have central holes this will not have much effect on the final image, because all such holes are situated over or under larger spaces in other layers of the lattice.

The interpretation of the structure of the crystalline plates seen in the electron micrographs is now obvious. They are evidently the (001) planes of tetragonal crystals. The edge of the square mesh was measured and was found to be approximately 100 Å, a value that was based on the previously measured magnification of the microscope. This would indicate that the centres of the disks are about 140 Å apart and that a certain amount of interpenetration or deformation has taken place during the formation of the crystals. The tetragonal habit is, of course, consistent with the 16-fold structure of the disks, and it is also consistent with the presence of the angles of 90° and 135° which are such a prominent feature of the edges of the crystals (Fig. 1). A diagram of the way in which the disks are packed in the unit cell is shown in Fig. 4. This unit cell contains two sets, probably two pairs, of disks, in which case it would have a molecular weight of about 1·6 million. For obvious reasons it was not possible to estimate the dimension of the *c* axis of the unit cell directly, but in the dried crystals it is probably about 70–100 Å, an estimate based on measurements of pairs of disks seen from the side.

Although most of the crystals exhibit a square lattice some have a rectangular one, and very thick crystals may only exhibit parallel lines (Fig. 5). This might be due to a slight deviation of the crystal lattice from true tetragonal form, but we are inclined to attribute it to minor distortion in the specimens and substrate.

The crystals appear to be formed during the dialysis of the alkaline solution of the '*A*' protein as an alternative to the polymerization to give the stacked disks and helical rods which are also to be found in the suspensions, and though small and thin are large enough to be seen under the optical microscope by phase contrast. The precise conditions favouring the formation of three-dimensional crystals at the expense of the two rod-shaped forms are not known, but are probably a function of the *p*H and ionic strength of the solutions. This is being investigated further.

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AGE AND SIGNIFICANCE OF DIABASE DYKE SWARMS OF THE CANADIAN SHIELD

By W. F. FAHRIG and R. K. WANLESS

Geological Survey of Canada, Ottawa

BASALT is the only abundant magma type thought to have been derived more or less directly from the Upper Mantle. In recent years the understanding of the petrology of this rock type has been enhanced through the study of basaltic provinces and through experimental work, particularly at the Geophysical Laboratory in Washington[1]. Diabase dykes of the Canadian Shield are thought to be chiefly tholeiites and representatives of one of the most common basaltic magma types. These dykes are so extensive in time and space that a broad survey of these intrusive rocks will provide additional information that will be useful in the integration of experimental and natural studies. The initial phase of the study of diabase dykes is concerned with the geometric outline of the swarms, with the age of the swarms and with the relationship of these features to the broad tectonic development of the continent. The first report of a potassium–argon study specifically aimed at the determination of the age of diabase swarms of the Canadian Shield is that of Burwash *et al.*[2].

The potassium–argon age measurements of diabase dykes used in this work were made on separated mineral fractions, chiefly biotite, and on whole-rock specimens of chilled dyke contacts and coarser-grained parts of dykes. Specimens from the chilled contacts generally yielded potassium–argon ages in excellent agreement with those of separated biotites. In one instance, a whole-rock potassium–argon age on coarse-grained material is 25 per cent greater than the best reasonable estimate of the age of the intrusion. Some of the averages reported here may be modified by additional work.

For convenience of reference the dyke swarms herein discussed have been numbered 1 to 13 on Figs. 1 and 2, proceeding roughly from the oldest to the youngest. The preliminary results of this dating programme are shown in Table 1.

Table 1

Dyke swarm	Material analysed	Average potassium–argon age
1	1 whole rock	2,485
2	4 whole rock 1 biotite 1 hornblende	2,150
3	2 whole rock 1 biotite	2,165
4	4 whole rock	2,090
5	4 whole rock	1,675
6	2 biotite	1,230
7	4 biotite 1 whole rock	1,315
8	2 biotite 2 whole rock	1,220
9	2 whole rock	1,030
10	2 whole rock	875
11	1 whole rock	790
12	3 whole rock	700
13	2 whole rock	640

These results indicate that diabase dyke swarms have intruded the Shield frequently throughout the span of Precambrian time.

The dimensions of some swarms and their changes in trend are so spectacular that they warrant special comment. Swarm No. 7, for example, extends over an area more than 1,000 miles long and locally more than 600 miles wide. Individual dykes of the swarm exceed 150 miles in length. The greatest concentration of dykes occurs in the Mackenzie District. Noteworthy changes in dyke orientation and concentration occur in this swarm as it passes from one structural province to another. The number of dykes in swarm No. 7 decreases in passing from the western parts of the Slave Province into the Bear Province, and the trend of dykes present in the Bear Province is more northerly. As a result, the swarm of dykes appears to converge and focus in a zone of dense intrusion and anomalously high gravity readings. South-east of Great Slave Lake dykes of swarm No. 7 show a change in trend as they enter an area shown by a dashed line on Fig. 2 where most of the potassium–argon ages are in the Hudsonian range of 1,700 million years but where occasional older dates have been reported[3]. Dyke swarm No. 8 is also spectacular in size, and north of Lake Superior the dykes of this swarm show a sweeping change in strike that suggests deflexion of the pattern of fracture around the boundary of the Southern Province.

The intrusion of dyke swarms Nos. 7 and 8 were igneous events of major significance in the middle Proterozoic history of the Shield. We suggest that many of these dykes penetrated the Earth's surface and vast outpourings of plateau basalt accompanied their intrusion. Probably hundreds of thousands of square miles of the Mackenzie District and the densely intruded areas of Ontario were covered by these outpourings. The detritus from the erosion of these flows would form a significant component in sediments being deposited in developing mobile belts adjacent to the craton.

If we accept the hypothesis that dyke swarms of regional extent were feeders to surface flows or near surface sheets, then periodically a blanket of tholeiitic lava was present

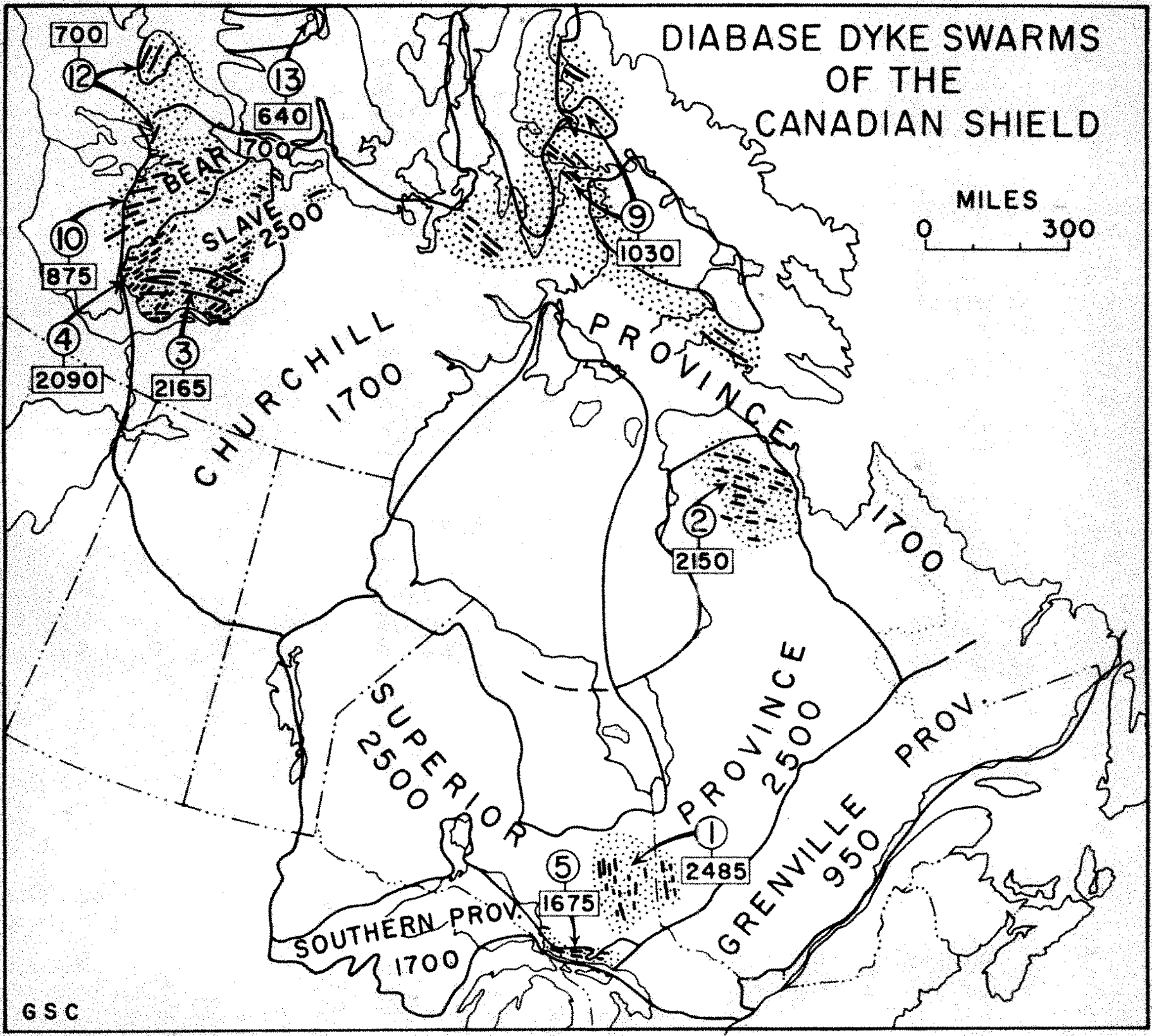

Fig. 1

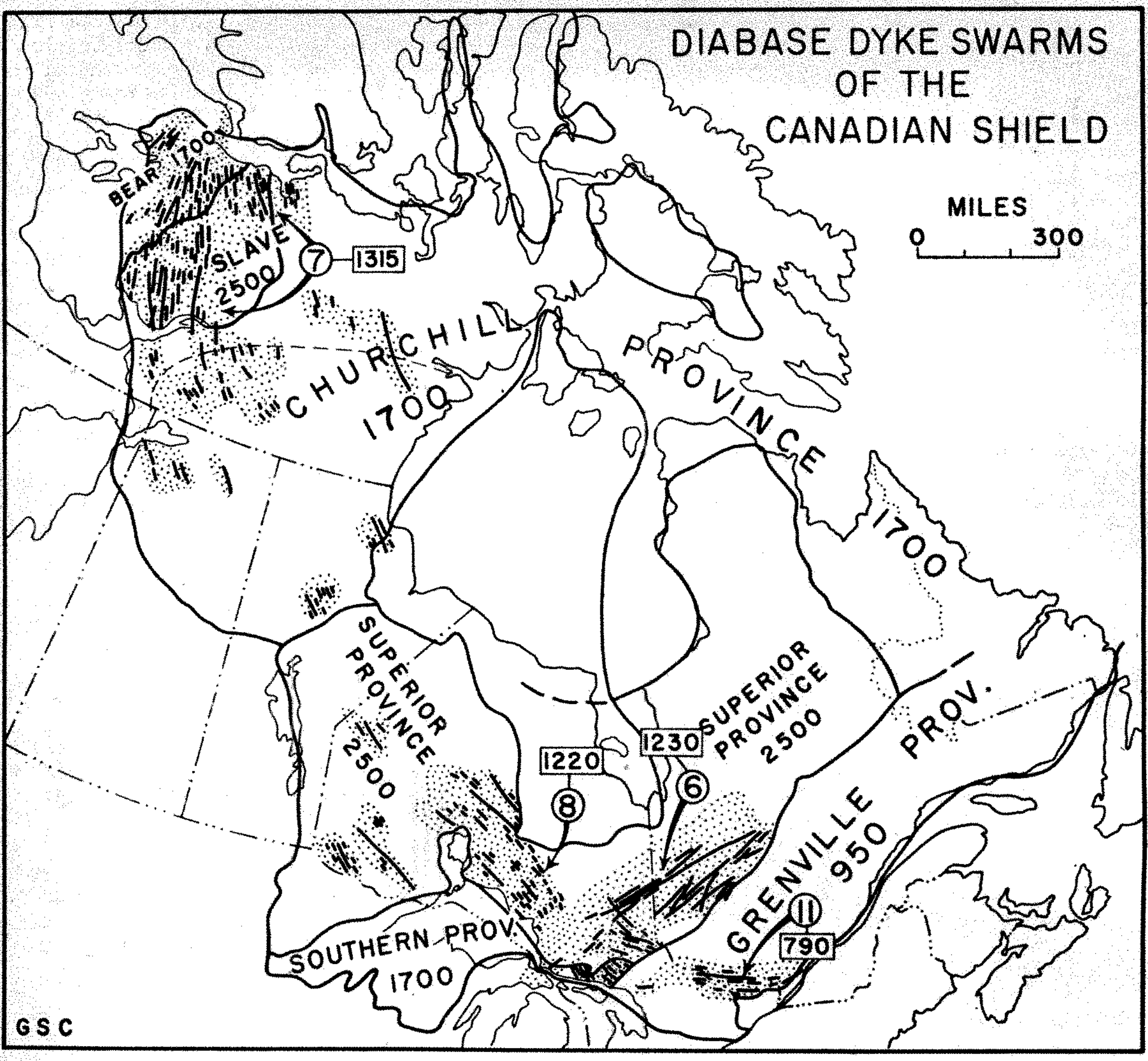

Fig. 2

at or near the surface of the Shield. Significant amounts of sialic crustal material could then be derived from the craton underlying these flows or sheets only after they had been removed by erosion. Basaltic cover rocks believed to possess a low rubidium–strontium ratio may therefore have contributed significant quantities of clastic materials to marginal basins and these could therefore influence the observed strontium-87/-86 ratio now found in younger metamorphic terrains.

In addition to this effect, under suitable climatic conditions, the erosion of the basaltic cover rocks or near-surface diabase sheets would release large quantities of more soluble material for precipitation in surrounding miogeosynclines. The great Precambrian deposits of iron-formation in the Shield may derive their iron not from the contemporaneous volcanic extrusions of the newly forming orogenic belt, but chiefly from the erosion of somewhat older tholeiitic lavas or near-surface sheets fed through fissures on the neighbouring craton. These outpourings probably represent the terminal activity of a previous orogenic cycle and in a tectonic sense are fundamentally different from the basalts of the developing eugeosyncline. Furthermore, in comparison with dyke-fed tholeiitic flood basalts, the surface areas of older infolded basalts of the Shield, for example, the Keewatin-type basalts, would be insignificant. This idea is particularly attractive as it appears to deal successfully with the two major problems in the genesis of this type of sedimentary iron-formation, namely, the banding of the iron-formation and the virtual restriction of this type of iron-formation to the Precambrian. If the iron is released by sub-aerial weathering of plateau basalt, the banding can be related to the yearly weathering cycle, and the rarity of iron-formation of this type in the Phanerozoic can be ascribed to a relative paucity of flood basalts on the craton during Phanerozoic time or to inappropriate climatic conditions or to both. The sub-aerial weathering of tholeiitic basalt would, as compared with the weathering of the average Shield rock, result in a much lower proportion of resistates. This is compatible with the character of Precambrian iron-formation.

It is interesting to speculate on the possible large-scale tectonic significance of dyke swarms. In some instances the trends of the swarms appear to be perpendicular to the directions of compression present during the orogenic episodes that immediately preceded dyke intrusion. For example, swarms 2 and 3 are roughly parallel to structural trends, and perpendicular to the direction of compression in the Superior province. Swarm 6 probably lies perpendicular to the direction of compression of the crust in this area during the Hudsonian orogeny. Swarms 7 and 8 parallel the western and southern boundary of the Shield and probably lie perpendicular to a middle Proterozoic compression.

It is suggested that elastic rebound of the crust after a period of crustal compression may result in low-pressure belts into which basaltic magma is introduced. We suggest that intense folding and compression in the orogenic belt and less intense compression within the craton results from sub-crustal currents. If relaxation occurs during or after a major orogenic cycle, as a result of diminution in size of sub-crustal currents, diabase dyke intrusion may occur both in the orogenic zone and in the adjacent stable craton If a period of tension is succeeded by additional mountain building accompanied by folding, metamorphism and granitic intrusion in the orogenic belt, diabase dykes related to the preceding period of tension will be preserved or recognizable only in the stable craton A series of dyke swarms in the craton may reflect more intense rhythmic compression and relaxation in neighbouring developing mobile belts

The examination of dyke swarms promises also to be of value in the interpretation of certain geochronological events in the development of the Shield For example, it is noted that dykes of swarms Nos 2 and 3 are the same age within limits of analytical uncertainty and they exhibit concordant trends If these trends are projected, the dyke swarms of approximately 2,200 million years could pass through the main body of the Churchill Province, in an area where potassium–argon dates are generally within the 1,700 million year range of the Hudsonian orogeny Stockwell[4] has reported that, within the Churchill Province between Nonacho Lake south-east of Great Slave Lake and eastward toward Hudson Bay, a number of potassium–argon ages ranging from 1,900 to 2,460 million years are present among more common Hudsonian potassium–argon ages of 1,700 million years He has interpreted these as survival dates from the Kenoran orogeny which affected this area about 2,500 million years ago Within this part of the Churchill province the younger dykes of swarm No 7 at 1,315 million years are prominent, but metamorphosed segments of older east–west dykes are also relatively common These may represent the extension of the pre-Hudsonian swarms Nos 2 and 3 through the Churchill Province If this is the case, doubt is cast on the idea that the Slave and Superior Provinces were originally isolated continental nuclei

We thank the Director of the Geological Survey of Canada for permission to publish this article

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OBITUARY

Prof. Jacques Hadamard, For.Mem R S

JACQUES SALOMON HADAMARD was born in Versailles on December 8, 1865 His mathematical abilities very soon became evident when he won the entrance examination to the École Polytechnique, perhaps the stiffest mathematical entrance examination in the world, with a record of 1,875 marks out of a total of 2,000 After graduation he obtained a teaching post at the University of Bordeaux, but soon returned to Paris as a professor at the Sorbonne In 1897 he went to the Collège de France, from which he retired in 1935 The Second World War forced him to go to the United States, where he found a temporary home in Princeton In 1945 he visited Great Britain and gave a charming lecture on personal recollections as a mathematician, which is still remembered with pleasure by many of his British colleagues He soon returned to Paris, leading a very active life in spite of his old age, until he died on October 17 in his ninety-eighth year

Most of his work in pure mathematics was done in the 1890's on analysis Hadamard, desiring to investigate the Riemann ζ-function, developed a theory of integral functions of a complex variable which has been the foundation of all the subsequent work on the subject In 1896 he proved the prime number theorem which had been conjectured by Gauss and Legendre a hundred years earlier let $\pi(x)$ be the number of primes not exceeding x, then $\pi(x) \sim x/\log x$ The same result was proved simultaneously by de la Vallée-Poussin Though a lot of work has since been done on the prime number theorem, Hadamard's proof is still the simplest and most natural proof of the theorem It must be a unique event in the history of science that a major break-through is achieved simultaneously by two scholars who each survived their achievement by two-thirds of a century

A few other results can be briefly mentioned

(1) Hadamard's inequality for real determinants

$$\|a_{ik}\|^2 \leqslant \prod_{i=1}^{n} \sum_{k=1}^{n} a_{ik}^2$$

(2) Hadamard's "3 circle theorem" let $m(r)$ be the maximum of the absolute value of a regular complex function in a circle with fixed centre and radius r Then $\log m(r)$ is a convex function of $\log r$

(3) Hadamard's gap theorem for a Taylor series of the form

$$\sum_{n=0}^{\infty} a_n x^{b_n}$$

Every point on the boundary of the circle of convergence is a singular point of the function if $b_{n+1} - b_n \rightarrow \infty$

His work in applied mathematics was mainly concerned with wave propagation and is of classical importance No standard text on the fluid mechanics of high-speed flows, for example, would be complete without basic reference to it, nor would any account of Huygens's principle Its influence permeates present-day thinking on solutions of the wave equations and allied problems Starting from the work of Riemann, Kirchhoff and Volterra, he developed in 1903 (ref 3) and in his later work in 1923 and 1932 (refs 4 and 5) the theory of hyperbolic linear differential equations for the general case of n independent variables, distinguishing between the cases when n is odd and even Here in these books are set out the tools in the form of the theory of characteristics and bicharacteristics, the principle of descent and the concept of the finite part of an infinite improper integral, without any of which an understanding of high-speed flows would be impossible Hadamard was not only interested in existence and uniqueness properties, but also, as Friedrichs remarks, in the property of the continuous dependence of the solution on the data, a property which is required if the problem is to be called correctly posed With the advent of modern computational techniques the application of the method of characteristics has become a standard method of solution not only for linear but also for quasi-linear hyperbolic differential equations, and three-dimensional aerofoil theory leans heavily on the concept of the finite part of an infinite integral

Hadamard's name must also be linked with those of Stokes, Earnshaw, Riemann, Rankine, Hugoniot and Rayleigh in developing the ideas of non-linear wave motion which led to the present theory of shock waves and their formation

Hadamard's merits as a mathematician were fully recognized at home and abroad He held many honorary doctorates and was an honorary member of many learned societies, in particular a foreign member of the Royal

Society and an honorary member of the London Mathematical Society.

Hadamard published the following books: (1) *La série de Taylor et son prolongment analytique* (Paris, 1901), (2) *Notice sur les travaux scientifiques de M. Jacques Hadamard* (Paris, 1912), (3) *Leçons sur la Propagation des Ondes* (Paris, 1903), (4) *Lectures on Cauchy's Problem in Linear Partial Differential Equations* (Yale, 1923, also Dover Publications, 1952), (5) *Le problème de Cauchy et les équations aux derivées partielles linéaires hyperbolique* (Paris, 1932), (6) *An Essay on the Psychology of Invention in the Mathematical Field* (Princeton, 1945).

H. HEILBRONN
L. HOWARTH

NEWS and VIEWS

Electronics at the University of Southampton:

Prof. E. E. Zepler

E. E. ZEPLER became the first head of the Department of Electronics on its creation in the then University College of Southampton shortly after the end of the Second World War. In 1949 he was elected to the newly established chair of electronics at the College, thus becoming the first professor of electronics with an independent department in Britain. Zepler's earlier experience with Telefunken in Germany (1925–35), where he became head of receiver and direction finder design, with the Marconi Co. in England (1935–40) and at the Cavendish Laboratory during the War, fitted him admirably for the post. The present time sees him leaving a thriving department with strong postgraduate teaching and research schools as well as undergraduate courses designed both for scientists and for engineers. His keen interest in electronics teaching found a further outlet in his associations with the British Institution of Radio Engineers, the educational policy of which was formed largely under Zepler's influence. He was elected president of the Institution in 1959–60 and still serves on the Council of the Institution as a vice-president, as well as on several of its committees. Zepler is also well known as the author of *Radio Receiver Design* and more recently as the co-editor (with S. W. Punnett) and part author of two further books concerned with *Electronic Circuit Techniques* and *Electronic Devices and Networks*. His publications also include, however, several books concerned with chess and chess problems. He is an outstanding chess player, having represented both Essex and Hampshire in tournaments since settling in Britain in 1935. Zepler's lively personality and his friendliness to all who have come into contact with him will not easily be forgotten, and it is a source of great pleasure to his many friends that in retirement he will continue to be associated with the University as a Senior Research Fellow in the Institute of Sound and Vibration Research.

Prof. G. D. Sims

DR. G. D. SIMS of the Department of Electrical Engineering, University College, London, has been appointed to succeed Prof. Zepler. Dr. Sims studied in both the Physics and the Mathematics Departments of the Imperial College of Science and Technology from 1945 until 1948, obtaining both a B.Sc. in physics and an M.Sc. in mathematics. From 1948 until 1954 he was at the Hirst Research Centre of the General Electric Co., where he worked on a number of projects, including the use of the H_{01} waveguide mode for long-distance transmission and some problems of magnetron operation. During this period he also worked part-time at Imperial College with Prof. D. Gabor on magnetron instability phenomena and presented a Ph.D. thesis on this subject. From 1954 until 1956 he was with the Theoretical Physics Division at the Atomic Energy Establishment, Harwell, and was attached to the Particle Accelerator Group (now the Rutherford High Energy Laboratory). Two years later he joined the staff of the Electrical Engineering Department at University College, London, and, while taking a full part in the teaching of the Department, continued his interest in research by supervising a number of postgraduate research projects. These interests included plasma problems, the direct conversion of heat to electricity using thermionic diodes, and a number of microwave valve investigations. Dr. Sims took up the chair of electronics at the University of Southampton in October.

Physics at the University of Leeds:

Prof. E. C. Stoner, F.R.S.

PROF. E. C. STONER retired on September 30 from the Cavendish Chair in Physics in the University of Leeds after nearly forty years' service in the University. Born at East Molesey in 1899, he went up to Cambridge from Bolton School, took firsts in both parts of the Natural Science Tripos and within little more than three years from graduation had published a valuable experimental paper (with L. H. Martin) and a theoretical one ("The Distribution of Electrons among Atomic Levels") which was to play a decisive part leading towards the enunciation of the 'exclusion principle'. In the years to follow, his most important contributions were in the field of magnetism, here his two books were to be recognized as standard works, while of his many notable original papers those in 1938–39 on collective electron ferromagnetism were perhaps outstanding. In recent years he has encouraged a wide-ranging approach in his laboratory to problems of electronic band structure in metals. Stoner went to Leeds as a lecturer in 1924, was appointed to a readership in 1927, to a newly instituted chair of theoretical physics in 1939 and to the Cavendish chair in 1951. He held a fellowship of his college, Emmanuel, from 1928 until 1931, and was elected a Fellow of the Royal Society in 1937. Throughout his service in Leeds he taught actively, in recent years in stimulating lecture courses in quantum mechanics and statistical mechanics. He played a leading part in the formulation of post-war university policy and in all the ensuing quinquennial surveys, served as a vice-president of the [then] Institute of Physics and, very effectively, on the Post Graduate Training Awards Committee of the Department of Scientific and Industrial Research.

Isidore Geoffroy St. Hilaire Medal for 1963

MISS PHYLLIS BARCLAY-SMITH has been awarded the Isidore Geoffroy St. Hilaire Medal for 1963—so far as we are aware, the first occasion that it has been given to a woman. This Medal is awarded annually by the Société National de Protection de la Nature et d'Acclimatation de France to a person whose nationality is not French and who is distinguished for work in this field. The Medal is being presented at a general meeting of the Society, which will be held on December 8 in the Grand Theatre of the Museum National d'Histoire Naturelle, Paris. Miss Barclay-Smith has a long record of various public activities, mainly on behalf of bird preservation. She has been the secretary of the International Council for Bird Preservation since 1946 and is a member of the Home Office Advisory Committee on Protection of Birds for England and Wales. As honorary secretary of the Co-ordinating Advisory Committee on Oil Pollution of the Sea, she took an active part in the promotion of international legislation to prevent the discharge of oil and oily waste products into the sea.

Technical Teacher Training

A POLICY statement entitled *Technical Teacher Training*, issued by the Association of Teachers in Technical Institutions and based on a report prepared by the Association's Education Committee, concludes that untrained teachers have been too readily accepted in the past and that the training of teachers is essential to ensure effective use of expenditure on further education (Pp. 24. London: Association of Teachers in Technical Institutions, 1963). It recommends that, as soon as possible, all new entrants to technical teaching, both full-time and part-time, should receive professional training and normally a full-time one-year course. A recognized professional qualification of technical teachers is recommended and suitable training schemes should be developed for regular part-time teachers, whose salary scales should provide financial recognition of professional training. Experienced members of staff should be given the responsibility of guiding and assisting new entrants, and grants to trainee teachers should be closely related to what they may reasonably expect to earn on appointment. Refresher courses should be provided for certain teachers and for those courses subsistence grants should be paid, while supplementary courses in general education should be provided to help meet the demand for teachers for craft and technician courses. Better liaison facilities are required between technical colleges and technical teacher training colleges, and the salaries paid in the latter must be sufficient to attract some senior staff from the former. While there is room for experiment with sandwich courses in teacher training, more experience is required for a final judgment. Existing teachers should be encouraged to improve their qualifications, and emphasis is laid on the need for much more research into problems of further education. Educational fellowships in all types of educational institutions are advocated to enable teachers to undertake educational research. Besides further extension of the existing technical teacher training colleges, at least two new colleges are required, one in the north-east and one in the south-west of Britain, and it is suggested that training departments should be opened in colleges of technology and some technical teaching training given in university departments of education. The new 'staff college' should cater for all fields of further education and for those concerned with educational administration and industrial training.

The Advancement of Science in India

THE Indian Science Congress Association has issued as a reprint the text of Dr. D. S. Kothari's presidential address, "Science and the Universities", given at the fiftieth session of the Congress at Delhi during October 7–10, 1963 (Pp. 17. Calcutta: Indian Science Congress Association, 1963). Dr. Kothari begins by quoting the Prime Minister's statement on scientific policy five years earlier and then discusses the implications of what he terms the 'current scientific revolution', in which the doubling period of science is about fifteen years in terms of co-operation and the unpredictability in the march of events. Not merely does it mean that some 90 per cent of scientists who have ever lived are alive to-day, but also that the time-gap between basic discovery and its application is continually diminishing. The gap between the richer and poorer countries, the developed and the underdeveloped countries, is widening rapidly, so that it is increasingly difficult for the developing countries to import the capital equipment and machinery needed to raise their production. This situation is detrimental to the interests of both developed and underdeveloped countries, but it is possible for the developed country to make major contributions to the rapid development of a newly developing country by supplying the initial nucleus of technical exports and material resources. Dr. Kothari recognizes that research and development expenditure is not an entirely satisfactory index of the development of science in a country, but there is a close and direct connexion between the percentage of national income spent on research and development and the number of scientists and technologists expressed as a percentage of the population. Stressing then the contribution of the universities to scientific advance, both in teaching and in research, Dr. Kothari refers to the unity of science and the danger of fragmentation, the imperative importance of a climate of free enquiry, the growing significance of team-work and the problems presented in the allocation of resources. These are the considerations which indicate where the universities can contribute most decisively to the scientific interests of a country, and finally he emphasizes the value, in a country of limited resources like India, of the centres of advanced study which the University Grants Commission was seeking to establish.

Higher Education in India

THE report of the University Grants Commission to the Government of India for the year 1961–62 records an increase in the number of universities from 33 to 46 during the Second Plan period, and with the five established during the year the total is now 51, of which 35 are affiliated, 14 unitary and 2 federal in character (Pp. v+36. New Delhi: University Grants Commission, 1963. Rs. 1.50; 3s. 6d., 54 cents). Several steps were taken in the development and expansion of engineering and technological education in the universities, and a Committee was appointed to make a thorough study of standards prevailing generally, while another Committee is considering the selection of some twenty centres of advanced study in various universities. The Commission continued to be concerned with examination reform and measures for promoting general education, and has offered assistance in organizing summer schools, institutes, conferences and seminars in different subjects. Great importance is attached to the development of the affiliated colleges in which about 86 per cent of the university students in the country are enrolled, and where the organization of postgraduate teaching presents problems. Some twenty-two State universities are now participating in the Commission's scheme for raising salary-levels to that of the central universities. The number of students increased by 11 per cent to 1,156,000, the increase of women students, now 186,030, being particularly notable, and the overall increase was shared by all faculties, the percentage increase being greatest in agriculture (78.6), engineering and technology (56.8), medicine (48.2) and education (46.7). Postgraduate students increased in number from 63,000 in 1960–61 to 72,859 (22 per cent of whom were studying science subjects): the total has almost doubled in the past five years. The percentage of failures in 1960 varied from 57 per cent in the B.A. examination, 50.8 per cent in the B.Com., 49 per cent in the B.Sc., and 46.6 per cent in the M.B.B.S. to 13.7 per cent in the B.Sc. (Tech.), but many successful candidates secured only minimum marks.

Organization of Scientific Research in Poland

IN a brief survey of the development of scientific research in Poland, published in *Polish Facts and Figures* (No. 729, October 19, 1963), Prof. I. Malecki refers to considerable changes in the scope and organization of research in Poland since 1952. Between 1950 and 1963 industrial production increased 3.7 times and there are now 172,000 students in 73 universities and specialized colleges, which employ 16,300 scientific workers, while the Polish Academy of Sciences employs 6,200 persons (including 500 professors and assistant professors), compared with 2,000 in 1953; 21 research institutes and 56 laboratories are now attached to the Academy. The two institutes of nuclear research, while under the scientific supervision of the Academy, are controlled by the High

Commissioner for Nuclear Energy The institutes attached to the Academy are mainly responsible for oriented fundamental research, while in the universities emphasis is laid on free basic research Applied research and technological development are conducted mainly in the industrial research institutes, the research staff of which has increased seven-fold in the past decade. The Polish Academy of Sciences is responsible for planning and co-ordinating fundamental research, and especially for long-term plans for developing science The general co-ordination of applied research and technical development is the responsibility of the Committee for Science and Technology established in 1963, which is also responsible for the finance of research and for the development of scientific manpower Scientific co-operation with foreign countries is regarded as an important element in the contemporary development of science, and the Academy's institutes have established numerous contacts with scientific institutions abroad In 1962, 94 Polish scientific workers went to Britain on behalf of the Academy and 74 British scientific workers went to Poland at the Academy's invitation

The American Academy of Arts and Sciences

THE *Records of the American Academy of Arts and Sciences, 1962–63*, comprises the usual lists of officers and committees, lists of members by classes and alphabetical, including new members elected on May 8, 1963, the statutes, records of meetings 1962–63, and the annual reports of committees (Pp 152 Boston, Mass American Academy of Arts and Sciences, 1963) The last-mentioned section includes one from the Committee on Pugwash Conferences on science and world affairs The sub-committee for international conferences has been reconstituted as a new full Committee on Pugwash Conferences on Science and World Affairs with a Sub-Committee on Informal International Conversations among Scientists

The National Museums of Southern Rhodesia

THE National Museums of Southern Rhodesia include three museums, in Bulawayo, Salisbury and Umtali The year 1962 witnessed the completion of the building programme of the Museums of Southern Rhodesia inaugurated in 1960 (Report of the Trustees and Directors of the National Museums of Southern Rhodesia for the year ended December 31, 1962 Pp 22 Salisbury and Bulawayo National Museums of Southern Rhodesia, 1963) At the present time, there are new museums in all three centres The full programme has cost £450,000 and the raising of this amount in a time of financial stringency has been no mean achievement The generous assistance of the big Mining Corporations, the State lotteries and Municipal contributions made this possible Pre-occupation with the building projects has of necessity meant the curtailment of the usual field-work and research activities Many of the recommendations of the three Fulbright Scholars who were at the Museum in 1961 have come to fruition These include the re-stocking of the National Parks where wild-life no longer existed, the utilization of wild-life as a source of protein, and the establishment of a postgraduate course on wild-life conservation at the University College

Simplified Gas Chromatography

THE 'Junior' gas chromatograph produced by Messrs Gallenkamp is a robust instrument designed specifically for teaching purposes but with a performance adequate for any routine industrial applications The new apparatus is expected to satisfy all the requirements of universities and technical colleges in which the theory of gas chromatography is combined with practical work Both gases and liquids with boiling points up to 80° C can be analysed The sample is introduced by syringe on to an unheated, general purpose, coiled silicone column The thermistor katharometer is powered by a long-life battery and is very stable and extremely sensitive Peaks may be plotted either by using a galvanometer or by the recommended 6-in strip chart recorder The detection limit of the katharometer is about 10^{-7} g and chromatograms can be obtained from about 10 µl liquid or 0 5 ml gas

Radioactivity in Drinking Water in the United Kingdom

THE report entitled *Radioactivity in Drinking Water in the United Kingdom. 1962 Results* gives measurements made under the Government's programme for monitoring radioactive fall-out in drinking water (Pp 24 London H M S O, 1963. 1*s* 9*d* net) Like the previous four reports in the series, it contains the radiostrontium and radiocæsium results obtained, and, in addition, details are included of investigations into the levels of other fission products in drinking water Because of the nuclear weapon tests during 1961, the amount of strontium-90 in drinking water rose above the 1961 level to about the 1959 level (which reflected the results of tests made during 1958) The concentrations of strontium-90 and of cæsium-137 were measured regularly in twenty-four public drinking waters Practically no concentration was found in deep well waters at Brighton and Wolverhampton and low concentrations in the Folkestone supply In the river-derived waters the average level of strontium-90 was 0 36 pc /l, but in the water derived from the chalk stream of the River Itchen at Southampton the concentration was again much lower, 0 065 pc /l. The average daily ingestion of strontium-90 and cæsium-137 is estimated at 0 41 pc per person and 0 16 pc per person, respectively These amounts represent less than 5 per cent and 1 per cent, respectively, of the average daily intake of these radionuclides in the diet It has been confirmed that strontium-90 is the most important of the fission products in drinking water to be determined and that when the levels of iodine-131 rose in milk during 1961 and 1962, following the commencement of nuclear tests, the rise in drinking water was negligible

Assay of Strontium-90 in Human Bone in the United Kingdom

IN its *Monitoring Report Series*, the Medical Research Council publishes, at six-monthly intervals, the results of analyses of strontium-90 in human bone samples, mainly from subjects who have died during the period under review The analyses are carried out by the Atomic Energy Research Establishment and by research workers in Glasgow and Cambridge No 6 in the Series surveys results for the first half of 1962, and No 7 for the second half (No 6 *Assay of Strontium-90 in Human Bone in the United Kingdom · Results for 1962* Part 1, with some Further Results for 1961 Pp iii+15 No 7 *Results for 1962* Part 2 Pp ii+17. London H M S O, 1963 1*s* 6*d* net each part) In No 6 there are some results for 1961 representing the first instalment of a new project designed, in collaboration with the West London Coroner's office, to give information about the population of London and its environs During 1962 there was no survey of bone samples obtained through the Coroner, but analyses have been resumed during 1963 and will be reported next year The average amounts of strontium-90 in bone were approximately the same for all age groups during the first half of 1962 as previously, but an increase in the level was observed in samples from new-born (including still-born) infants and children aged 0–4 years in both the Atomic Energy Research Establishment and the Glasgow records for the period July–December 1962 The rise, which has been delayed longer than expected, is a result of the nuclear weapon tests carried out in the autumn of 1961 For the infants the average amount of strontium-90 was 1 37 pc /g calcium, that is, 1 37 strontium units and 2 5 for children in the 0–4 age group, compared with 0 78 and 1 34, respectively, for the corresponding period in 1961 The highest value recorded during the year was 7 5 strontium units, but this value is suspect, and excluding this result the highest value is 3 6 units compared with 3 5 of the previous year At the end of July 1962 analyses of bone at Cambridge were discontinued The reports contain summary tables giving details of the samples and the results of their analyses, together with the types of counter and counting methods used

Relief-radiography of Bladder Tumours

A METHOD of relief-radiography of bladder tumour by means of a 76 per cent solution of 'Urografin' has been described by Prof Naotomo Oka and Tadashi Kato, of the Department of Urology, Nagoya City University Medical School, Japan (*Nagoya Medical Journal*, 8, 3, December 1962) The method is simple to operate and the shadow or contour of the bladder tumours is clearly shown up The method is suitable for displaying other disorders of the bladder, such as prostatic tumours, trabeculation and irregularities after operations

Experimental X-ray Crystallography

AN international summer school in "Experimental X-ray Crystallography" will be held in Castel Tenno, Riva del Garda, Italy, during August 31–September 12, 1964 It will consist of a course of lectures and demonstrations intended for crystallographers concerned with single crystals Only a slight acquaintance with the subject will be assumed and all subject-matter will be treated in a fundamental manner Attention will be directed to the orientation of crystals by various photographic and diffractometer methods For this purpose the theory and application of the stereographic projection and of the reciprocal lattice will be dealt with The treatment will start from the simplest and finish with the most general problems The theory of the use of diffractometers, particularly the main automatic types, will be explained, and in the demonstrations the student will interpret data obtained from such instruments and work out programmes for transfer to digital computors These programmes will include the various corrections which are essential The use of photographic methods for the examination of the intensity of X-ray reflexions will be discussed and also the possibilities and limitations on the use of microdensitometers Students will not use X-ray diffraction apparatus though some instruments will be available for purposes of demonstration The lectures will be given by Dr W A Wooster, formerly of the Department of Mineralogy and Petrology, Cambridge, and he will be assisted in the demonstrations by Dr Nora Wooster, Mr A M Wooster and Mr G A Wooster The lectures will be given in English, but provision will be made for those who indicate their wish to use French or German The inclusive charge for the course of lectures and demonstrations will be 115 dollars, or 110 roubles, or 70,000 It lire, or £40 This sum does not include hotel charges Those interested should apply to Dr W A Wooster, Brooklyn Crystallographic Laboratory, Bottisham, Cambridge, and a second notice will be sent to all who indicate their wish to join such a course not later than January 7, 1964

University News·

Birmingham

THE following appointments have been announced *Lectureships* Dr G M Butler (botany), Mr D A. Jones (genetics), Dr A Macdonald (chemistry), Dr C J Beevers (physical metallurgy), Dr H O Berktay (electrical engineering), Dr R J Bland (electrical engineering); Mr M A Brebner (computing in the Department of Mathematical Physics), Dr H Kaliszer (mechanical engineering); Dr R Lazenby (computing in the Department of Mathematical Physics), Mr H C Wilkie (pure mathematics), *Research Fellowships* Dr N Baggett (Imperial Chemical Industries Research Fellow in the Department of Chemistry), Dr C A Barson (Senior Research Fellow in the Department of Chemistry), Dr P S Dobson (physical metallurgy), Mr J W Edington (physical metallurgy), Mr W J Feast (chemistry), Mr C I Harris (biochemistry), Mr I R Harris (physical metallurgy), Dr M H B Hayes (Senior Research Fellow in the Department of Chemistry), Mr B E Job (chemistry), Mr C D. Lewis (engineering production), Dr G S Prosser (chemistry), Mr J W G Wilson (electron physics), Mr D A Woodford (physical metallurgy), Mr G A Young (geology).

St. Andrews

THE following appointments to lectureships have been announced (1) St Salvator's College, P E Gibbs (botany), Dr R B Thomas (theoretical physics), Dr J R MacCallum (chemistry), Dr H C McGregor (zoology) (2) Queen's College, Dr C R Allen, Dr W K Busfield, Dr J W Falconer, Dr P A Warsop and Dr T J R Weakley (chemistry), R J Jarvis and Dr R M Dicker (mathematics) Dr R Foster has been appointed senior lecturer in chemistry at Queen's College

Announcements

THE following have been appointed trustees of the British Museum (Natural History) Prof O M B Bulman, professor of geology, University of Cambridge, Prof H G Callan, professor of natural history, University of St Andrews, and Sir Landsborough Thomson, chairman, Executive Committee, Council for Nature

A SYMPOSIUM on the "Utilization of Metallurgical Wastes" will be held at the National Metallurgical Laboratory, Jamshedpur, during February Further information can be obtained from the Director, National Metallurgical Laboratory, Jamshedpur 7

THE fourth Rankine Lecture of the British National Society of Soil Mechanics and Foundation Engineering entitled "Long-term Stability of Clay Slopes" will be delivered by Prof A W Skempton at the Institution of Civil Engineers on February 5 Further information can be obtained from the Secretary, British National Society of Soil Mechanics and Foundation Engineering, Institution of Civil Engineers, Great George Street, Westminster, London, S W 1

A SYMPOSIUM on "Neutron Dosimetry for Radiological Purposes", arranged by the Institute of Physics and the Physical Society on behalf of the Joint Health Physics Committee, will be held at the Royal Aeronautical Society, London, on January 24 The symposium will include five main sessions the interaction of neutrons with tissue, flux and energy measurement, determination of absorbed dose in tissue, measurement of dose equivalent (formerly RBE dose), dosimetry of high-energy particles Further information and forms of application can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S W 1

ERRATUM Certain errors were made in the obituary notice of Prof J F Piccard (*Nature*, **199**, 229, 1963) Prof Piccard's widow, Dr Jeannette Piccard, writes as follows "Jean received an honorary Dr Sc degree from Jamestown College, North Dakota, in 1957 He also received an honorary Dr Sc from the University of Southern Illinois in 1959 He never received an honorary degree from the University of Strasbourg nor the United States Distinguished Service Award Auguste Piccard, Jean's twin brother, received the degree from Strasbourg in 1932 Jacques Piccard, the son of Auguste, received the U S Distinguished Service Award in 1960 The American Meteorological Society honoured Jean, not for his scientific balloon flight but for the invention design, and construction of the modern plastic balloon so widely used as a research tool to-day He did this with a research grant of 600 00 dollars from the University of Minnesota in 1936 It is easy to confuse the many accomplishments of the different members of the Piccard family "

MARINE ECOLOGY AND PALÆOECOLOGY

"TO provide discussion between botanists, zoologists and palæontologists on subjects of mutual interest by comparing analogous living and fossil assemblages" was the principal purpose of a symposium on marine ecology and palæoecology which was held by the Systematics Association in the Department of Zoology and Wadham College, Oxford, during September 24–26. The meetings were attended by about 130 people, of whom just more than half were palæontologists, and there was useful interchange between disciplines that enjoy far too little contact.

The first paper was by Prof. O. M. B. Bulman (Cambridge), who discussed the mode of life of the extinct non-dendroid graptolites. A pelagic habit is immediately suggested by (1) their wide distribution (in some cases almost world wide), (2) their occurrence in all types of matrix, (3) their most characteristic occurrence in black shales which seem to represent deoxygenated bottom mud lacking in benthonic organisms. There appeared to be very little concrete evidence of attachment of the 'true' (non-dendroid) graptolites to floating weed, or of common floats for synrhabdosomes which had been postulated by Ruedemann. A variety of buoyancy devices had been attributed to them, such as vesicles, webs between the branches and, in some, triple-vaned structures at the end of the nema. Prof. Bulman suggested that, of the various possible buoyancy mechanisms, the most likely in the majority of graptolites was the presence of gas bubbles in the soft tissue which is now generally accepted as covering the graptolite skeleton in life.

In the discussion which followed, Mr. C. W. Wright (London) suggested that the three-vaned structures, said by some to be buoyancy devices, were more likely to be attitude-maintaining devices, particularly since the posture of most graptolite rhabdosomes appear to have been highly important.

Similar evidence of a pelagic mode of life was presented by Dr. R. P. S. Jefferies (British Museum (Natural History)), for the Jurassic lamellibranch '*Posidonia*'. He considered two species, *Bositra buchi* (= *Posidonia ornati* et *P. alpina* auctt.) and '*Posidonia*' *radiata* (= *P. bronni magna* auctt.). Like graptolites, they commonly occur in black shales with few benthonic associates, but also occur sporadically in limestones and sandstones with much benthos. This strongly suggests they were not benthonic.

Granted this, Dr. Jefferies thought that both were probably pelagic swimmers, rather than living attached to flotsam. Thus, in *B. buchi* there was an anterior and posterior gape, suggesting swimming ability by analogy with Recent relatives, no sign of attachment in the shell outline at any growth stage and a shell thickness (60μ) comparable with Recent pteropods.

A nekto-planktonic mode of life for '*P.*' *radiata* was suggested by Hauff's observation that it became abundant when normal benthos disappeared but that it was then much more abundant than such 'benthos' as *Ostrea*, *Inoceramus dubius* and *Pentacrinus* that occurred attached to driftwood.

Swimming ability might be primitive in scallops, since Recent *Lima* and *Pecten* both swim in much the same way and also early Pectinacea were probably active as they have few epizoa and the shape of the shell sometimes suggested swimming ability.

After the paper, Prof. C. M. Yonge (Glasgow) commented that Recent scallops could swim only intermittently. Dr. Jefferies replied that experiments suggest that a pelagic mode of life for '*Posidonia*' was as feasible as for a Recent pteropod.

Mr. N. Tebble (London) was sceptical of the evidence for a pelagic habit in '*Posidonia*', remarking that there was just as much evidence that the sand-living species *Tellina tenuis* is planktonic, which it certainly is not. Dr. Jefferies replied that it was facies that showed that '*Posidonia*' was not benthonic.

Dr. J. A. Allen (Cullercoats) pointed out that there was often an epifauna on Recent *Chlamys septemradiata*, a good swimmer. Dr. Jefferies replied that perhaps early Pectinacea were even more active than the species cited.

Turning to a study of living forms which is proving of particular interest to palæontologists, the meeting heard a paper by Dr. E. J. Denton and Dr. J. B. Gilpin-Brown (Plymouth, and the University of Auckland, New Zealand) on the buoyancy of *Sepia* and *Nautilus*. They showed that *Sepia* changed its specific gravity according to the light, and was more buoyant at night time. This buoyancy is controlled by changes in the relative gas and liquid spaces in the chambers of the cuttle bone. In the older chambers the gas pressure is always about 0.8 atmospheres whatever the depth, but the liquid inside the chambers becomes less saline as depth increases, indicating an adjustment of osmotic pressure as a buoyancy control. In adult *Nautilus*, the gas pressure in all chambers is always about 0.9 atmospheres, and the buoyancy control is effected by varying the liquid content of the last-formed chambers. The problem remained as to how the liquid was removed from these chambers, since the septa are impermeable and in the newest chambers the siphuncle is not in contact with most of the liquid when the animal is in its normal swimming position. Dr. Denton and Dr. Gilpin-Brown suggested that the explanation lay in a special wettable layer on the inside walls of the chambers. This layer functions in the manner of blotting paper, holding liquid which once touches it and transporting it to the living tissue of the siphuncle which then pumps it, by way of the circulation, out of the chambers. The liquid almost all disappears by about the fourth or fifth chamber behind the animal proper. Further work is needed on the pumping mechanism, since some cephalopods, such as *Spirula*, can certainly go deeper than 240 m, which is the limit set by a pump using simple osmosis.

A complementary paper on fossil cephalopods was given by Mr. C. W. Wright (London), who discussed present-day knowledge of ammonite ecology and systematics. He emphasized how great were the gaps in our knowledge of what was thought of as a 'well-known' group: even their attribution to the Tetrabranchiata could not be regarded as proved. Mr. Wright pointed out that modern cephalopods occupy a wide range of ecological niches and there was reason to think that the ammonites may have done the same. Although generally supposed to be free-swimming, buoyant forms, there were even examples of ammonites with asymmetrical sutures and siphuncles which may well have lain on one side on the bottom like flat-fish.

Recent evidence from the United States of assemblages of up to 400 individuals in isolated doggers suggests original gregarious associations, and Mr. Wright compared this with the breeding swarms of certain living cephalopods which migrate *en masse* and are very sensitive to temperature and salinity. Such a tendency for associations in 'schools' might help to explain the problem of the rapid evolution of the ammonites, since a mechanism was required for the isolation of parts of a population as an agent for speciation.

Mr. Wright contrasted the supposed two main stocks of Mesozoic ammonoids the smooth, thin-shelled, 'open-sea' forms such as *Phylloceras* and *Lytoceras*, and the ornamented, thick-shelled, 'inshore' forms which constitute the majority The slow evolution of the former might be due to random oceanic distributions, with little splitting up of the populations, while the more rapid evolution of the inshore forms might have been permitted by a combination of the diversity of habitats available in the inshore regions and the habit of associating in 'schools'

Rapid evolution and the repeated production of certain morphological types, such as the oxycones which turn up in many families, necessitate a fragmented classification Mr Wright concluded by pointing out that ammonite classifications, which can at best be only expressions of the present state of knowledge, must be regarded as experiments, to be tested against new information and material

A second important theme of the meeting was the relationship between the distribution of various marine animals in present-day seas and that in the fossil record Mr Norman Tebble (British Museum (Natural History)) described the distribution of certain present-day zooplankton, notably among the pteropods and polychætes, which particularly show a specificity to certain water masses He emphasized that temperature and salinity were among the principal controlling factors, with the greatest changes in distribution occurring at the surface hydrological boundaries, the Sub-Tropical Convergence, the Secondary Polar Front and the Antarctic Convergence He demonstrated a marked asymmetry in the distributions For example, among the pelagic polychætes of the Atlantic Ocean three were endemic to the Antarctic waters, sixteen extended from the Sub-Tropical Convergence to the Secondary Polar Front and two of these continued into the high boreal waters of the North Atlantic where there were no endemic species

In discussion, Dr W J Fry (London) remarked that the distribution of the pycnogonids, entirely benthonic, appeared, from available records, to parallel very closely the distribution of polychætes in oceanic waters, and he thought this similarity again underlined the caution necessary in ascribing particular types of aggregation to particular modes of life

Dr C. Edwards (Millport) also described modern distributions, with particular reference to the amphipod crustacea, notably '*Gammarus*' (sens lat), which have spread into brackish and fresh water Lake Baikal, the Caspian and the Black Sea were probably populated with representatives of this genus from the open sea during the Tertiary era They have numerous endemic species, but these are still morphologically close to marine forms On the other hand, the well-known freshwater shrimp *G pulex* has probably had a longer freshwater history The re-opening of the Black Sea to marine conditions has had the effect of driving the endemic species back into brackish water lagoons, deltas and river-mouths *Chaetogammarus tenellus* has ascended the Volga, the Danube and the canals of eastern Europe, and descended the Vistula to Gdansk becoming adapted *en route* to freshwater conditions, but will not descend into the brackish range at the mouth of the Vistula Perhaps competition with native brackish species in the Baltic limits its distribution *G locusta*, which is purely marine around the British Isles (where the brackish habitats are occupied by two other species), does go into the lower salinities of the Baltic, it is probably ill-adapted to fluctuating salinities but can adapt to constant low salinities

Dr Edwards suggested that it was a general rule that if species have become adapted to lower salinities they would not re-adapt themselves to fully marine conditions again if they could avoid doing so by entering brackish localities such as river-mouths and lagoons. After the paper there was some discussion about how this rule applied in the fossil record, particularly among the invertebrates Dr Edwards considered that an important factor was the longevity of marine environments compared with short-lived local freshwater habitats

A third paper which concerned itself with distribution through time as well as space was one by Dr F T Banner (British Petroleum Co , Ltd) on heterochronous homeomorphy in the Globigerinacea These wholly marine planktonic Foraminifera range in age from Lower Cretaceous to the present day and are found in all parts of the world Dr Banner produced numerous examples of Recent forms in which the tests are homeomorphic with those of distantly related Tertiary species Homeomorphy occurs particularly in species of *Globigerina* and *Globorotalia*, the geographical distribution of fossil forms was often parallel to that of their present homeomorphs, a relationship that may be of palæoecological significance Thus, unkeeled *Globorotalia* (*Turborotalia*) *scitula*, of widespread Recent occurrence, parallels the wide geographic distribution of fossil *G* (*T*) *fohsi barisanensis*, while heavily keeled *G.* (*G*) *tumida*, which is recorded only within 20° N and S of the equator, is equally similar to the Miocene *G* (*G*) *lobata robusta*, a form of limited geographic range within the tropics Bandy has suggested that keeled Globigerinacea may be indicative of tropical and warm areas in the Cretaceous as well as in the Cainozoic, and noted an apparent relationship between the distribution of keeled *Globotruncana* and palæotemperatures as estimated by Urey and others More recent discoveries of heavily keeled species of *Globotruncana* in the Maestrichtian of Denmark and Sweden do not, however, appear to support his hypothesis of such indications of a general climatic cooling at the end of the Cretaceous

Another example of homeomorphy was provided by the development of chamber-elongations in almost all groups of the Globigerinacea independently Eocene *Hantkenina* and Cretaceous *Schackoina*, both possessing superficially similar narrow spine-like processes, had a wide geographical distribution, but other homeomorphic groups, such as the clavate *Hastigerinella* (Recent) and *Schackoina* (Cretaceous), appear to have a restricted geographic distribution Generalizations, although hazardous, might be made to suggest that, relative to a morphologically simple and geographically widespread ancestral stock, the more elaborate the test, the more restricted became the habitat of the morphologically most advanced species

Combining his investigations of both ancient and modern brachiopods, Dr M J S Rudwick (Cambridge) discussed the development of protective devices in various articulate groups As with the Globigerinacea, Dr Rudwick was able to produce numerous examples of deceptively close convergence He quoted the classic case of the Middle Triassic spiriferoid *Tetractinella* and the Upper Jurassic terebratuloid *Cheirothyris* In this case he suggested that the prominent projections on the shells served to carry the mantle margin outwards as an 'early warning' device This fitted in with observations on the extreme tactile sensitivity of the mantle edge in modern brachiopods Similarly, the hollow spines of such rhynchonelloid genera as *Acanthothiris* might have been another early warning system

Dr Rudwick then described his extension of the pre-war work of Herta Schmidt on the zigzag margins which are present in many brachiopods, and the probability of their having functioned as filters to prevent the entry of over-large grains into the shell These zigzag margins developed in several different ways during ontogeny, suggesting that they were evolved independently in different groups One variety, which is associated with a distinctive pattern of shell ornament, was itself apparently evolved three times, in the Devonian genus *Nayunella* (= *Yunnanella* auctt), the Jurassic *Rimirhynchia* and some species of the Cretaceous *Cyclothyris* All varieties of zigzag margins disappeared at the end of the Mesozoic and are unknown in Cainozoic or living brachiopods Dr Rudwick mentioned, however, that a few living species possess another kind of protective device the edge of the shell is 'guarded' by rows of sensitive setæ. Grooves along the margins of many fossil brachiopods probably indicate the positions of setæ which had the same function.

The final morning of the meeting was devoted to papers and discussion of ancient and modern reefs. The first paper in this session was by Dr. S. A. Wainwright (California) on modern reef coral associations. He started by emphasizing the need for more quantitative field studies of associations. He discussed the various organisms which directly affect coral morphology, such as boring green algae, symbiotic zooxanthellae and predators among the polychætes, gastropods, asteroids, cirripedes, crabs and fishes. One particularly good example was the parrot-fish which eats living coral on the open parts of the reef, and Dr. Wainwright suggested that palæontologists should look out for such features as the teeth marks of fish and gastropod scars (which are often specifically determinable) in fossil coral reefs.

Dr. Wainwright next considered the distribution of corals on the reefs, taking Fanning Island, in the Central Pacific, as an example. He showed graphically the increase in number of species and growth-rate from the reef flat to the edge of the reef slope, at about 10 fathoms, though with a marked break over the reef ridge, where only algae survive in the heavy surf. Normally, in areas of heavy surf many corals are broken and rolled about, and the consequent abrasion provides many a habitat for boring sponges, gastropods and, of course, new corals. Coral fragments and even blocks up to a ton or so are carried up the reef and thrown on to the beach by wave action. On the slope immediately below the ridge there are only massive encrusting corals, but these pass down into the more fragile branching types at the bottom of the slope.

Dr. Wainwright ended by appealing to biologists to examine reefs and their associations in even greater detail so that not only would the dynamics of reef-building and reef ecology be more clearly understood but also the sort of information available to the palæontologists would be greatly enlarged. From the palæontologists' point of view, what is desired is more information about coral associations in present-day seas, the biological role of their association and the effects of the associations on the growth pattern of the coral skeleton. The weaknesses inherent in the act of extrapolating from living to extinct organisms are greatly reduced when one deals with species-specific associations the manifestations of which are equally specific, or associations which are 'area specific' in relation to the whole coral reef.

Mr. G. F. Elliott (Iraq Petroleum Co.) turned to the other important constituent of reefs in his paper on past and present calcareous algae. He pointed out that calcifying algae at the present day are distributed among many groups and that some fossil ones are similar to living forms which are not calcified. Their maximum development is found in warm waters and they show a great variety of form. There are the delicately beaded types with light calcification, characteristic of the green algae; there are the thread-like masses within the sediment of the blue-green algae; and there are the massive, heavily calcified structures of the red algae. The fronded forms all occur in sheltered waters. The very common green alga *Halimeda*, for example, is very abundant in lagoons and also occurs (though less commonly) on the lower part of the reef slopes, down to considerable depths. On the other hand, the massive red algae, for example *Lithothamnium*, are particularly important on the exposed parts of modern reefs. These algae also perform an even more important function in binding together reef debris. Their tremendous growth-rate makes up for breakage by the surf, and Dr. Wainwright, in discussion, added that it has been estimated that algae extract calcium carbonate from the sea-water up to 100 times faster than corals.

In the fossil record, Mr. Elliott pointed out, an important consideration is the rapid diagenesis of algal calcifications. This is partly due to the fact that the carbonate is laid down as fine particles (not in laminæ, as in other calcareous organisms) and also due to the high proportion (up to 18 per cent) of magnesium carbonate sometimes present, which leads readily to the process of dolomitization: many beds of dolomite are probably algal in origin.

Mr. Elliott then summarized the fossil record of calcareous algae from Precambrian times onwards. He directed particular attention to certain points, notably the importance of the blue-green algae in the early part of the record, the rarity of algae in the Devonian reefs and the great importance of algae in Carboniferous and Permian reefs as compared with the Mesozoic. The coralline algae appeared in the Upper Cretaceous and came to dominate the reef environment in Tertiary times.

Prof. M. Lecompte (Brussels) discussed the building organisms of the Devonian reefs of Belgium, their ecological characteristics and bathymetrical distribution. He confirmed Mr. Elliott's observations on the rarity of algae in Devonian reefs, apart from the problematical *Stromatactis*, but he demonstrated clearly the regular succession of forms which are seen repeatedly in both bioherms and biostromes, and which can be related to the depth and turbulence of the water. He emphasized the long-term nature and wide extent of reefs in Belgium and adjacent areas, clearly the result of a rhythmic epeirogenic control, which repeatedly brought the sea-floor to the right depth for reef growth. The reef periods alternate with periods of shale deposition, and each time a succession can be traced through: (1) a 'deep-water' zone; (2) a 'quiescent' zone; (3) an 'infra-turbulent' zone; (4) a 'sub-turbulent' zone; (5) a 'turbulent' zone. Each of these is characterized by a particular suite of fossils. The deepest zone shows a dominance of brachiopods, notably stunted variants of forms which occur also in other bathymetric zones. These are followed upwards by the appearance of first solitary and then massive corals. Tabulate forms, such as *Alveolites* and *Thamnopora*, are the main builders in the 'infra-turbulent' zone, but several of them apparently could not live in the rougher water. Many other forms also appear, such as bryozoans and sponges. Lamellar stromatoporoids come in very strongly in the 'sub-turbulent' zone and are replaced by massive stromatoporoids in the 'turbulent'. Stromatoporoids were apparently excluded from muddy environments, but the pure limestones of the 'turbulent' zone are largely composed of these organisms, associated with a wide variety of other groups, such as corals, brachiopods, crinoids (which are usually underestimated) and *Stromatactis*. The last-named was probably very important, but is usually not preserved.

The final paper of the meeting was by Dr. W. S. McKerrow (Oxford) on some local Jurassic coral reefs. This was an introduction to an excursion that was held in the afternoon. Dr. McKerrow described two horizons at which corals were found in life position. In the Upper Oxfordian 'Corallian' beds (Upper Jurassic) branching corals were to be seen thus near Faringdon, Berkshire. The reef rock around Oxford, known as the Coral Rag, is contemporaneous with the detrital Wheatley Limestone which is composed of reef debris. The other coral occurrence was in the Great Oolite Series (Middle Jurassic) at Witney, Oxfordshire. The corals in these earlier beds have been attributed to five different genera, belonging, in the latest classification, to three different sub-orders. Dr. McKerrow showed by means of actual specimens that there were transitions between the different types. The simple *Montlivaltia* clearly passes into the branching *Thecosmilia*, and among compound massive types *Isastraea* (with walls between the corallites) passes into *Thamnasteria* (in which the walls have disappeared), and the latter (with solid septa) passes into *Microsolena* (with perforate septa). These passages could be seen in the same colonies, with the septa in direct continuation. It appeared that the taxonomy of these forms was based (at least in this case) on phenotypic rather than genotypic characters

The excursion in the afternoon, led by Dr McKerrow and Dr H G Reading, visited first an exposure of the Great Oolite at Crawley Road Quarry, Witney, where members saw a Bradford Fossil Bed reef in the Forest Marble The presence in this reef of remains of all animal groups except cephalopods suggests a shallow lagoon in which the water was too turbulent for cephalopods The second quarry visited showed a 'Corallian' reef at Shellingford Cross Roads Quarry, near Faringdon, where members saw patches of massive corals in early layers give way to mainly loosely-branching corals higher up, suggesting an increase in the depth of the water at this particular locality with time The detrital Wheatley Limestone in this quarry was seen below the Coral Rag

In the course of the symposium special thanks were expressed to Prof J W S Pringle for entertaining the meeting at Oxford

D V Ager
D Nichols

FOREST RESEARCH

MANY factors influence the growth of forest trees, and these factors cover a particularly wide range when the practice of forestry is largely concerned with afforestation and the use of exotic trees Thus it is easy to understand why forest research in Britain is so comprehensive This is certainly shown by the numerous projects described in the annual report of the Forestry Commission for the year ending March 1962[*]

Where so much dependence is placed on seed supply for ensuring the large planting programme provenance and genetics are very important Not only are vigorous trees wanted but also especial attention is being directed to those provenances which show resistance to frost So that the forester may know where to obtain tree seed in Britain of the best of the existing varieties and cultivars, the *Register of Seed Sources* has been brought up to date and it now contains 535 classified seed sources totalling 8,833 acres Associated with this has been the work of converting the best seed sources into seed stands and thus improving the genetic quality of seed collected in Britain

Much attention is being directed to the study of soil moisture Deep peat remains a problem with its poor drainage, the physical instability of the trees, the difficulties of timber extraction and not enough being known about the water and nutrient régimes of the trees There is another drainage problem on heavy clay soils, but some hope is seen in improved types of mechanical drain diggers

Exposure is a limiting factor to afforestation on many sites in Britain, and it is not easy of assessment However, an inexpensive technique has been developed by using the rate of tatter of standard cotton flags set up for a period on the sites being studied An account of this method is to be published soon Wind damage is being examined from various aspects, and one project uses a wind tunnel to determine the relationship between wind velocity and the forces acting on a tree Another project utilizes the 'tree-pulling' technique to test the effect of direction of pull on tree resistance Both series of experiments should bring out useful information, but they do not seem to take into consideration that trees in a forest are often subjected to gusts and turbulence and not to a steady force of wind

The annual report for the New Zealand Forest Service[†], covering the period January 1–December 31, 1962, shows an economic flavour Emphasis is laid on the quality of the end-product so that utilization will be the most profitable possible Hitherto, New Zealand's high-grade timber has largely come from its indigenous forests, but these resources are now approaching exhaustion The tools at the disposal of the sylviculturist in order to obtain a better proportion of good-grade timber from the exotic forests are pruning and thinning and, therefore, present investigations are attempting to relate pruning and its effect on grade enhancement and the relationship between green crown depth and the density of the stand However, the importance of protection forestry is not being forgotten More watershed surveys have been carried out, and some reveal conditions which are rather disturbing Active erosion and the resulting outpourings of detritus into streams may be largely due to red deer, goats and opossums destroying the protective vegetation cover In some of the more accessible areas, hunters appear to exert sufficient control on the deer numbers and so the vegetation cover is in reasonable condition to give adequate protection against soil erosion

C J Taylor

[*] Forestry Commission Report on Forest Research for the year ended March, 1962 Pp ix+194+12 plates (London H M S O , 1963) 13s net

[†] New Zealand Forest Service Forest Research Institute Report of the Forest Research Institute, Rotorua, for the period January 1 to December 31, 1962 Pp 86+9 plates (Wellington Government Printers, 1963)

RETURN OF THE NENE TO HAWAII

FROM the beginning of the Wildfowl Trust's attempts to rear the Hawaiian geese, *Branta sandvicensis*, in 1950, it had been hoped that it would become possible to return the Hawaii geese reared at Slimbridge The growth of the stock has been slow and some of the birds raised have been dispersed to other collections in Europe and North America in order to increase the chance of maintaining and developing flourishing stocks in captivity By 1960, however, enough geese had been reared to allow the return of some of the nenes to Hawaii It was not until the summer of 1962 that this somewhat complicated and expensive operation could actually be carried out[*]

The Division of Fish and Game, Department of Land and Natural Resources of the State of Hawaii, proposed that the Slimbridge-reared geese should be used in an attempt to re-establish the species on the Island of Maui, where it had been extinct for many years A survey of the existing habitat on Maui in June 1960 showed that there were 9,000 acres of excellent nene habitat and a further 30,000 acres likely to be of some value A release site was selected near Paliku within the Haleakala National Park There were strong reasons for this choice the area at the upper end of the Kampo Gap provides excellent nene habitat with food throughout the year, it is remote, ensuring a minimum of disturbance to the birds in the release pen, suitable accommodation exists for the people needed to care for the birds

In June 1962 thirty geese were despatched by air from Slimbridge to New York They were taken to the U S Federal Quarantine Station at Clinton, New Jersey, where they were held for 21 days They were then sent on by air freight to Honolulu There they were kept in the Zoo for three days to recover from the journey and were

[*] The Wildfowl Trust Fourteenth Annual Report, 1961–62 Edited by Hugh Boyd Illustrated by Peter Scott Pp 180+42 photographs (Slimbridge The Wildfowl Trust, 1963) 17*s* 6*d* net

given individually distinctive plastic leg bands On the twenty-sixth day they travelled by air to Kahului, on Maui

The Slimbridge birds consisted of 10 juveniles, 7 one year old, 10 two years old, 2 three years old and 1 four years old 17 were females and 13 males At Kahului they were joined by five juvenile females reared by the State's Pohakuloa propagation project on Hawaii The 35 birds were put in light-weight cardboard boxes and taken by lorry up to the rim of Haleakala Crater Here the boxes were loaded on pack boards and were carried on the backs of 23 Boy Scout volunteers 8 5 miles down into the Crater and across to the release pen at Paliku on the other side

The release pen at Paliku encloses about an acre of good grass, on the edge of an ancient 'aa' lava flow The geese were released in the late afternoon of July 26 As they were removed from the boxes, their clipped primaries were extracted so that new feathers would start to grow immediately The birds were given constant care and supplied with native berries and scratch feed daily They were also treated for coccidiosis and cæcal worms—these parasites having been detected in the fæcal droppings of the flock There was much pecking and other signs of social adjustment for a week or so, but the birds soon settled down

The geese began making short flights within the pen early in September The first flights out of the pen were seen on September 12, two Hawaiian-reared birds being the pioneers The first Slimbridge bird did not fly out until September 17 By the end of the month, 27 out of the 35 were flying a few hundred yards out of the pen, returning at dusk The Hawaiian and English birds, though remaining in distinct flocks within the pen, mixed very well outside it

Earlier experience with four separate releases, of 87 birds in all, on the Island of Hawaii, made with similar release pens, has shown that the geese will generally remain in the vicinity of the pen for several months It is planned to make annual releases of nenes from the same pen in Haleakala Crater for at least two more years, a total of 100 birds being the aim

DAMAGE TO LIVESTOCK FROM RADIOACTIVE FALL-OUT

IN the event of nuclear war, one major problem for civil defence authorities would be the availability and maintenance of food supplies One aspect of this problem, the effect of fall-out on domestic animals, is considered in a report, entitled, *Damage to Livestock from Radioactive Fallout in Event of Nuclear War*, prepared by the Subcommittee on Livestock Damage of the Advisory Committee on Civil Defence, National Academy of Sciences—National Research Council, Washington*

The object of the report is to present estimates of the biological effects of various levels of radiation exposure These estimates could be used by civil defence planners, whose responsibility would be, first, to feed a population during emergency conditions immediately following a nuclear attack, secondly, to sustain a population during the subsequent recovery period, and, thirdly, to ensure an adequate maintenance of animal populations No attempt is made to present maximum permissible levels of exposure, since this concept is considered irrelevant under conditions of national emergency where famine and other hazards would far exceed those normally found in peacetime The report therefore deals mainly with the lethal effects of radiations received from external sources or from radioisotopes within, or on, the bodies of animals On the effect of external radiation, animals investigated include cattle, pigs and poultry, and details are given of the clinical responses of these animals following radiation exposure Estimates are also presented of the mortality and possible utilization of food animals at different times after brief exposures to various doses of radiation For mammals, it is estimated that a dose of 350 rads would have only a negligible effect on salvageability, following a dose of 750 rads, most animals could be salvaged during the first three days after exposure, but by the fourteenth day all animals would be unfit for use Estimates for poultry suggest that these animals are able to withstand slightly higher radiation doses than mammals

The ingestion of radioisotopes may damage the health of food animals, but may also render them unfit for human use because of the radioactive contamination. The report suggests that contamination should not be significant immediately after a nuclear attack but may become serious if food animals receive protracted exposure to contamination This factor might be more important for isotopes like cæsium-137, with a general distribution in the body, than for iodine-131 and strontium-90 which are concentrated in specific sites Iodine-131 in milk, however, may represent a hazard, particularly to young children An assessment of the suitability of contaminated animals for human consumption would require knowledge of the amount and distribution of fall-out The report describes formulæ which could be used to estimate the probable human hazard on the basis of radioactive contamination of the food intake of animals, it also suggests the use of certain foods, such as poultry, eggs and some marine animals, which would not be highly contaminated

On the problem of stock maintenance, the report considers that radiation exposure would not be seriously harmful Data from a wide variety of adult animals showed no effect on fecundity from radiation exposures of 400 rads One gap in the data, however, concerns the radiation sensitivity of young animals Irradiation of pregnant animals produces abnormalities in the fœtus, but this would be of minor importance in stock maintenance Similarly, gene mutations would be unimportant, particularly in view of the high level of selection normal to animal husbandry

Final chapters in the report contain recommendations on methods for handling animals which have been exposed to fall-out and suggestions on areas of investigations in which research is urgently needed A series of appendixes present the basis on which estimates are made throughout the report and a comprehensive list of references is also included

C E PURDOM

[*] National Academy of Sciences—National Research Council, Washington, Publication 1078 *Damage to Livestock from Radioactive Fallout in Event of Nuclear War* Pp vi+93 (Washington, D C National Academy of Sciences—National Research Council, 1963) 2 dollars

MUCUS IN INVERTEBRATES

A GROUP of scientists interested in investigations on "Mucus in Invertebrates" met on August 27, 1963, in Washington, D C, during the sixteenth International Congress of Zoology The session was organized for the Division of Invertebrate Zoology of the American Society of Zoologists by Dr Sophie Jakowska, Food and Drug Research Laboratories, Inc, Maspeth, New York

Twelve speakers, representing Australia, Brazil, Canada, Denmark, Sweden and the United States, discussed various aspects of slimy secretions of lower animals The subjects discussed by the speakers included: adhering surfaces in *Hydra* and starfish (Dr. Chaet, American University, Washington, D.C.), mucus-like materials involved in eggshell formation of trematodes (Dr. Clegg, Australian National University, Canberra), mucus of the giant slug, *Ariolimax columbianus* (Dr. Taylor, Albert Einstein Medical Center, Philadelphia), regeneration of mucus-producing cells in the slug (Dr. Arcadi, Whittier College, California), mucus gland in the octopus (Drs. Gennaro and Lorincz, University of Florida), mucous secretions in ophiuroids (Dr. Fontaine, Victoria University, British Columbia), and in crustaceans (Dr. Dahl, University of Lund, Sweden, and Dr. Steeves, University of Alabama). Mucous substances of protochordates were described by Dr. Norrevang (University of Copenhagen), Dr. Olsson (University of Stockholm) and Dr. Sawaya (University of São Paulo, Brazil). The papers listed on the programme which were not presented included investigations on turbellarians (Dr. Pedersen, University of Copenhagen), on effects of photoperiod on mucus secretions of gastropods (Dr. Segal, San Fernando Valley College, California), and mucopolysaccharides in *Molgula* (Mrs. Bonnelly deCalventi, University of Santo Domingo, Dominican Republic).

The participants emphasized the great variety of structures in which mucoid substances originate, and their role under normal and experimental conditions. Opinions were expressed that, although there is relatively little information on the chemistry of such materials from invertebrates, it is to be expected that they probably differ in chemical composition from each other, and from the typical salivary secretions of man and mammals which so far have received considerable attention. The ultrastructure of mucus-secreting surfaces was demonstrated by several speakers.

The chairman urged the participants to exploit their familiarity with the widest variety of mucus-secreting organisms as natural working models of normal and abnormal conditions which cannot be examined with equal ease in higher animals. These investigations may shed further light on the physico-chemical properties of pathological mucous secretions, for example, such as those occurring in cystic fibrosis.

During the discussion, a number of speakers remarked on the apparently high concentration of iodides in marine invertebrates which normally secrete copious mucus, as suggested by the characteristic odour. Dr. Anna M. Bidder (University of Cambridge) pointed out the value of excised octopus gut for *in vitro* investigations on mucus secretion. Dr. Fontaine (Victoria University, British Columbia) mentioned a species of starfish, commonly found along the Pacific Coast, and valuable for the abundant mucus they secrete and collect in a pouch-like structure. Other living systems of interest (for example, hemichordate proboscis) were suggested as models for investigating rheological properties of mucus. The utility of common invertebrates noted for their slimy secretions (slugs, snails) was also stressed.

Since this informal symposium represented only a session of related papers during the Congress of Zoology, full manuscripts are not published as part of the Congress *Proceedings*. The chairman was, therefore, instructed to seek the most suitable means of publication in form of collected papers on mucus in invertebrates. Additional papers and brief communications have been pledged by other authors. The papers have been submitted to the New York Academy of Sciences for publication in the form of *Annals of the New York Academy of Sciences*. A previous monograph dealing with mucous secretions, including a few papers on mucus in invertebrates, appeared in the *Annals* in March 1963.

It is hoped that publicizing this meeting and the forthcoming publication will encourage others to work in this important field. A future meeting is planned for 1965 or 1966. Further information can be obtained from Dr. Sophie Jakowska, Food and Drug Research Laboratories, Inc., Maspeth, New York.

NATURAL OCCURRENCE OF AMINO-ACIDS IN DOLOMITIC LIMESTONES CONTAINING ALGAL GROWTHS

By Dr. J. S. HARINGTON*

Pneumoconiosis Research Unit, South African Council for Scientific and Industrial Research, Johannesburg

AND

Dr. P. D. TOENS

Department of Geology, West Rand Consolidated Mines, Ltd., Transvaal

THE detection of fluorescent oils containing polycyclic aromatic hydrocarbons and amino-acids in crocidolite and amosite asbestos, but not in chrysotile, has been reported by Harington[1,2] and an organic origin for these materials has been suggested[3].

This article concerns the detection of amino-acids and hydrocarbons in dolomitic limestones underlying the Pretoria Series in which the crocidolite asbestos horizons are present in the north-western Cape Province, South Africa.

The Dolomite Series is composed almost entirely of carbonate rocks[4]. It is about 10,000 ft. thick in the north-western Cape and 4,000–7,000 ft. in the western Transvaal[4], and has an estimated age of about 2,000 million years[5].

Recent investigations[4] have postulated that deposition of the Dolomite Series occurred under warm, shallow and saline conditions. To allow carbonates to precipitate, the *p*H of the water must have been about 8 (± 0·5). Reducing conditions would also have been required for the preservation of organic matter and the formation of pyrite. Various types of algae, of which fossil remains have been found, probably flourished in these quiet conditions, forming a skin over the surface of the sediments.

The siliceous rocks of the overlying Pretoria Series must have been deposited under mildly oxidizing conditions to permit the formation of iron oxides. These conditions would probably not have enabled organic growths to be preserved within the rock, although the detection of amino-acids[2] does suggest that organisms of some sort were present.

*Cyclo*hexane and ethanolic extractions of three samples of pulverized, primary, dolomitic limestones were carried out by methods described earlier[1,2]. Precautions were taken to avoid contamination of the rocks before and during analysis.

Specimen 1: *Dolomitic limestone containing stromatolites* (carbon content 0·15 per cent). These algal growths exhibit distinct and rather elaborate branching, and closely resemble the algal heads and domes which are forming in the Great Bahama Bank at the present time[6]. The zones in which these structures are present vary in

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* Present address: Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3.

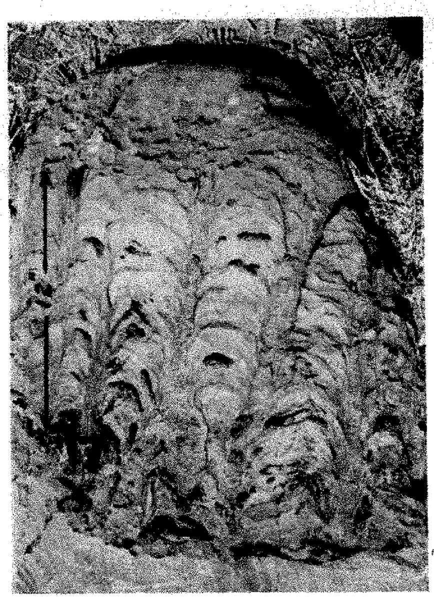

Fig. 1. Dolomitic limestone containing algal growths (from Danielskuil, N.W. Cape, South Africa)

thickness from 6 in. to 10 ft. or more. The growths consist of a number of superimposed cones which are composed of fine algal filaments (Fig. 1).

Specimen 2: *Algal limestone underlying the Pretoria Series* (carbon content 0·20 per cent). About 100 ft. of impure algal limestone are present immediately below the Pretoria Series. The rocks are dark grey and have a distinctly fetid odour on fresh fracture (? hydrogen sulphide). Extremely contorted banding is present, and is caused by the wavy nature of the algal filaments preserved in the rock. Minute specks of pyrite are abundant and occur within the algal banding.

Specimen 3: *Shaly limestone from Silverstreams.* This specimen was derived from a narrow band of fetid, dark, shaly limestone lying below a layer of chert in a quarry. Extremely contorted shaly bands are visible in thin section and are probably due to movement which has forced the shale into cracks and along bedding-planes in the fine-grained limestone. Within the shale numerous crystals show zonal and patchy distribution of calcite and dolomite. Algal banding was not identified unequivocally, but was suspected of being present in some samples.

The results of analyses for amino-acids obtained by a semi-quantitative, paper chromatographic method are given in Table 1. Values for crocidolite asbestos, obtained earlier[2], are included for comparison.

Table 1. AMINO-ACID COMPOSITION OF ORGANIC EXTRACTS OF DOLOMITIC LIMESTONES CONTAINING ALGAL GROWTHS (μg/1,000 G ROCK)

Amino-acid	Crocidolite fibre (ref. 2)	(1) Stromatolite	(2) Algal limestone	(3) Shaly limestone
Serine	950	3·3	2·5	2·0
α-Alanine	380	13·3	3·8	4·0
Glycine	350	3·3	1·3	2·0
Aspartic acid	240	—	0·6	—
Threonine	170	—	0·6	2·0
Leucine	140	—	0·6	20·0
Isoleucine	140	—	—	20·0
Valine	140	—	—	—
Phenylalanine	100	—	0·6	4·0
Glutamic acid	70	1·5	0·6	2·0

Analyses of the three specimens for oils and 3 : 4-benzpyrene[1] showed that both were present in No. 2, and neither in No. 1 nor No. 3. In all three cases, the yields after *cyclo*hexane and ethanolic extraction were, in general, insignificant (mg/100 g dust): 0·3 and 7·4; 0·4 and 1·8; 0·1 and 3·2, for samples 1, 2 and 3 respectively. A sample of algal dolomite from the western Transvaal (2,226 g), investigated because it contained very well-preserved algal growths, and because the overlying Pretoria Series does not contain asbestos in this area (Klerksdorp), gave no detectable yields after *cyclo*hexane and ethanolic extractions and neither 3 : 4-benzpyrene nor amino-acids was detected by analysis. Such negative results serve as useful control checks against accidental contamination of the samples before and during analysis.

The detection of amino-acids in carbonate rocks of the Dolomite Series, together with the presence of algal structures, suggests that organic processes were active during the deposition of these rocks about 2,000 million years ago, and that the Dolomite Series has undergone very little metamorphism since then.

We thank Dr. J. J. le R. Cilliers for advice, Mr. Paul Baily for performing the analyses and Miss Marianne Smith for assistance.

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MAGNETO-HYDRODYNAMIC GENERATION AND NUCLEAR REACTORS

By J. K. WRIGHT

Fundamental Studies Section, Central Electricity Research Laboratories, Leatherhead, Surrey

THE problems of the design of both high-temperature nuclear reactors and magneto-hydrodynamic (MHD) generators are being actively investigated by large research teams in various parts of the world. The purpose of this article is to discuss some of the problems of using a MHD generator in conjunction with a nuclear reactor.

The early commercial nuclear reactors have low gas temperatures and consequently the electricity is generated at low thermodynamic efficiencies. For example, the Berkeley Nuclear Power Station, with a reactor gas exit temperature of 345° C, has an overall thermodynamic efficiency of only 24·6 per cent, whereas the Dungeness reactors when built will have an exit gas temperature of 410° C resulting in an overall thermodynamic efficiency of 32·9 per cent. Both these systems are graphite moderated gas-cooled reactors with magnox cans. The purpose of more advanced designs is to increase the operating temperature and, consequently, thermodynamic efficiency. Advanced gas-cooled reactors will operate with reactor gas temperatures in the region of 625° C, and the *Dragon* reactor is designed to have an exit gas temperature of 800° C, which might be extended to 1,000° C with future developments.

More advanced types using ceramic fuel elements might be developed to operate at still higher temperatures. In this range the use of steam as a thermodynamic working fluid becomes less favourable and other systems would be used. One method of converting the heat energy in the reactor coolant gas into electrical energy is to use an MHD generator.

As well as effort being placed into raising the operating temperature of nuclear reactors, methods of lowering the minimum operating temperature of an MHD generator have been suggested. This minimum temperature of the working fluid is determined by the need to obtain adequate electrical conductivity. Using a favourable working medium, such as helium, seeded with about 1 per cent of cæsium, and relying on the temperature of the working fluid to produce the required ionization, minimum temperatures of the order of 1,700° C are obtained

This minimum temperature may possibly be reduced by some non-equilibrium ionization technique Proposed methods include external ionization by an auxiliary discharge or by injecting electrons and relying on the low recombination cross-sections of the inert gases to maintain a high degree of ionization and therefore electrical conductivity Another proposed method is to use the induced MHD currents to break down partially the gas and thereby increase the electrical conductivity Since this works essentially by preferentially heating the electrons in the gas, the method is known as 'elevated electron temperature' This method has considerable potential although it has yet to be proved experimentally in an actual MHD generator It is also not yet known at how low a gas temperature it will work

A crucial question at this time is whether it is worth attempting to develop high-temperature reactors and the associated low-temperature MHD plant In the following example it is shown that an MHD generator-reactor system operating at a very high temperature is not necessarily more efficient than an equivalent gas turbine-reactor system operating at a somewhat lower temperature

Consider a nuclear reactor with an exit gas temperature T_1 °K feeding an MHD generator or gas turbine of stage efficiency η_1 and having an exit gas temperature T_2 °K The gas is then cooled in a heat exchanger to a temperature T_3 °K and compressed from P_0 to P_1 in an isothermal compressor of efficiency η_2 before being heated to a temperature T_4 °K by the heat extracted from the previous heat exchanger Fig 1 shows the scheme

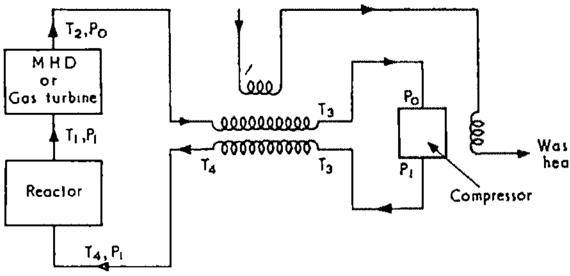

Fig 1

Pressure drops across the reactor and heat exchangers are ignored and it is assumed the circulating gas has a constant specific heat at constant pressure C_p

Then for unit mass of gas

Heat gained in reactor = $C_p(T_1 - T_4)$

MHD or gas turbine output = $C_p(T_1 - T_2)$

Work done in isothermal compressor =

$$\frac{\gamma - 1}{\gamma} \frac{C_p T_3}{\eta_2} \ln P_1/P_0 = \frac{C_p T_3}{\eta_1 \eta_2} \ln T_1/T_2$$

Since the pressure ratio across the gas turbine or MHD duct is

$$\frac{P_1}{P_0} = \left(\frac{T_1}{T_2}\right)^{\gamma/\eta_1(\gamma - 1)}$$

Hence the cycle efficiency is

$$\frac{T_1 - T_2 - \dfrac{T_3}{\eta_1 \eta_2} \ln \dfrac{T_1}{T_2}}{T_1 - T_4}$$

For the sake of illustration, values have been chosen of $T_3 = 350°$ K (77° C) and $T_4 = T_2 - 200°$ representing the drop across the heat exchanger and any losses These figures are typical but are not intended to be optimized in any way

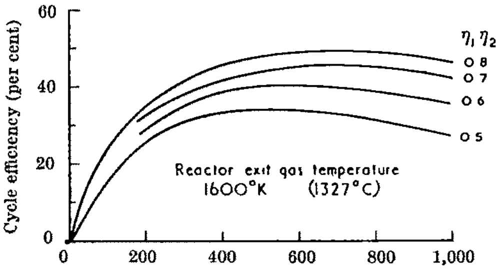

Fig 2

Fig 2 shows the way the cycle efficiency varies with MHD or gas turbine exit temperature for a particular reactor exit gas temperature of 1,600° K Notice how the efficiency decreases with the product of turbine and compressor efficiencies $\eta_1\eta_2$ Efficiencies approaching 90 per cent are typical for gas turbines and compressors, so a value for $\eta_1\eta_2$ of 0 8 might be typical for a gas turbine generator system Since there are no experimental data on the performance of MHD generators using non-thermal ionization it is difficult to assess the turbine efficiencies that may be obtained in practice, but values of $\eta_1\eta_2$ of 0 6 are probably realistic Notice also the significant temperature drop across the MHD duct for optimum working

Fig 3 is a diagram of system efficiency against reactor gas temperature The upper curve shows the best performance of a gas turbine system with $\eta_1\eta_2 = 0\ 8$ The middle curve is for the optimum efficiency of an MHD generator with $\eta_1\eta_2 = 0\ 6$ The lower curve shows the performance of an MHD system when the MHD exit stagnation temperature is restricted to 1,200° K (929° C) This may correspond to a temperature of the flowing gas at the exit of the duct as low as 800° C It is not known at how low a temperature the non-thermal ionization processes will operate, but the limit of 800° C (which may be optimistic) has a pronounced effect on cycle efficiency at reactor gas temperatures below about 1,400° C

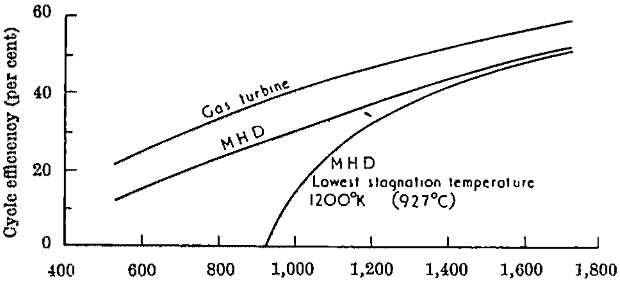

Fig 3

The foregoing example is, of course, a particular, simple and idealized thermodynamic cycle However, the conclusions that it is necessary to work with an MHD exit gas temperature well below the reactor exit temperature and that gains in MHD 'turbine efficiency' can be as important as reductions in minimum working temperature are probably generally valid In particular, it should be noted that gas turbines which should be capable of operating at reactor exit gas temperatures of the order of 1,000° C in a helium atmosphere may lead to a cycle as efficient as one using MHD with a reactor exit gas temperature of the order of 1,300° C A much more detailed investigation of MHD performance using non-equilibrium ionization and of reactor performance and costs at various temperatures must be made before any final conclusions can be drawn about the usefulness of MHD for nuclear reactors

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LOW-LEVEL ASSAY OF IODINE-131 IN BIOCHEMICAL RESEARCH

By Dr C. F. BARNABY and Dr L. G. PLASKETT

Medical Research Council Department of Clinical Research, University College Hospital Medical School, London, and Department of Biochemistry, University of Edinburgh

IODINE-131 ($Z = 53$, $N = 78$) was first used as a biological tracer by Hamilton and Soley in 1939 (ref. 1). Iodine-131 can be cheaply produced by the reaction ^{130}Te (n, γ) ^{131}Te, ^{131}Te β/25 min ^{131}I, and is also a fission product. Because of this, and its convenient half-life (8.05 days), it has become widely used. Furthermore, the energies of its β- and γ-radiations are such that, with sources of high or medium activity, assay is a relatively simple matter, requiring no very specialized equipment.

The daughter product of the decay of iodine-131 is stable xenon-131, the present accepted decay scheme is shown in Fig. 1.

In addition to making iodine-131 the isotope of choice in the examination of both intrathyroidal and peripheral iodine metabolism, these properties have induced many workers to use it for examining systems in which iodine is not normally involved. Fat[2], protein[3] and carbohydrate[4] labelled with iodine-131 have found a place in biochemical research, and the isotope has also been utilized in the double isotope derivative technique for the analysis of amino-acid mixtures[5]. In spite of the ease with which iodine-131 may be assayed, some of these applications involve low-level counting problems. It is the purpose of this communication to examine the nature of these problems as they occur in the examination of iodine metabolism and to suggest ways in which they can best be solved. Details are provided of a counting system which has proved its value in the scanning of low-activity paper chromatograms, and we discuss the results which have been obtained in analysing the iodine compounds of extrathyroidal tissues.

Need for low-level counting in experiments involving administration of ^{131}*I-iodide.* Experiments are commonly designed in which radioactive iodide is administered to an experimental animal or human subject. Its fate is then followed by external counting of parts of the body, by assaying the radioactivity of the excretions and body fluids, or, after death, by determining the distribution of radioactivity among the organs and tissues. It is also usually desirable to investigate the chemical nature of the labelled material in the tissues and body fluids, and this necessitates fractionation procedures. It is the introduction of fractionation which most frequently poses the low-level counting problems. The fractionation technique which has achieved greatest success for the separation of iodinated compounds is paper chromatography[6,7], in which the sample size is always very small.

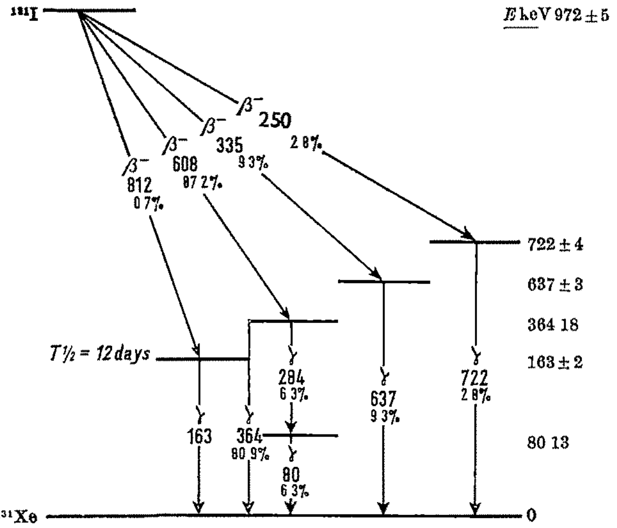

Fig. 1. Decay scheme of iodine-131

Analysis of thyroid. In the analysis of thyroid tissue the problems of low-level counting are least apparent. The thyroid gland concentrates injected ^{131}I-iodide, and therefore, in equilibration investigations such as those of Pitt-Rivers and Rall[8], there is usually no problem in estimating the radioactivity of even very minor components of thyroid tissue, autoradiography of paper chromatograms, although relatively insensitive, is quite applicable. The same is true of the analysis of thyroid tissue several hours or several days following a single dose of iodine-131, because uptake is high and the iodine pools of the thyroid are approaching isotopic equilibrium. One situation in which low-level counting becomes important is in kinetic experiments in which it is necessary to examine the labelled compounds of the thyroid gland a few minutes after a dose of iodine-131. In this situation thyroid uptake is usually a fraction of 1 per cent of the dose, and the radioactivity of a minor component with slow turnover, such as triiodothyronine, may be a small fraction of 0.1 per cent of the radioactivity of the gland[9]. Either low-level counting must be used or large doses of iodine-131 must be used which may constitute a hazard to the experimenter.

Analysis of peripheral tissues. A completely different situation exists in the investigation of peripheral iodine metabolism. In this case equilibration investigations result in rather low concentrations of radioactive iodine in body fluids and in tissues such as liver, kidney and skeletal muscle. It is no solution to this problem to increase the quantity of radioactivity injected, since this would subject the thyroid tissue to high doses of radiation and would affect thyroid function. The maintenance of normal levels of iodine compounds in the peripheral tissues clearly depends on the integrity of thyroid function. Feller, Chaikoff, Taurog and Jones[10] investigated the radiation dose-levels to thyroid tissue which would produce thyroid damage in the rat. Functional damage, causing changes in the plasma protein-bound iodine-levels, and impairment of the ability of the gland to concentrate and retain iodine, were observed after radiation doses to the thyroid tissue exceeding 100,000 reps. Histological change seemed to be a more sensitive index of radiation damage, and was seen in one experiment in which the dose was 46,000 reps. In the rat, in experiments lasting for a few days following administration of iodine-131, 24 μc appeared to act as a tracer dose, causing no radiation damage, whereas 300 μc always caused radiation damage.

It is possible that biochemical functions which have not yet been tested for radiation sensitivity may be more sensitive than those which have been used so far. For example, Anbar and Inbar[11] suggest that much lower radiation doses may damage the rat thyroid, because they found an increase of butanol non-extractable protein-bound iodine in the peripheral blood after such doses. Moreover, Basu, Lahiri and Chandhuri[28] have observed depression of thyroid function in the rat following doses ranging from 10 to 100 μc. Therefore, the discussion which follows will consider an experiment in which no more than 10 μc is administered to the rat. If the maximum uptake is 20 per cent of the dose, then at the time of maximum uptake the thyroid contains 2 μc iodine-131 If the thyroid weight is 10 mg this represents a concentration of 200 μc /g tissue The concentration present in the peripheral tissue varies a great deal from experiment to experiment, but is of the order of 10^{-4} of the thyroid concentration, that is, about 0 02 μc /g The largest amount of homogenized tissue which can be analysed by direct chromatography on paper, with or without prior pancreatin digestion[12], is about 1 mg, the chromatogram would thus contain a total of 20 μμc , which, after development, is distributed over a large area of the paper If the scan is to be of any real use it must be possible to measure the radioactivity of peaks amounting, at most, to 10 per cent of this, that is, 2 μμc In a source of this size there will occur only 4 4 disintegrations per minute The scanning of such chromatograms constitutes a low-level counting problem Only a counter with very high efficiency and a very low background is effective Such counters are not generally available and for this reason thyroid is the only tissue which can normally be analysed for radioactive iodine compounds by direct application of homogenates or hydrolysates on to paper chromatograms In practice, concentration techniques such as butanol extraction[13–15] or adsorption on to resins[16–18] are almost always used in the analysis of extrathyroidal tissues In these techniques there is a danger of deiodination[19] and esterification[20,21], moreover, recoveries are very rarely complete, so that some of the tissue iodine is unaccounted for We have constructed low-level counting systems in order to avoid the use of such concentration techniques in the analysis of non-thyroid tissue

Need for low-level counting in experiments involving administration of labelled iodine compounds While only the danger of radiation damage limits the amount of iodine-131 which can be administered in the form of carrier-free ^{131}I-iodide, the administration of labelled iodine compounds is often limited by their specific radioactivity It is not possible to produce samples of labelled amino-acids in which all of the iodine is present as iodine-131 Therefore, injection of the labelled compounds usually involves administration of a chemically appreciable amount of carrier along with the labelled material Care is necessary to ensure that the metabolism of the experimental animal is not altered by the administration of this carrier For example, in a commonly used technique for the preparation of labelled thyroxine[22] 0 68 mg diiodo-L-thyronine and 10 mc iodine-131 are used as the starting materials, and the product has a specific activity of 4 μc /μg The injection of only 4 μc of this material into the rat involves administering as much thyroxine as normally circulates in the blood of the rat, the level of protein-bound iodine-127 is probably doubled temporarily following the injection, so that the results may reflect physiological conditions which have been altered by the experiment The specific activity could only be improved by using less diiodothyronine or more radioactivity, and in an ordinarily equipped laboratory either measure presents difficulties Special methods have been utilized by Roche *et al* [23] and by Plaskett[24] to obtain thyroxine of much higher specific activity, but even if thyroxine containing 100 μc iodine-131 per μg is available, it is doubtful if it is advisable to administer more than 10 μc of it to the rat (As with the limitation of dose by radiation damage, the maximum dose which may safely be given is hard to define precisely Until more is known about the effects of giving carrier, the choice is unavoidably somewhat arbitrary) As in the analysis of extrathyroidal tissues after giving ^{131}I-iodide, low-level assay must be carried out if concentration techniques, with their many disadvantages, are to be avoided

Clinical investigations Finally, in clinical investigations with the human subject, in which tracer doses of iodine-131 are usually limited to about 20 μc , the results which can be obtained are very severely limited by problems of low-level assay

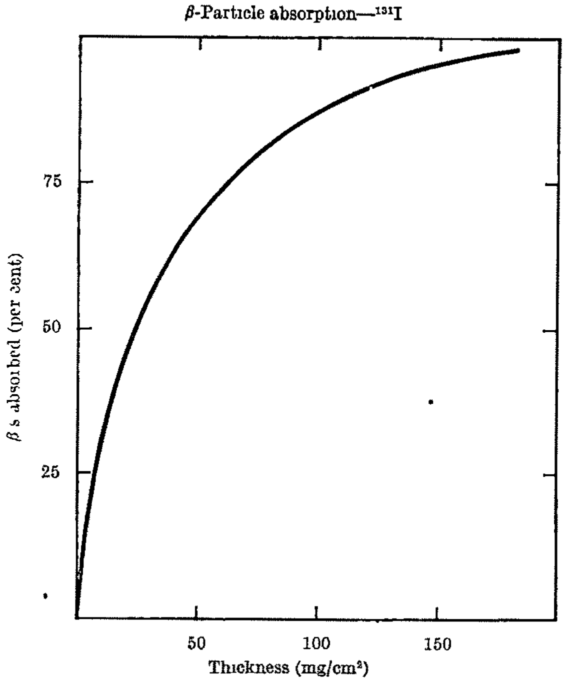

Fig 2 Absorption curve for iodine-131 β-particles

Nature of low-level sources In any of these applications of low-level counting the sources usually take the form of liquid samples or segments of chromatography paper Chromatography paper is sufficiently thin for a useful fraction of the β-particles to emerge from the source, and use can therefore be made of thin end-window Geiger–Muller counters Window thicknesses of just over 1 mg/cm^2 can be readily obtained (for example, 20th Century Geiger–Muller tube type *EW*2C), and since Whatman No 1 chromatography paper is about 10 mg/cm^2 this window thickness represents an unimportant addition to the absorption of the β-particles (Fig 2) An apparatus using such counters detects β-particles with high efficiency but has a very low efficiency for γ-rays

In liquid sources and other thick sources the β-particles are almost completely absorbed Such sources are most frequently assayed by detecting the γ-radiation in a well-type NaI crystal scintillation counter

Choice of Counting System The Relative Importance of Low Background and High Efficiency

For low-level counting systems the parameters to be considered are the background and the counting efficiency. If the background rate is R_2 counts per minute and the efficiency is ε, a source in which D atoms disintegrate per minute, each disintegration producing one β-particle will give a count rate due to the source alone of εD counts per minute The total count rate, including the background, will thus be $R_2 + \varepsilon D (= R_1)$ In practice R_2 is determined by obtaining n_2 counts in t_2 min and R_1 by obtaining n_1 counts in t_1 min The source rate (r) is then given by $R_2 - R_1$ The error in $R_2 - R_1$ is given by

$$\delta r = \sqrt{\frac{n_1}{t_1^{\,2}} + \frac{n_2}{t_2^{\,2}}} = \sqrt{\frac{R_1}{t_1} + \frac{R_2}{t_2}}$$

Differentiation shows that the best use of the total counting time, T, occurs when

$$t_1/t_2 = \sqrt{R_1/R_2}$$

These quantities can be related to the percentage error $x\left(x = \dfrac{100\delta r}{R_2 - R_1}\right)$ by the equation

$$T = \frac{10^4(\sqrt{R_2} + \sqrt{\varepsilon D + R_2})^2}{x^2\,\varepsilon^2\,D^2}$$

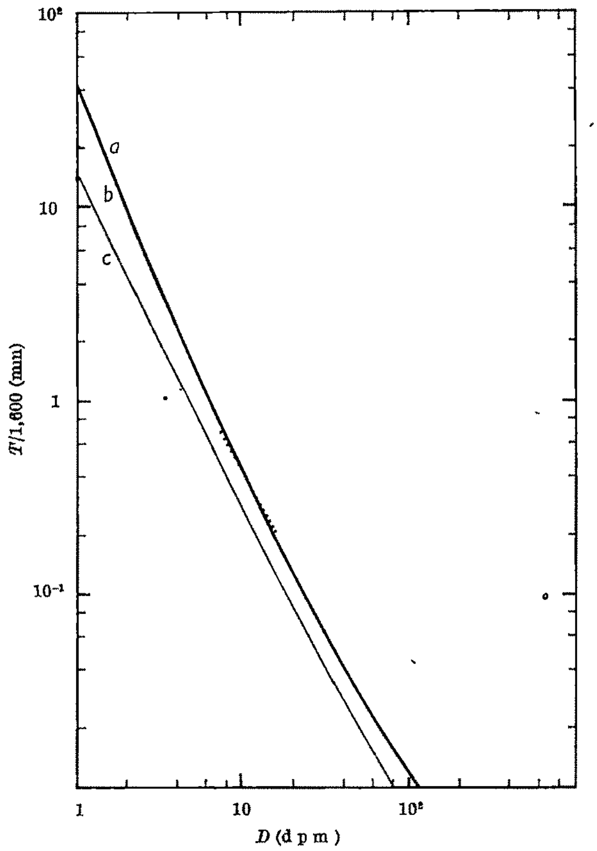

Fig. 3. T versus D curves for (a) efficiency = 0·5, background = 10 c.p.m.; (b) efficiency = 0·25, background = 1·5 c.p.m.; (c) efficiency = 0·5, background = 3·5 c.p.m.

which can be used to determine the properties of any counting system.

Components of the background. The importance of reducing the background of the counter is clear. By far the major components of this background are (1) the γ-rays from environmental radioactive contamination external to the apparatus, (2) cosmic ray mesons, (3) radioactive material in the counters themselves, and (4) spurious events arising in the electronic circuits associated with the system. For the thin window 20th Century Geiger–Muller counter type *EW2C*, a cylindrical counter about 2 in. long and 1 in. in diameter, the total background in a typical laboratory is of the order of 30 counts per min.

The first, and largest, component (environmental radiation) can be reduced by surrounding the counter with a high-density shield, usually lead, the background of the *EW2C* surrounded by 4 in. of lead is about 5 counts per min.

The cosmic ray mesons are normally reduced by surrounding the counter with a number of cosmic ray counters arranged in a semicircle above the counter and operated in anticoincidence. Since the μ meson intensity in any given direction is proportional to the square of the cosine of the angle to the vertical the most important counters are those nearest the top of the ring and the ones making large angles with the vertical are often dispensed with. The μ meson rate in the counter under discussion is about 3·5 per min and so the background in the shielded counter run in anticoincidence with cosmic ray counters is reduced to about 1·5 counts per min.

The component due to radioactivity in the counter itself must be accepted and the spurious pulses in the associated circuits and mains pick-up can be eliminated by proper design and construction.

Efficiency. The efficiency of the counter for iodine-131 uniformly distributed on a small piece of Whatman No. 1 chromatography paper is about 25 per cent. Only 50 per cent of the betas go in the direction of the counter and about half these are absorbed before they enter the sensitive volume of the counter. Some β-particles will, of course, have path-lengths in the paper considerably longer than the thickness of the paper, since they will travel at a steep angle through it.

The efficiency of the system is immediately doubled if two counters are used, viewing opposite sides of the paper. To illustrate how performance is related to efficiency and background let us consider two systems, one using two counters face to face, shielded by 4 in. of lead, and the other having one similarly shielded counter but surrounded by an anticoincidence ring of cosmic ray counters. We have seen that the efficiencies and backgrounds of these will be 0·5 and 10 counts per min and 0·25 and 1·5 counts per min respectively. The variations of the total counting time, T, for a 5 per cent error, with the amount of iodine-131 in the source, D, is shown in Fig. 3. It will be seen that at $D = 12$ disintegrations per min (that is, about 5 μμc.) the two systems are equivalent, for sources containing less than this amount of activity the system with the lower background and efficiency is superior, and for sources of more than 12 disintegrations per min the higher background and efficiency is best. It is therefore desirable to choose the system according to the amount of activity to be counted.

Low-level scanning of paper chromatograms. The double counter system has been routinely used by us for scanning paper chromatograms. It is surrounded by an efficient anticoincidence shield which reduces the background to 3·5 counts per min, the efficiency remains at 0·5. The curve for this arrangement is shown in Fig. 3, and it will be clearly seen that for very low activities the low background system is very advantageous, but the difference between the double-counter, with and without anticoincidence, quite rapidly lessens with increasing D. An advantage of the lower-background system is that the occurrence of spurious pulses is more easily detected. It is always very difficult to verify the absence of such events, but if the background, and consequently the standard deviation of the background, is low, the presence of such pulses becomes more apparent. The background of the double-counter system with anticoincidence is somewhat greater than twice the background of the single counter with anticoincidence. This is because a significant fraction

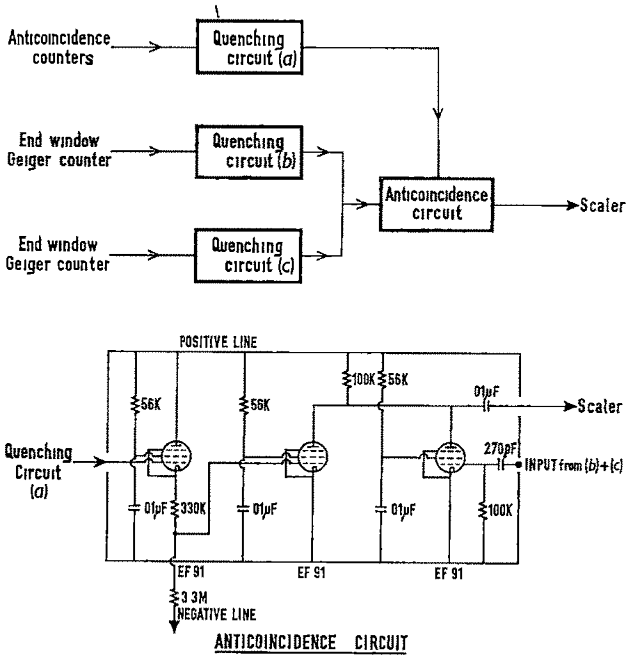

Fig. 4. Electronic arrangement for low-level counter

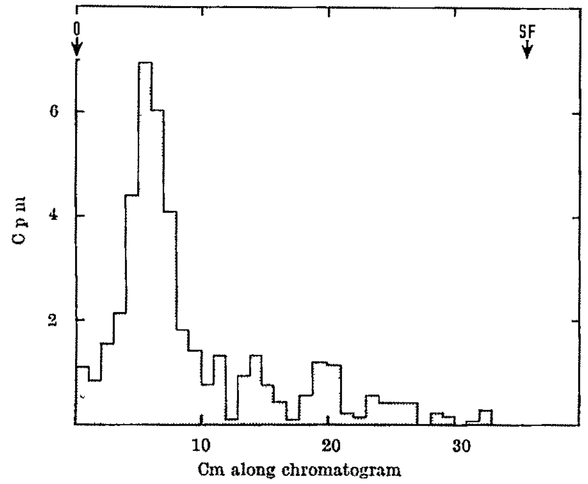

Fig 5 Chromatogram scan of a pancreatin digest of liver homogenate from a rat killed after injection of ^{131}I-iodide

of the radioactive contamination in the counter occurs in the mica window With two counters, radiation from one window travelling in the direction of the other counter can trigger it off, and since there is twice the amount of activity present the background from this source, compared with the single counter, may be greater by a factor of up to 4 From measurements on several samples of mica it was concluded that this source accounted for almost half the background in the double counter system

Electronic circuits which have given satisfactory service over a long period in a double-counter system with anticoincidence are shown in Fig 4 The anticoincidence array consisted of 12 *G*60 Cintel Geiger–Muller counters arranged in a semicircle The quenching circuits were of a standard type One valve of the Rossi-type anticoincidence circuit was cut off permanently by a standing bias. Positive square pulses from the quenching circuit attached to the anticoincidence ring lifted this bias and no pulse was passed to the scaler when pulses arrived at the grids of both Rossi valves simultaneously (that is, when a meson passed through the apparatus) In the absence of a pulse to lift this bias a pulse at the grid of the first valve gave an output pulse to the scaler The pulses from the quenching circuit feeding the first valve were delayed for a short time to ensure efficient anticoincidence operation There is some loss in efficiency due to the paralysis time of the quenching circuit associated with the anticoincidence ring, but this should not exceed 1 per cent The anticoincidence device can be made nearly 100 per cent efficient if the counters are slightly overlapped to prevent mesons leaking through spaces between counters

Experimental work using this counting system has already been published[9,25] in connexion with investigations on intrathyroidal iodine metabolism Since the greatest test of low-level counting methods occurs in the examination of peripheral iodine metabolism, a chromatogram scan is presented in Fig 5, which was obtained by chromatographing directly a pancreatin digest of liver homogenate from a rat killed 44 h after injection of ^{131}I-iodide Most of the radioactivity was located in a single peak with a low R_F value, which, in the solvent system used, butan-1-ol/dioxan/2 N ammonia (4 1 5), corresponds in position to the iodothyronine glucuronides The position and shape of the peak have been accurately defined even though the total radioactivity of the chromatogram did not exceed 30 μμc It was possible to elute the radioactivity of this peak from the chromatogram and to show that after hydrolysis with a *Patella vulgata* β-glucuronidase preparation it exhibited the chromatographic properties of thyroxine The compound, therefore, resembles a thyroxine conjugate previously isolated from mouse kidney by Galton and Pitt-Rivers[26]

Measurement of thick sources For liquid sources and other thick sources the most common method of measurement involves the use of a sodium iodide (Tl) well-type crystal Commonly a crystal of about 2 in diameter and 2 in deep is used with a well $\frac{5}{8}$ in × $1\frac{1}{2}$ in The mean path-length for γ-rays in the crystal will be about 1 in and the total number of interactions from iodine-131 γ-rays (photo-electric plus Compton) will be about 47 per cent of the number of disintegrations In practice the 364-keV photoelectric peak is found to contain about 50 per cent of the total number of events, which agrees with the theoretical prediction that about 24 per cent of the disintegrations will produce 364-keV photoelectrons A good crystal should, in practice, give a 364-keV photopeak resolution (ratio of width of peak at half-amplitude to peak position) of about 12 per cent Assuming a photoconversion efficiency of 36 eV/photon for sodium iodide and a photocathode efficiency of 10 per cent for the photomultiplier, a resolution of about 8 per cent is theoretically possible This is never realized in practice, probably owing to losses of photons by absorption and on reflexion from the sides of the crystal

It is necessary to consider whether it is advantageous to use single channel analysis to count only the photopeak, thereby reducing the background count The background in the photopeak region of a typical sodium iodide crystal of the dimensions mentioned and shielded by 3-in thick lead is about 25 counts per min If the bias of the apparatus is set so that all events above an energy of 50 keV are recorded, then the background will increase to about 150 counts per min We have, then, to compare a system with an efficiency of 0 25 and background of 25 counts per min with one having an efficiency of 0 47 and background 150 counts per min The curves obtained for these situations are shown in Fig 6 It will be seen that for low activities (< 100 μμc) single channel pulse-height analysis is preferable, but that, in the range con-

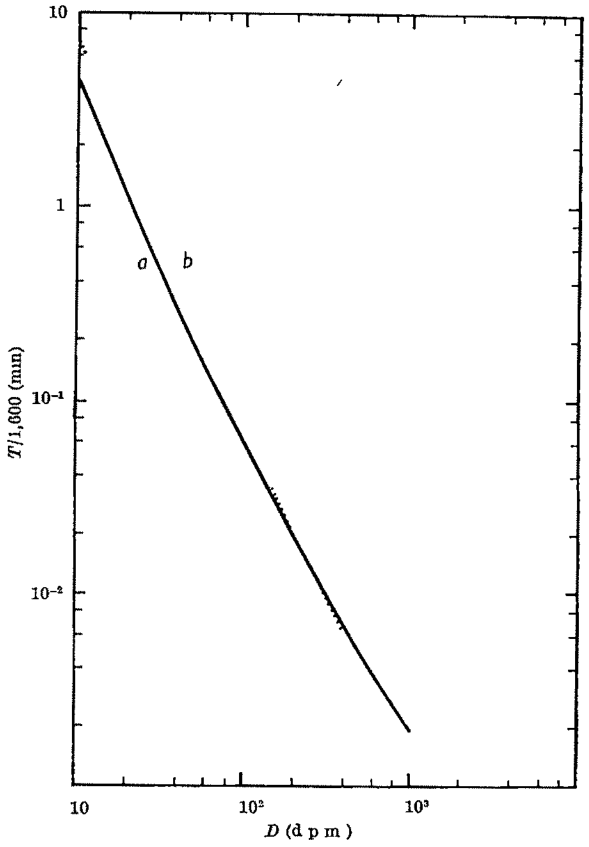

Fig 6 *T* versus *D* curves for (*a*) efficiency = 0 25, background = 25 c p m , (*b*) efficiency = 0 47, background = 150 c p m

sidered, the two systems are roughly equivalent above this level

Newly developed counting methods The systems described here are now sufficiently well developed to be in regular use. In another, less well developed method, thin sources of iodine-131 can be measured by counting the β-particles with a thin plastic phosphor To absorb completely the β-particles a phosphor about 3 mm thick would be required A typical plastic phosphor, such as *p*-terphenyl in polystyrene, must absorb about 200 eV from the energy of a β-particle to produce a photon of light A typical photomultiplier has a photocathode efficiency of about 10 per cent and, therefore, an energy of 2 keV is required to produce one photoelectron at the photocathode, assuming that all the photons are collected In practice, it is extremely difficult to collect every available photon and it is probably more realistic to base calculations on a figure of 3 keV/photoelectron It would be necessary to bias the apparatus to cut out the photomultiplier noise and, since a standard photomultiplier produces a significant number of noise pulses of up to about 5 electrons the bias would have to be set at a point which would eliminate the measurement of β-particles entering the phosphor with energies less than 15 keV This is an optimistic figure since the bias would most likely have to be set higher to allow for temperature variation in the noise This system in its simplest form of a thin phosphor, viewed by one photomultiplier and silvered on the back face to reflect light into the photomultiplier, is therefore, from the point of view of efficiency, fundamentally worse than the Geiger–Muller counter system To compare with the best Geiger system the background in the phosphor would have to be significantly lower than is, in practice, attainable Some improvement could be made by using a double system and running the photomultipliers in coincidence The elimination of photomultiplier noise would allow the bias to be set lower and the β-particles from both sides of the source would be counted Fast, reliable, coincidence circuits are, however, difficult to design and maintain It would also be difficult to devise a simple system to introduce the source between the two phosphors—the method used would have to combine the requirements of light tightness and minimum absorbing material between source and phosphor If these difficulties can be solved, then the double phosphor system would offer an attractive method for counting thin sources of iodine-131, especially since the dimensions of the phosphor could be matched to those of the source, thus minimizing the background

Another technique for counting low-activity sources of iodine-131 involves the use of a sodium iodide crystal working in coincidence with a thin-window Geiger–Muller counter Pulses are registered when the 364-keV γ-ray is recorded by the scintillation counter and the 600-keV cascade β-particle is recorded simultaneously by the β-counter. Using a single channel pulse-height analyser with the sodium iodide counter, very low backgrounds can be obtained With counters of 1 in diameter operating face to face, the source being placed between them, we have obtained efficiency of about 8 per cent with a background of 1 or 2 per hour These figures could be improved with a more favourable geometry This counter has an obvious application for the rapid determination of the presence of activity, even if the efficiency cannot be made large enough to allow quantitative estimates of radioactivity to be made in a time short enough to compete with other methods

Finally, mention must be made of the future use of solid conduction counters for chromatogram scanning These counters are now being rapidly developed[27] and will, no doubt, eventually provide an excellent method for the low-level counting of iodine-131 Because of this thinness of the active volume of the counter the backgrounds are potentially very low, but unfortunately the noise pulses in the presently available counters are so large that the bias has to be set at a level which cuts out a significant fraction of the pulses from the lower energy β-particles The efficiency of the available counters is therefore low but great efforts are being made to improve this

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2,5-DIHYDRO-2-FUROIC ACID: A PRODUCT OF THE ANAEROBIC DECOMPOSITION OF ASCORBIC ACID

By I. M. COGGIOLA

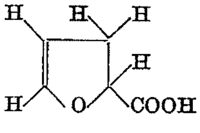
C.S.I.R.O. Division of Food Preservation, North Ryde, New South Wales, Australia

THE anaerobic decomposition of ascorbic acid was studied by Huelin[1], who identified the products furfural and carbon dioxide His report that loss of ascorbic acid from an unbuffered aqueous solution involved an increase of *p*H was not confirmed—the formation of other acids resulted in little net change in *p*H

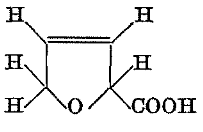
A 5 per cent (w/v) solution of ascorbic acid in glass-distilled water was incubated at 100° C under an atmosphere of carbon dioxide for 10 days until decomposition was practically complete The acids were separated by displacement ion-exchange chromatography[2] The fractions containing the predominant acid were combined and freeze-dried, to give a brown semi-crystalline material The methyl ester was prepared by reaction with diazomethane

The *p*H titration curve of the acid showed the presence of one carboxyl group (*p*K, 3 2) The absence of carbonyl groups was shown by its failure to react with 2,4-dinitrophenylhydrazine Hydrogenation of the methyl ester indicated the presence of one double bond The infra-red spectrum of the acid showed a carboxyl group at 1,727 cm^{-1} and the infra-red spectrum of the methyl ester after gas chromatography showed that hydroxyl groups were absent As further purification of the acid was unsuccessful, and as its purity was inadequate for elementary analysis, the *cyclo*hexylamine salt was prepared as colourless needles m p 160°–163° C (decomp) Analysis of this salt gave C, 62 2, H, 9 2, O, 22 5, N, 6 5 per cent Calc for $C_{11}H_{19}O_3N$ C, 61 9, H, 9 0, O, 22 5, N, 6 6 per cent This would give a formula of $C_5H_6O_3$ for the isolated acid (calc equiv wt 114, found, 116) As two of the oxygen atoms are involved in the carboxyl group, the remaining one, which belongs neither to a carbonyl nor to a hydroxyl group, must be involved in an ether linkage The foregoing evidence indicates the possibility of a dihydrofuroic acid

Sublimation under high vacuum with the application of heat was attempted as a means of purification, but resulted in decomposition, with the formation among other products of 2-furoic acid, m p 132°–133° C The infra-red and nuclear magnetic resonance spectra were identical with those of an authentic sample of 2-furoic acid. Analysis gave C, 53 6, H, 3 7, O, 42 5 per cent, calc for $C_5H_4O_3$ C, 53 6, H, 3 6, O, 42 8 per cent From this it could be assumed that the carboxyl group is in the 2-position The acid could have one of the structures I, II or III



(I)



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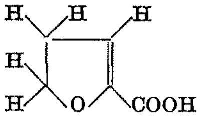


(III)

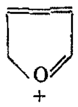
The nuclear magnetic resonance spectra of the acid and its methyl ester were determined at 60 Mc/s in deuterochloroform solution Signal positions are given in p p m from tetramethylsilane as internal reference The spectrum of the acid consisted of multiplets at 4 8 p p m (2 protons, essentially an *AB* quartet with J_{AB} approximately 12 c/s and split further by several protons), 5 3 p p m (1 proton), and 6 0 p p m (2 protons, essentially an *AB* quartet with J_{AB} = 6 3 p p m and further splitting due to interaction with several protons), and a singlet at lower field (position variable with concentration), which disappeared on treatment with deuterium oxide[3] and must be due to the carboxylic hydrogen

The spectrum of the ester showed multiplets at 4 8 p p m (2 protons), 5 2 p p m (1 proton), and 6 0 p p m (2 protons), all having the same structure as the corresponding signal in the spectrum of the acid, and a singlet at 3 7 p p m (3 protons) which must be due to the ester methoxyl

The position and principal coupling constants of the two two-proton multiplets are consistent with the presence of a di-substituted *cisoid* double bond and a methylene group with non-equivalent protons (for example, in a ring) The position of the resonances[4] and the presence of additional splitting, due to long-range interaction[5], are entirely consistent with structure II for the acid and ester respectively

The presence of a signal due to two vinylic protons in the nuclear magnetic resonance spectrum (multiplet at 6 0 p p m) excludes structure III, which is also inconsistent with the ultra-violet spectrum, which showed no strong absorption The long-range interactions causing the fine splitting in the nuclear magnetic resonance spectrum are consistent with structure II In particular, the signal attributed to the proton on C-2 (at 5 3 p p m) would be expected to be much less complicated in structure I than in II due to the absence of homoallylic interactions[6] The position of the resonance assigned to the ring methylene protons (4 8 p p m) is consistent only with structure II, in structure I this resonance would be expected to occur much farther upfield[4]

The mass spectrum of the ester was determined on an Atlas-Werke model *CH*4 spectrometer and showed only one main peak at m/e 69 which is attributed to the resonance stabilized ion IV The absence of the parent peak, even at low energy, is entirely consistent with structure II (ref 7)



(IV)

The predominant acid isolated is considered to be 2,5-dihydro-2-furoic acid

I thank Drs J S Shannon and S Sternhell, of the C S I R O Division of Coal Research, for the mass and nuclear magnetic resonance spectra respectively, and for their interpretation

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ORIGIN OF SODIUM CONCENTRATION PROFILE IN THE RENAL MEDULLA

By Dr G G. PINTER*

Department of Physiology, School of Medicine, University of Maryland

AND

Dr J L. SHOHET

Department of Electrical Engineering, Johns Hopkins University, Baltimore

In recent years, mathematical models have been described with the purpose of simulating the function of the Henle loops on one hand and the role of the vasa recta on the other Hargitay and Kuhn[1] and Kuhn and Ramel[2] presented the countercurrent multiplier hypothesis for the operation of the Henle loops These authors took the presence of the collecting ducts into consideration, but omitted the vasa recta as an integral part of the system Similarly, Gunzler[3] derived equations for the concentration profile in the vasa recta without paying attention to the presence of the Henle loops

* Holder of U S Public Health Service research career development award

These models have been successful in predicting most of the experimental results obtained by chemical analysis of the medullary tissue and by micropuncture studies It appears however, that they do not readily offer an explanation for the sodium concentration profile found in the renal medulla of well-hydrated animals[4] As reported by Levitin *et al*[5] and by Boylan and Asshauer[6] in water diuresis, the concentration of tissue sodium may show an increase from the cortico-medullary border to an internal layer of the medulla whereupon it may decrease toward the papilla Furthermore, the hypotheses dealing separately with the Henle loops and vasa recta do not eliminate the doubt expressed by Smith[7] that a sudden change in sodium-transporting activity and water permeability of the cells lining the thin segment of the Henle loop at the turning point is not warranted by the histological picture

In this article we propose an integrated model which takes into account the interaction between Henle loops and vasa recta The operating unit of our model is a small segment of the medullary interstitium into which are immersed one Henle loop and one vas rectum (Fig 1) For the sake of simplicity, we disregard momentarily the presence of the collecting ducts, the influence of which will be considered later Apparently, lymph vessels are not present in the medulla[8]

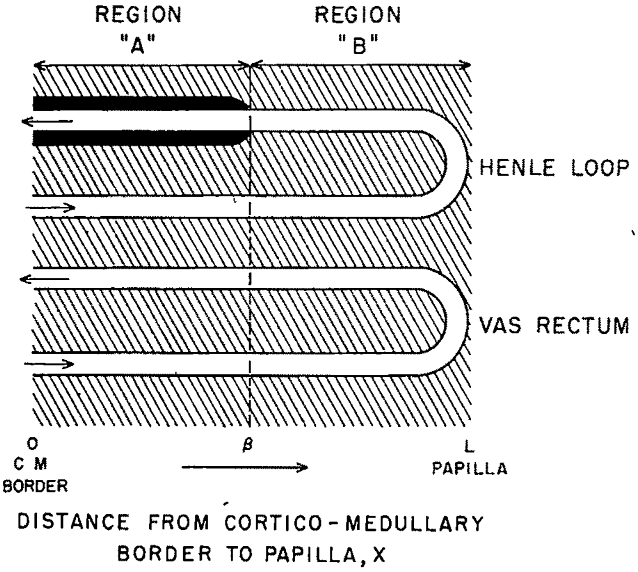

Fig 1 Operating elementary unit

We assume that sodium moves across the wall of the vasa recta and that of the thin segment of the Henle loops according to the concentration difference between the lumen and interstitium, while the wall of the thick segment of the ascending Henle loops transports sodium out of the lumen into the interstitium at any point at a rate which is proportional to the sodium concentration in the lumen In other words, in this model the countercurrent multiplier action of the Henle loop is restricted to the region where the ascending limb is thick walled (region *A* in Fig 1) The function of the thin segment of the Henle loops and of the entire vasa recta is to wash the sodium from the interstitium of the countercurrent multiplier region toward both cortex and papilla depending on the direction of flow in these channels It is further assumed that the diffusion of sodium in the medullary interstitium is predominantly perpendicular to the course of the Henle loops and vasa recta, that is, the process of sodium diffusion in the cortico-papillary direction may be neglected Again, the system is in equilibrium, that is, the amount of sodium moving into equals the quantity moving out of our operating elementary unit

The following boundary conditions are assumed (1) The input concentrations in both tubes are known and constant (2) Flow rate and output concentration in both tubes are interdependent variables affecting the shape of the concentration profile in the medulla

The system is divided into two regions (Fig 1) Region *A* will be the area between the entrance to the system and point β Region *B* will be the area between point β and the turning point of the tube Two sets of coupled equations can then be written for region *A* and region *B* In all, ten interdependent differential equations describe the concentrations prevailing in the ascending and descending Henle loops and vasa recta as well as in the interstitium, 5 in region *A* and 5 in region *B* The equations for region *A* are

$$\frac{\partial c}{\partial x} = k(j - c) \qquad (1)$$

$$\frac{\partial c'}{\partial x} = k(j - c') \qquad (2)$$

$$\frac{\partial c_h}{\partial x} = k_h(j - c_h) \qquad (3)$$

$$\frac{\partial c_h'}{\partial x} = k_h' c_h' \qquad (4)$$

$$\frac{\partial j}{\partial x} = k(c - j) + k(c' - j) + k_h(c_h - j) + k_h' c_h' \qquad (5)$$

where c is the concentration in the descending limb of the vas rectum, c' is the concentration in the ascending limb of the vas rectum, c_h is the concentration in the descending part of the Henle loop and c_h' is the concentration in the ascending part of the Henle loop j is the interstitial concentration, k the 'catch-all' membrane coefficient for the vasa recta, k_h the coefficient for the Henle loop and k_h' the coefficient for the thick-walled segment of the Henle loop x, the distance, is measured from the cortico-medullary boundary towards the papilla

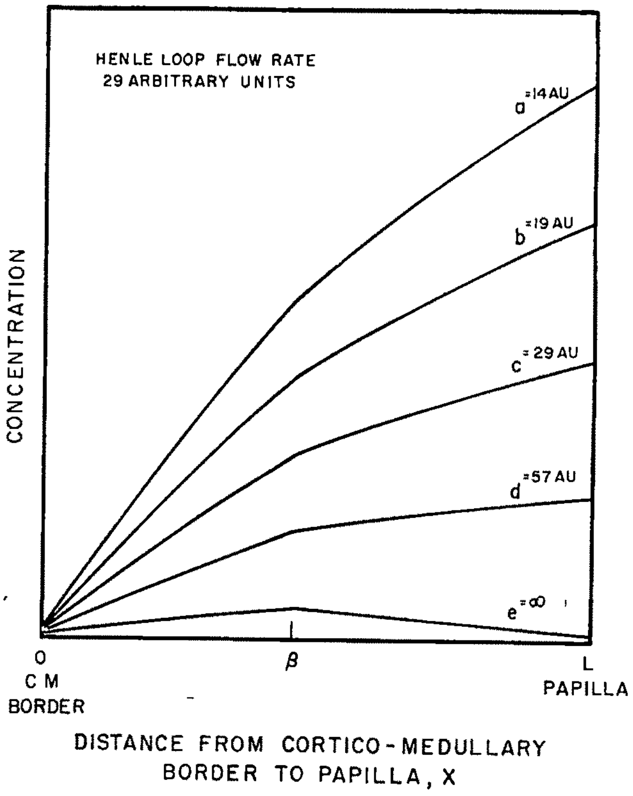

Fig 2 Interstitial concentration in the renal medulla with vasa recta flow rates of 14, 19, 29, 57, and infinite number arbitrary units (AU) and Henle loop flow rate of 29 arbitrary units

The corresponding equations for region B are identical to those for region A except for (4) and (5) which are now

$$\frac{\partial c_h'}{\partial x} = k_h(j - c_h') \qquad (6)$$

$$\frac{\partial j}{\partial x} = k(c - j) + k(c' - j) + k_h(c_h - j) + k_h'(c_h' - j) \qquad (7)$$

The system of equations may then be solved with the appropriate boundary conditions and the requirements that the solutions in region A and region B must match at point β, and that at the turning points c must equal c' and c_h must equal c_h' It will also be assumed that the rate of change of concentration with respect to x in the input of both tubes and at $x = 0$ in the interstitium are identically equal to zero

The equation for c gives

$$c = A + Be^{p_1x} + Ce^{p_2x} + De^{p_3x} + \frac{c_{h_0}'}{4}\left[\frac{k_h'^2}{kk_h} + \frac{k_h'}{k} + \frac{k_h'}{k_h} + 1\right]^{-1} e^{l_h'x} \qquad (8a)$$

in region A, where p_1, p_2 and p_3 are found from the solution of the determinental equation

$$\frac{p^3}{k^2k_h} + \left[\frac{2}{k^2} + \frac{4}{kk_h}\right]p^2 + \left[\frac{6}{k} + \frac{3}{k_h}\right]p + 4 = 0$$

and c_{h_0}' is the output concentration of sodium from the Henle loop and

$$c = \bar{A} + \bar{B}e^{\bar{p}_1x} + \bar{C}e^{\bar{p}_2x} + \bar{D}e^{\bar{p}_3x} \qquad (8b)$$

in region B, where p_1, p_2 and p_3 are found from the solution of the determinental equation

$$\frac{\bar{p}^3}{k^2k_h} + \left[\frac{3}{k^2} + \frac{4}{k_hk}\right]\bar{p}^2 + \left[\frac{8}{k} + \frac{3}{k_h}\right]\bar{p} + 5 = 0$$

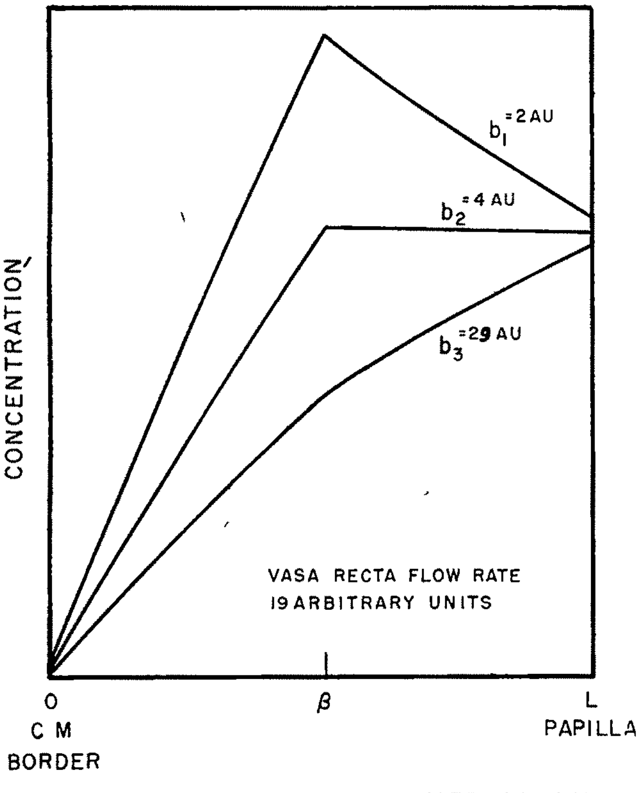

Fig 3 Interstitial concentration in the renal medulla with Henle loop flow rates of 2, 4, and 29 arbitrary units and vasa recta flow rate of 19 arbitrary units

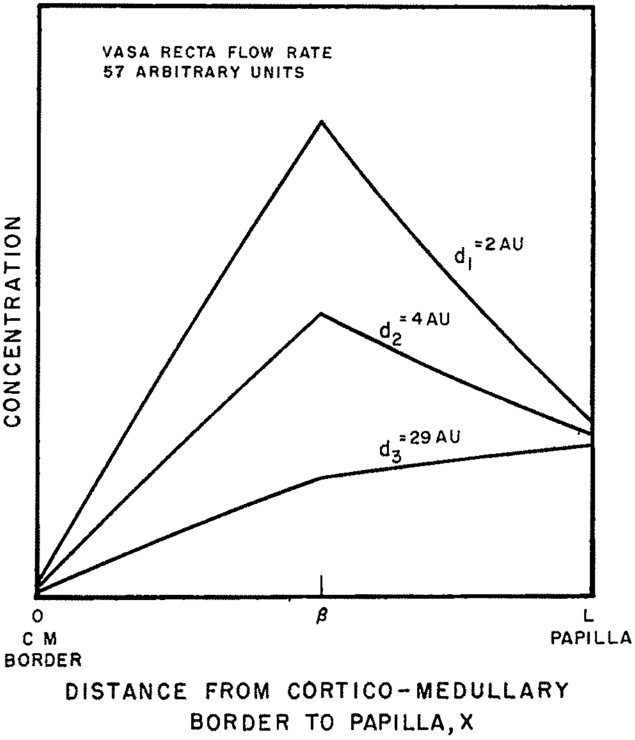

Fig 4 Interstitial concentration in the renal medulla with Henle loop flow rates of 2, 4, and 29 arbitrary units and vasa recta flow rate of 57 arbitrary units

The coefficients A, B, C, D, $\bar{A}$, $\bar{B}$, $\bar{C}$ and $\bar{D}$ are evaluated from the boundary conditions

The solution for c' is identical in form to that of c, except for different coefficients A', B', C', D', $\bar{A}'$, $\bar{B}'$, $\bar{C}'$ and $\bar{D}'$ The exponents are identical

The results for c_h are

$$c_h = A_h + B_he^{p_{h_1}x} + C_he^{p_{h_2}x} + \frac{c_{h_0}'}{4}\left[\frac{k_h'}{k_h} + 1\right]^{-1} e^{l_h'x} \qquad (9a)$$

in region A, where p_{h_1} and p_{h_2} are evaluated from the solution of

$$\frac{p_h^2}{kk_h} + \left[\frac{3}{k_h} + \frac{2}{k}\right]p_h + 4 = 0$$

and

$$c_h = \bar{A}_h + \bar{B}_he^{\bar{p}_{h_1}x} + \bar{C}_he^{\bar{p}_{h_2}x} + \bar{D}_he^{\bar{p}_{h_3}x} \qquad (9b)$$

in region B, where the exponents are found from the solution of

$$\frac{\bar{p}_h^3}{k_h^2k} + \left[\frac{3}{k_h^2} + \frac{4}{kk_h}\right]\bar{p}_h^2 + \left[\frac{8}{k_h} + \frac{3}{k}\right]\bar{p}_h + 5 = 0$$

c_h' is found to be

$$c_h' = c_{h_0}' e^{k_h'x}$$

in region A, and of exactly the same form as c_h in region B with coefficients $\bar{A}_h'$, $\bar{B}_h'$, $\bar{C}_h'$ and $\bar{D}_h'$.

Finally, j, the concentration in the interstitium, is found directly from equation (1) since the solution for c is known It is then

$$j = A + B\left(1 + \frac{p_1}{k}\right)e^{p_1x} + C\left(1 + \frac{p_2}{k}\right)e^{p_2x} + D\left(1 + \frac{p_3}{k}\right)e^{p_3x} + \frac{c_{h_0}'}{4}\left[1 + \frac{k_h'}{k}\right]\left[\frac{k_h'^2}{kk_h} + \frac{k_h'}{k} + \frac{k_h'}{k_h} + 1\right]^{-1} e^{k_h'x} \qquad (10a)$$

in region A, and:

$$y = \bar{A} + \bar{B}\left(1 + \frac{\bar{p}_1}{k}\right)e^{\bar{p}_1 x} + \bar{C}\left(1 + \frac{\bar{p}_2}{k}\right)e^{\bar{p}_2 x} + \bar{D}\left(1 + \frac{\bar{p}_3}{k}\right)e^{\bar{p}_3 x} \qquad (10b)$$

in region B. Note that the solutions to all these equations depend on the membrane coefficients, the flow rates when the boundary condition of equilibrium is applied and the input concentrations. The actual solution of the equations for the concentration at any point depends on the membrane coefficients and flow rates in both channel systems. It was assumed that the parameter which is the most readily regulated physiologically is the flow rate in the vasa recta and in the Henle loops. The equations were put on an analogue computer connected to an X–Y plotter whereby the behaviour of the concentration profiles in ascending and descending Henle loops, vasa recta and in the medullary interstitium could be observed under changing flow rates in the vascular and tubular channels. Figs. 2, 3 and 4 represent results from the computor for the concentration profile in the medullary interstitium under varying flow rates in the vasa recta and in the Henle loop. On these graphs the abscissa represents the site in the medulla from the cortico-medullary border to the turning point of the channels and the ordinate is measured in terms of Na concentration.

In Fig. 2, the Henle loop flow rate was held constant at 29 arbitrary units and the vasa recta flow rate was varied as follows: curve *a*, 14, curve *b*, 19, curve *c*, 29, curve *d*, 57, curve *e*, a very large number (∞) arbitrary units. It may be pointed out that under the conditions of finite flow specified in Fig. 2 the Na concentration continues to rise throughout the entire length extending from the cortico-medullary border to the papilla. A continuous rise in sodium concentration which extended from the cortico-medullary border up to the papilla was also observed in both limbs of the Henle loop and vasa recta. To our knowledge it is this finding, namely, that in hydropenic animals the increase of sodium concentration extends up to the papilla, which leads to the inference that the Henle loop operates as a countercurrent multiplier in its entire length. From the graphs it can be seen that such an inference is not necessary in a model in which the countercurrent multiplier operation is limited to the portion of the Henle loops where the ascending limbs have thick walls. Such a model may create continuously increasing sodium concentration if its action is co-ordinated with the function of the vasa recta. In Figs. 3 and 4 the vasa recta flow rates were held constant and the Henle loop flow rates were varied. These graphs indicate that our model may duplicate conditions in which the highest sodium concentration is found in a middle layer of the medulla. Our model predicts that this layer will be one in which the beginning of most of the thick segments of the ascending Henle loops is found.

It may be assumed that the collecting duct contributes a constant amount of solute and/or water to the medullary interstitium along its entire length. This operation shifts the concentration profile upwards or downwards, respectively, by an amount proportional to the product of the amount added times the longitudinal distance. If the rate of transport of water or solute across the wall is not constant, the influence is more complex, but it is not expected that it would basically change the shape of the concentration curve.

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LABILE FLAVIN ADENINE DINUCLEOTIDE OF HEART MUSCLE PREPARATIONS

By DR. TERENZIO CREMONA*, DR. EDNA B. KEARNEY, DR. JAMES SALACH
and DR. THOMAS P. SINGER

Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit

MASSEY and Swoboda[1] recently reported that on storage at 4° of heart muscle preparations isolated by a modification of Keilin and Hartree's method[2] there is a gradual decrease in the acid-extractable FAD content and corresponding increase in the FMN content. While the supporting results presented are convincing, so far as this preparation is concerned, the claims of these authors that the same breakdown occurs in Keilin–Hartree preparations isolated by mechanical grinding and differential centrifugation[3] are less convincing. Massey and Swoboda concluded that (*a*) within experimental error FAD is the only acid-extractable flavin present in fresh heart mitochondria, (*b*) that any enzyme isolated from this source which contains FMN is likely to be an artefact. Conclusion (*a*) agrees with the earlier report of Ziegler *et al.*[4] that more than 90 per cent of the acid-extractable flavin content of heart mitochondria is FAD. For conclusion (*b*), Massey and Swoboda quoted the example of DPNH-cytochrome reductases isolated from heart, all of which have now been shown to contain only FMN (refs. 5 and 6) and to be degradation products of the DPNH dehydrogenase of the respiratory chain[6,7]. Since purified preparations of DPNH dehydrogenase had been reported to contain both FAD and FMN (ref. 8), Massey and Swoboda[1] further suggested that even this preparation may be modified to some extent.

According to as yet unpublished reports, Merola and Coleman[9] and Nickel *et al.*[10] could not confirm these results and affirmed that the FMN present in certain heart muscle preparations is not an artefact due to ageing. The former authors based their conclusions on ageing of beef heart mitochondria at −20°, while Nickel *et al.* examined the effect of storage at 0°–4° on Keilin–Hartree preparations isolated by mechanical grinding and differential centrifugation. Thus the conditions used by both groups deviate from those utilized in most of the experiments of Massey and Swoboda and, hence, do not bear on the experimental findings, although they are relevant to these authors' conclusions.

In view of the obvious interest of the phenomenon and its implications on many problems concerning respiratory enzymes, including the question of the prosthetic group of DPNH dehydrogenase[1], we wish to report that we have confirmed fully the findings of Massey and Swoboda as regards acid precipitated Keilin–Hartree preparations but have not been able to observe a similar breakdown of FAD in mitochondria or in Keilin–Hartree samples prepared at neutral pH.

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* On leave of absence from the International Institute of Genetics and Biophysics, Naples.

Table 1 STABILITY OF FAD IN BEEF HEART MITOCHONDRIA

Exp	Preparation	Acid-extractable flavin (mμmoles/mg protein)	FAD/total acid-extractable flavin (per cent)	Total acid-extractable flavin (μmoles)	Total FAD (μmoles)	Total FMN (μmoles)
1	Fresh BHM, rapid preparation (2–3 h after killing)	0 32	84			
2	Normal, fresh BHM (5–6 h after killing)	0 38	85			
3	Frozen stored BHM (same as Exp 2, after 3 days at −15° in sucrose–PO$_4$)	0 39	85			
4	Mitochondria stored at 4° in sucrose–PO$_4$					
	Immediately after preparation	0 33	85			
	24 h later	0 38	86			
	92 h later	0 32	82			
5	Fresh BHM	0 38	85	15 4	12 8	2 60
	First centrifugation residue	0 42	84	14 1	11 8	2 26
	,, ,, supernatant	0 24	71	1 34	0 96	0 38
	,, ,, sum			15 4	12 8	2 60
	Second centrifugation supernatant	0 18	81	0 40	0 32	0 075
	,, ,, residue (ETP)	0 28	69	0 93	0 64	0 291
	,, ,, sum			1 34	0 96	0 37

Beef heart mitochondria (BHM) and ETP were prepared exactly as per Ringler *et al* (ref 8) Total flavin, FAD, and FMN were analysed by differential fluorometry in 5 per cent TCA extracts Protein was determined by the biuret method with the coefficient of 0 095 Samples were stored in 0 25 M sucrose–0 01M phosphate, *p*H 7 4 In Exp 5 40 5-g fresh BHM were processed all the way to the ETP stage without storage The supernatant solution from the first centrifugation (7 min at 19,000*g*) is recentrifuged at 106,000*g* for 30 min, the neutralized pellet is the ETP

Table 2 STABILITY OF FAD IN KEILIN–HARTREE PREPARATIONS

Prepared at neutral *p*H					Acid precipitated preparation				
Hours after killing	Acid extractable flavin (mμmoles/mg protein) Total	FMN	FAD	FAD/total flavin (per cent)	Hours after killing	Acid extractable flavin (mμmoles/mg protein) Total	FMN	FAD	FAD/total (per cent)
4 6	0 302	0 100	0 231	77	7	0 358	0 072	0 328	92
28	0 303	0 102	0 230	76	27 5	0 362	0 091	0 310	86
52	0 302	0 100	0 199	77	51 5	0 370	0 123	0 283	77

The Keilin–Hartree preparation isolated at neutral *p*H was prepared by means of grinding in a mechanical mortar (at 4°) and differential centrifugation, following the previously described modification (ref 8) of King's procedure (ref 3) The average time for preparation was 4 h The acid precipitated sample was made exactly according to Massey and Swoboda (ref 1) The average time for preparation was 3 5 h

Table 1, Exps 1 and 2, shows that beef heart mitochondria, whether prepared rapidly or by the normal procedure, contain the same ratio of FAD to total flavin and (Exps 3 and 4) this ratio remains constant on several days' storage at 4° or at −15° Exp 5 demonstrates that in the preparation of ETP (the source of DPNH dehydrogenase[8]) no FAD is destroyed nor is FMN formed It may be concluded that the breakdown of FAD observed by Massey and Swoboda is not a characteristic property of mitochondrial flavoproteins Elsewhere[11] it has been shown that storage of ETP for 2 weeks at −15° causes no breakdown of FAD and that if the isolation of DPNH dehydrogenase is carried out within a period of 24 h of the killing of the animal, without interruption, the same FMN content is found in the resulting preparations as in samples purified over a period of several days from stored mitochondria Thus the observations of Massey and Swoboda do not seem to have any direct bearing on the flavin moiety of DPNH dehydrogenase

It should be noted that the FAD to total flavin ratio reported in Table 1 (82–86 per cent) for mitochondria is the value which has been found over many years of analyses of heart mitochondria in this laboratory and agrees very well[12] with the values reported by Massey and Swoboda[1] and Rao *et al* [5], but not with those of Merola and Coleman[9] or of King *et al* [13]

Table 2 demonstrates that while Keilin–Hartree preparations isolated at neutral *p*H show no breakdown of FAD, those prepared by Massey and Swoboda's technique[1] show a higher initial FAD and total flavin content and a tendency for a gradual decline in the ratio of FAD to total flavin on storage Again, the flavin composition agrees with the figures of Massey and Swoboda but not with those of King *et al* [13] We suggest that the agreement in FAD to total flavin ratio between the two types of preparation after storage is fortuitous and should not be taken to indicate that the sample prepared at neutral *p*H had already broken down to this ratio in the course of preparation, as proposed by Massey and Swoboda[1] The higher initial total flavin and FAD content of acid precipitated samples might indicate, instead, the inclusion of flavoproteins which are not recovered in the alternative procedure and are not normal mitochondrial constituents

It remains to be determined whether the interesting breakdown observed by Massey and Swoboda is caused by labilization of a flavoprotein during exposure to acid *p*H or is the result of the precipitation of a labile flavoprotein at *p*H 5 4 but not at neutral *p*H In any event, these results do not support the conclusion of Massey and Swoboda regarding the absence of FMN in mitochondrial enzymes Their suggestion that the presence of FMN in dehydrogenases isolated from heart mitochondria is diagnostic of preparative modification is also not supported by these results and is, further, unnecessary to explain known facts The DPNH-cytochrome reductase preparations from heart described in the literature are, indeed, degraded derivatives of DPNH dehydrogenase, but they have been shown to arise during extraction and are thus not formed by whatever process is responsible for the labilization of FAD in certain preparations[6,7,14] As to DPNH dehydrogenase itself, recent work in this laboratory has conclusively demonstrated that the prosthetic group is FMN (ref 15), in agreement with predictions in the literature and analyses of its degradation products[5,6]

This work was supported by grants from the U S National Science Foundation (*G* 20457), the U S National Heart Institute (*HE* 01995) and by contract No *Nonr* 1656 (00) between the U S Office of Naval Research and this Institute

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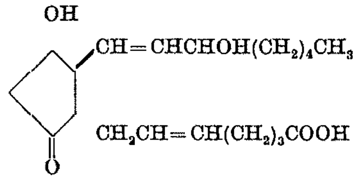
ISOLATION AND IDENTIFICATION OF TWO SMOOTH MUSCLE STIMULANTS FROM MENSTRUAL FLUID

By Dr G EGLINTON, Prof R A RAPHAEL, F.R.S, and Dr G N SMITH
Department of Chemistry, University of Glasgow
AND
Dr W. J HALL and Dr V R PICKLES
Department of Physiology, University of Sheffield

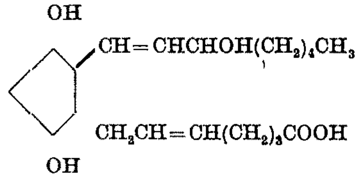
THE presence of a group of lipid smooth-muscle stimulants in menstrual fluid was first reported in *Nature* in 1957 (ref 1) Since then it has been shown that these substances, which are almost certainly produced by the endometrium in its secretory phase[2], are in part carried to the myometrium, and there stimulate the rhythmical contractions of normal menstruation. An excess of such an effect is probably an important factor in causing the intense uterine cramps of primary dysmenorrhœa[3]

Two physiologically active constituents of high potency have now been isolated from menstrual extracts and, by methods described here, have been identified as prostaglandins PGE_2 and $PGF_{2\alpha}$



PGE_2

(11a,15 dihydroxy-9-keto-prosta-5,13-dienoic acid)



PGF_{2a}

(9a,11a,15-trihydroxy-prosta-5,13-dienoi acid)

These substances are members of an important class of smooth-muscle stimulants originally detected by von Euler[4], who introduced the name 'prostaglandin', the recent outstanding investigations of Bergstrom and his colleagues have resulted in the separation and structural identification of five naturally occurring individuals of this closely related group of compounds[5,9,10,13,14]

The prostaglandins PGE_2 and PGF_{2a} are certainly not the only active constituents present in menstrual fluid, but they do account for the major part of the activity Although these two components are only present in minute amounts their high activity, detectable at a concentration of 10^{-10} g/ml, permitted the physiological effect to be discerned at an early stage[6]

Preliminary fractionation results gave some indication that the active materials were of the long-chain hydroxy-acid type[7] This result, together with the nature of the activity shown, gave the first inkling that the substances involved might be members of the prostaglandin series Accordingly, the separation techniques used so elegantly by Bergstrom's school in the isolation of the prostaglandins from more abundant sources were applied to the menstrual fluid derived from 3,675 specimens, collected and treated as described previously[7] Successive extractions with acetone and dichloromethane produced a crude menstrual lipid concentrate (about 75 g)

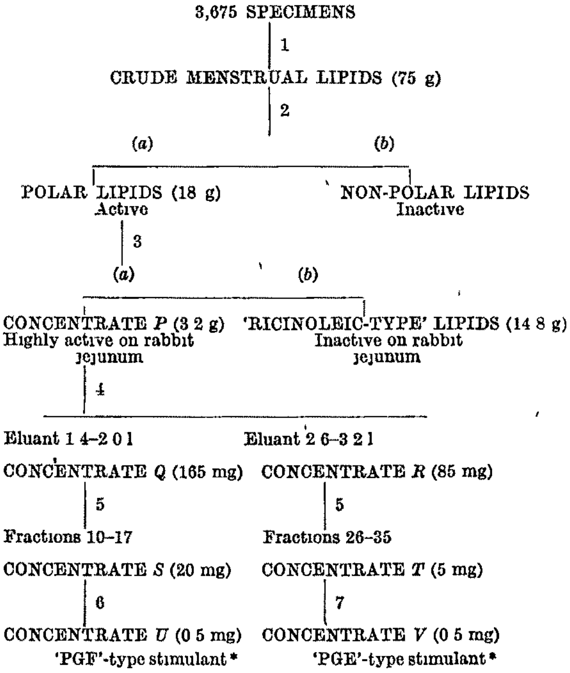


Fig 1 Fractionation scheme for the isolation of the active constituents from the menstrual specimens

(1) Acetone and dichloromethane extraction (ref 7) (2) Elution from hydrophobic 'Embacel' with (*a*) aqueous acetone, (*b*) acetone, ether (3) Reversed-phase partition chromatography on column of hydrophobic 'Embacel' impregnated with paraffin oil (ref 8) (*a*) 30 per cent aqueous acetone, (*b*) 50–100 per cent aqueous acetone (4) Reversed-phase partition chromatography using iso-octanol/chloroform//methanol/water, 1 1 9 10 v/v (see Fig 2) (5) Reversed-phase partition chromatography using iso-amyl acetate/chloroform/methanol/water, 4 6 35 65 (6) Reversed-phase chromatography using the system described in 4 except that a phosphate buffer of *p*H 7 2 replaced the water, followed by re-chromatography using system 4 (7) Extraction from chloroform solution with a phosphate buffer *p*H 7 2 followed by acidification and re-extraction into chloroform

* Biossay of the concentrates obtained at each stage on the guinea pig uterus and the rabbit jejunum indicated that the recovery of prostaglandin-like activity in the final concentrates was at least 90 per cent of that originally present

The treatment of this lipid concentrate is briefly outlined in Fig 1 Assay of the active constituents was carried out *in vitro* on preparations of guinea pig uterus human myometrium and rabbit jejunum Extraction of the polar lipids was followed by reversed-phase chromatography[8] to remove the compounds of 'ricinoleic acid type' polarity This produced a concentrate, *P* (3 2 g), containing all the components which were physiologically active on the rabbit jejunum Fractionation of *P* using Bergstrom's first system[9] (Fig 2) afforded two active fractions *Q* (165 mg) and *R* (85 mg) of contrasting physiological behaviour Fraction *Q*, a potent stimulant of the human myometrium (Fig 3), was highly active on the rabbit jejunum, but relatively inactive on the guinea pig uterus Fraction *R* either completely inhibited or feebly stimulated the contractions of the human myometrium (Fig 3), it was extremely active on the guinea pig uterus but only moderately stimulating to the rabbit jejunum

Separate fractionation of *Q* and *R* then followed using Bergstrom's second system[10], which is effective in discrimi-

(*Continued on page 993*)

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INSPIRATIONS OF ENRICO FERMI

The Collected Papers of Enrico Fermi
Vol 1 Italy, 1921–1938 Edited by E Amaldi, H L Anderson, E Persico, F Rassetti, C S Smith, A Wattenberg and E Segrè Pp xlii + 1043 (London and Chicago University of Chicago Press, Rome Accademia Nazionale dei Lincei, 1962) 15 00 dollars; 120*s*

"THERE can be no greater or more enduring monument to Enrico Fermi than his own work", and half that monument appears in Volume 1 of *The Collected Papers of Enrico Fermi*, which covers the period up to his departure from Italy to collect the Nobel Prize and non-return His papers were generally written in Italian in this period and are here reprinted without translation, but where he published the same paper in several languages the English text is given Because Fermi died at the relatively early age of fifty-three there are many contemporaries and collaborators who remember him well, and Volume 1 is probably unique in containing accounts of the circumstances which led to many of the investigations written by well-informed colleagues, and these are all in English Together with the long biographical introduction by Segrè, they make the whole volume most fascinating reading even without a knowledge of Italian

Fermi was a prodigy gifted with an exceptional memory so that he made rapid progress in mathematics and physics in his first university, Pisa, and had ample spare time to read widely, even in his third year he published original mathematical work on the effect of a gravitational field on electric charges A notebook written in undergraduate years shows with what very advanced topics he could deal with clarity and precision, and when even later in life he needed a powerful mathematical approach to a problem he could readily reconstruct it from his earlier achievements For his doctoral thesis, however, the subject had to be experimental, as theoretical physics was not an accepted subject in Italy in those days (1922) His colleague at Pisa, Rassetti, informs us that Fermi obviously enjoyed his first essays in experimental physics, which consisted of producing a curved crystal X-ray spectrometer which produced a focused image—no mean feat at that time

On returning to Rome he met Senator Corbino, the director of the Physics Institute, a man who gave the Fermi school such invaluable support at a later date Fermi's papers from Rome were theoretical, concerning gaseous phenomena, but after a year he was appointed to Florence, where he met Rassetti again and collaborated in experiments of some sophistication on the anomalous Zeeman effect in mercury vapour Fermi defined the experiment precisely and the two men successfully solved the problem under conditions of great physical hardship in the laboratory Rassetti recounts how Fermi as a theoretician was able to work at home burning the midnight oil and at this time produced very quickly his great paper on the theory of an ideal gas which would satisfy the Nernst principle at absolute zero and give the absolute entropy for low density and high temperature, the type of statistics used was developed at the same time by Dirac though Fermi's presentation to the Academy antedates Dirac's this paper won for him the chair at Rome in theoretical physics at the age of twenty-five and he was joined almost at once by Rassetti The "golden age of Fermi's life" had begun and modern physics in Rome was on the move

His work on the statistical distribution of electrons in an atom followed in 1927 that of Thomas in the Cavendish Laboratory, Cambridge, and at the Como conference on physics that year his contributions ranked with those of the great leaders of physics By 1928 his school in Rome had applied his statistics to a number of atomic problems which he reviewed in the Debye conference at Leipzig Segrè and Amaldi recount how smoothly he proceeded with complex calculations and taught the young school of theoreticians who were gathering around him, and in 1929 some of these went to laboratories outside Italy to strengthen their experimental activities, without these experiences the complex neutron research of later years in Rome would not have proceeded so well He continued theoretical work for three years on atomic and molecular problems and wrote the long review in *Revue of Modern Physics* 1922 on quantum theory of radiation known to all students of those days, but "the advent of quantum mechanics in Fermi's opinion signalled the completion of atomic physics the future lay in the exploration of the nucleus" (Segrè)

Segrè reminds us of the swiftly-moving events of 1932, unfortunately the Fermi review (paper No 72 of his works) on the nucleus presented to the fifth International Conference in Paris in July of that year is not reprinted in these collected papers, an error, in my opinion, in view of the volcanic upsurge which had just occurred Wertenstein confused the neutron just then born with the neutrino, a product of Pauli's hypothesis for β-ray decay but called by Pauli a "neutron", thereby unwittingly infringing Rutherford's 1920 name for a hypothetical particle of proton mass Fermi suggested the diminutive jokingly and it has remained for the β-decay particle from that day in October 1931 He worked on the β-decay theory and submitted an account to *Nature*, which was rejected as too remote from physical reality to be of interest to readers, so the full paper was published in Italy and has stood the test of time, alas, Fermi did not live to see a few neutrinos captured in thousands of gallons of water, the paper No 76 and fuller accounts are all reproduced

The young experimenters had returned to Rome and, with Rassetti, Fermi constructed nuclear instruments and prepared a strong polonium/beryllium neutron source Curie and Joliot had produced artificial radioactivity with α-particles, Fermi suggested using neutrons and almost instantly produced radioactive fluorine Rutherford was one of the first to congratulate him, and further to congratulate him "on your successful escape from the sphere of theoretical physics !" (one can see Rutherford made little sense of the β-decay theory !) Some difficulties were encountered with the experiments and Fermi decided to try filters made of paraffin wax instead of lead, strong activities were at once produced and in perplexity the experimenters went to lunch When they returned at 3 p m Fermi had the answer all worked out the fast neutrons had been slowed by collision with hydrogen atoms and were then more easily captured, thus raising the amount of artificial radioactivity produced by a very large factor Prof Corbino, who followed this work keenly, advised taking out a patent; this was instantly filed, the patent basic to the use of a moderator in a nuclear power reactor used on a massive scale in the huge reactors at Hanford, Washington, in 1944, for the production of uranium-239 which decays to plutonium, perhaps one of the most far-reaching patents of all time All the papers on neutron-induced radioactivity are reprinted, including the famous paper in the *Proceedings of the Royal Society* describing the enhanced effects of slow neutrons, and the suggestion that some of the activities of irradiated uranium samples might be due to "transuranic elements"

As Italy prepared for the Ethiopian war, the Rome school dispersed There followed a number of papers dealing with nuclear resonance levels, a tribute to Prof Corbino, and to Lord Rutherford "whose efforts had opened up one of the widest, and as yet unfathomable fields of investigation" (written 12 months before fission was discovered), and finally the Nobel Prize Address, December 10, 1938, after which Fermi sailed for the United States T E ALLIBONE

PLASMA PHYSICS AND RELATED TOPICS

Plasma Physics and Magnetofluidmechanics

By Ali Bulent Cambel (McGraw-Hill Series on Missile and Space Technology) Pp xiii+304 (New York McGraw-Hill Book Company, Inc , London McGraw-Hill Publishing Company, Ltd , 1963) 89*s*

An Introduction to Plasma Physics

By Dr W B Thompson. Pp. viii+256. (London and New York Pergamon Press, 1962.) 70*s* net.

An Introduction to Waves, Rays and Radiation in Plasma Media

By Dr J J. Brandstatter. Pp xiv+690 (New York McGraw-Hill Book Company, Inc , London McGraw-Hill Publishing Company, Ltd., 1963) 116*s* 6*d*

Proceedings of the Symposium on Electromagnetics and Fluid Dynamics of Gaseous Plasma, New York, N.Y., April 4, 5, 6, 1961

Edited by Jerome Fox, in association with Martha Crowell (Microwave Research Institute Symposia Series, Vol. 11) Pp xxiv+468 (Brooklyn, N Y Polytechnic Press of the Polytechnic Institute of Brooklyn, London John Wiley and Sons, 1962) 60*s*

Plasma Hydromagnetics

Sixth Lockheed Symposium on Magnetohydrodynamics Edited by Daniel Bershader Pp vi+146 (Stanford, California Stanford University Press, London· Oxford University Press, 1962) 36*s*.

Plasma Physics and Thermonuclear Research, Vol 2

Edited by C L Longmire, James L Tuck and W. B Thompson (Progress in Nuclear Energy, Series 11.) Pp viii+363 (London and New York. Pergamon Press, 1963) 100*s* net

THESE books on plasma physics consist of three text-books, two symposium reports and a review of plasma physics and thermonuclear research As is inevitable, since both the symposia and the review of thermonuclear research cover periods only up to the middle and end of 1961, much of the work arousing considerable interest at the present time is not included For example, in the review of plasma physics there is no mention of finite resistivity instabilities nor of the hybrid mirror containment systems of the Ioffe type However, these volumes enable some topics and papers, which would normally be scattered throughout the literature, to be brought together in such a way that a more uniform account of various aspects of the subject can be obtained Throughout all six books reference to the original contributions is facilitated by extremely extensive bibliographies

The text-books are respectively one of a fairly elementary nature, which could be used for an introductory course on magnetogasdynamics, an intermediate text giving an introduction to the dynamics of an idealized plasma, and an advanced text mainly concerned with the propagation of waves in anisotropic plasmas

Plasma Physics and Magnetofluidmechanics could be used as an introductory text for all interested in the dynamics of electrically conducting gases in the presence of applied electric and magnetic fields The subject-matter has a broad scope, but there is a distinct bias towards the engineering approach. Much of the early part of the book is an elementary review of the relevant electromagnetic theory, including a brief introduction to waves and wave propagation Properties of the ionized gas are then considered, including ionization and recombination, thermodynamics, and transport phenomena The flow equations of magnetofluidmechanics are obtained both from the Boltzmann equation and from a macroscopic point of view These lead to a discussion of Alfvèn and shock waves Various flow problems in the presence of magnetic fields, such as Couette and Poisseuille flow, are considered in detail and the results of these analyses are expressed in terms of a large number of similarity parameters The title is slightly misleading since there is no discussion of many of the usual problems of plasma physics, such as that of stability

A study of the dynamics of an idealized plasma in which inelastic processes (including ionization and recombination) are unimportant is presented in *An Introduction to Plasma Physics* Although intended as an introductory text some prior knowledge of both theoretical and plasma physics is essential, for at times the arguments in this book are difficult to follow This is possibly due to the large number of minor errors which are not always obvious and are sometimes misleading The whole book presents a very comprehensive account of plasma dynamics, and, since much of the material is not readily available elsewhere, it should be very useful in the understanding of specialized theoretical papers After introductory chapters, the book gives a full mathematical discussion of magnetohydrodynamics and magnetohydrodynamic stability The later chapters on plasma dynamics and particle motions, and the kinetic theory of plasmas, are by far the best and most fundamental Derivations are made of the Vlasov equation, the Fokker-Planck equation, and the magnetohydrodynamic equations, and there is included a good account of the dielectric properties of a hot plasma and an introduction to the concept of fluctuations

Magneto-sonic waves and low-frequency oscillations of a uniform magnetized plasma are dealt with in W B Thompson's book, but for a fuller account of waves and wave propagation one must recommend *An Introduction to Waves, Rays, and Radiation in Plasma Media*

This is an advanced text-book giving a very complete and unified treatment of the various factors which affect the propagation of waves, rays and radiation in anisotropic and inhomogeneous plasma media The fairly detailed mathematical formulation is of a mixed vector–tensor–matrix type, and throughout the basic physics is kept clearly in view There is a certain bias towards the propagation of electromagnetic waves and ionospheric application but a full account of other modes of propagation (such as magneto-elastic, magneto-acoustic, and shock waves) are included The latter are obtained from the magnetohydrodynamic equations, which incidentally are derived in detail Propagation is considered also in terms of a microscopic description for a low-density plasma obtained from the Boltzmann equation The sections on propagation of rays and radiation are almost self-contained The radiation considered is that due to collective motions in a homogeneous anisotropic plasma, bremsstrahlung and radiation characteristic of ionization processes are not included The section on ray propagation is probably the only such treatment conveniently available in text-book form

Some of the problems of wave phenomena are also considered in the *Proceedings of the Symposium on Electromagnetics and Fluid Dynamics of Gaseous Plasma* This is a collection of twenty-three papers presented in the

Microwave Research Institute Symposia Series in New York during April 4–6, 1961 This symposium brought together electromagneticists and fluidmechanicists to discuss topics of interest in the non-thermonuclear range On the theoretical side, attempts were made to unify the approach to plasma problems through both the moment equations akin to the macroscopic treatment of the fluid-dynamicists, and the microscopic treatment via the distribution functions and the Boltzmann equation There was some discussion on shock phenomena and magnetohydrodynamic channel flow The experimental studies included shocks, transmission and reflexion of microwaves and propagation properties concerned with communications problems, and the significance of plasmas for aerodynamic lift, thrust and drag To some extent the papers at this symposium had been condensed by their authors to such a degree that excessive reference has to be made to original contributions

This criticism cannot be made of the articles in *Plasma Hydromagnetics*, for each contribution tends to be self-contained In this book of the sixth Lockheed Symposium on Magnetohydrodynamics, held in December 1961, there are eight articles by prominent authors on diverse aspects, both theoretical and experimental, of the dynamics of plasmas These reviews are well presented and deserve a place on any plasma physicist's bookshelves

W B Thompson discusses particle interactions in a plasma and (using techniques of the same-type as those outlined in his book) calculates the electric microfield and afterwards obtains an expression for the scattering of radiation from a plasma This is extremely interesting in view of the scattering experiments using a laser which are being attempted at the present time R M Patrick and M Camac discuss an experimental investigation of collision-free shock waves in a magnetic annular shock-tube In view of charge-exchange effects, it is still not possible to say that the required dissipation in the shock is produced by non-linear interactions between magneto-hydrodynamic waves P A Sturrock is concerned with the energy and momentum associated with waves in plasmas R Lust reviews the application of plasma physics to problems in solar physics, and discusses the role of hydromagnetic waves in heating the solar chromosphere and corona R Karplus studies the absorption of low-frequency hydromagnetic waves in the lower ionosphere and related dispersion and dissipation phenomena leading to ionosphere heating In their article on hydromagnetic ionizing waves, W B Kunkel and R A Gross consider theoretical solutions for a magnetically driven ionizing shock propagating into an undisturbed magnetic field, and B Sonnerup deals with the theory of magneto-gasdynamic flow in slowly diverging two-dimensional channels in such a manner that compressibility effects are included Finally, R F Post gives a complete review of the nature of plasma instabilities which might be encountered in mirror machines, with the inclusion of a discussion of finite Larmor radius effects and some experimental results

Plasma Physics and Thermonuclear Research (Volume 2) contains, as the title indicates, reviews of work being carried out on controlled fusion The bulk of this book was prepared before the 1961 Salzburg Conference, and thus, in this case, the account by R F Post of the Mirror Machine Program (1958–1961) contains no mention of finite Larmor radius effects Other reviews on this volume are those on *OGRA*, the *DCX* Program, fast magnetic compression experiments, cusp containment, hard-core pinches and rotating plasma devices, together with articles on the problems of cyclotron radiation and acceleration of plasmas Since the first volume in this series was published in 1958, there has been some slowing down of the *tempo* of both theoretical and experimental investigations into confinement and other problems of thermonuclear research This volume is notable for indicating the increasing awareness of the difficulties of the thermonuclear problem, although some optimism (which still has not been rewarded) is shown Diagnostic techniques, too, have been considerably refined, for example, elegant continuum measurements have been made on *Scylla* using an X-ray crystal spectrometer

As a summary of the period 1958–1961 this volume cannot be improved, but since, in this case, most of this material (and much more besides) is now available in the proceedings of the 1961 Salzburg Conference (*Nuclear Fusion* 1962 *Supplement*), it is a pity that we had to wait until 1963 for this book J COOPER

A COMPENDIUM OF PHYSICS

American Institute of Physics Handbook

Edited by Dr. Dwight E Gray (Co-ordinating Editor) Pp xv+2118 Second edition (New York McGraw-Hill Book Company, Inc , London McGraw-Hill Publishing Company, Ltd , 1963) 231*s*

IN 1957 the American Institute of Physics brought out a 1,500-page *Physics Handbook*, and now, six years later, we are offered this second edition, enlarged by some 600 pages and brought very much up to date The first edition quickly established itself as a working reference and was highly appreciated by many engaged in research or in advanced teaching and by many preparing textbooks The content of a publication of this character can be reasonably judged on three grounds, namely reliability, coverage and accessibility Only time and detailed usage can reveal any errors in so formidably large a content The extent of the field covered is certainly very impressive, indeed no less than 127 authors have contributed to this compilation, which includes definitions, formulæ, tables and data in graphical form There are, however, undoubtedly some infelicities in this publication in connexion with the accessibility of the massive information packed between the covers

In this edition there are nine sections, one more than in the first edition, this dealing with solid-state, which also includes several items formerly dispersed within the other sections Despite the mere six years between the two editions this volume contains numerous changes, and not always additions Section 1 (computational), which formerly was merely a five-page list of available mathematical tables, has been expanded to a 56-page article describing and illustrating computors and their applications Section 2 (mechanics) remains largely as before, increasing slightly to 263 pp In the section on acoustics which follows, there is a marked reduction by 22 pp , to a total of 156 pp , largely through elimination of the former excessive 33-page section devoted to illustrations of impedance analogies This cut is a real improvement The section 4 on heat is doubled in length, now covering 330 pp , and a good deal of this arises through inclusion of tables for vapour-pressure and for heats of combustion Electricity and magnetism, in 291 pp , remains largely as before, although there are numerous changes in distribution of material and especially additions in connexion with magnetic properties of materials

The optics section is practically doubled now (239 pp) Many more refractive indices are included and a section on lasers is also incorporated The section entitled atomic and molecular physics, devoted very much to spectra, is covered in 229 pp and is very much as before Naturally enough, nuclear physics has expanded and now covers 326 pp The thoroughness of this section is illustrated by the fact that the list of accelerators in operation throughout the world is so detailed that this table alone fills 40 close-packed pages

The final new section, which is devoted to solid-state physics, occupies 160 pp In this there are extensive tables on crystallographic data, electrical properties of metals and semiconductors, extensive diffusion tables for ionic crystals and formidably detailed pages of references to original papers Included are compact sections on dislocations in crystals and on luminescence effects There is indeed a great deal in this new section

The book concludes with a 60-page index in which are perhaps some 6,000 items

The only real weaknesses in the volume are connected with indexing and with accessibility of information included One can indeed criticize several items The indexing is woefully inadequate For example, optical data of value are given in a table for 230 crystalline minerals, yet not a single one of the names of these minerals appears in the index, neither obscure minerals nor common minerals One might have hoped, as just one example, to find the name mica, or at least muscovite, somewhere in the index of a reference hand-book, but this is only one of hundreds of items, possibly thousands, which are missing Then again some real search difficulties arise through curious tabulation even of these very items just mentioned, for the minerals are arranged in order of increasing refractive index and not alphabetically This is a pure absurdity, in that it presupposes that a searcher already knows the refractive index of a mineral fairly closely when indeed he is seeking its value Not to have an alphabetical list of the minerals is an unforgivable oversight In any event, the separation of minerals into different tables for biaxials and uniaxials is no help, since this also implies previous knowledge of the optical character when beginning a search, no doubt a simple matter for a distinguished mineralogist, but certainly bewildering to a young research physicist

It is a matter for considerable regret that so magnificent a compilation of data should be marred by problems of accessibility The index should certainly be very greatly expanded in any future editions, which I very much hope will appear in due course As one has now come to expect from McGraw-Hill, the production of the volume is first rate both as regards format and typescript, diagrams and binding and the cost is by no means unduly high when account is taken of the formidable mass of content The initiative for the production of this valuable hand-book came from the American Institute of Physics, and the editor, D E Gray, is to be congratulated on a production which reflects a worthy sense of co-operation and team spirit This volume should be acquired by every physics laboratory whether devoted to research, to development or to teaching S TOLANSKY

AUTOMATIC CONTROL

Servomechanisms

By L A Stockdale Pp viii+295 (London Sir Isaac Pitman and Sons, Ltd, 1962) 35*s* net

Progress in Control Engineering

Vol 1 Edited by R H Macmillan, T J Higgins and P Naslin Pp viii+260 (London Heywood and Co, Ltd, 1962) 63*s* net

OF the many volumes that have appeared in the field of automatic control in recent years few have been written as, or been suitable for, student text-books The appearance of *Servomechanisms* helps to redress the balance in this respect, having been written specifically for technical college and university students by a practising teacher in the field

After a short introductory and non-mathematical chapter on the principles of control, three chapters are devoted to system 'hardware', including error detectors, motors and amplifiers System analysis commences at Chapter 5 with the Laplace method applied to second-order systems, followed by an early introduction to transfer functions These are used in the subsequent treatment of damping, phase-advance and integral compensation, and the effects of feedback and feedforward A chapter entitled "Transfer Function Analysis" includes the algebraic stability criteria and a brief mention of the root-locus method, making the valid point that computors and simulators are probably of greater value in designing for satisfactory response

Two chapters dealing with harmonic response and one describing the log-modulus method include analysis of the effect of phase advance and retarding circuits and a description of test equipment for these methods Three final chapters introduce the describing function and phase-plane methods for non-linear systems, and sampled-data systems

The content and arrangement of this book, which includes numerous examples at the end of each chapter, seem ideally suited for its purpose and deserve that it be widely used

Progress in Control Engineering is the first of a series of reviews of various aspects of the subject, similar to the reviews already appearing in several other fields Of the eight subjects treated four deal with general analytical methods while the others are concerned with more specialized topics In the first category are statistical methods (M. J Pelegrin), operational and transform techniques (B M Brown), time-lag systems (N H Chosky), and frequency response techniques applied to non-linear systems (P E W Grensted) Of the more specialized topics are "The Governing of Diesel Engines" (D B Welbourn), "The Human Operator in Control Instrumentation" (T B Sheridan), "Automatic Control by Pneumatics" (R Molle and J Rasquinet), and a contribution on the place of digital computors in control (M V Wilkes)

Each subject is developed from a point which demands very little in the way of specialized previous knowledge, and aims to bring the reader to the stage where he can "understand and appreciate both present-day work in the field and the lines along which future developments are to be expected" In the main the contributions achieve this objective, and this volume could well be the first of an interesting series E M DEELEY

CERAMICS AND THE 'HEAT BARRIER'

Special Ceramics 1962

Proceedings of a Symposium held by the British Ceramic Research Association Edited by P Popper Pp xiii+482 (London Academic Press, Inc (London), Ltd., New York Academic Press, Inc, 1963 Published for the British Ceramic Research Association) 105*s*

QUOTING from the cover to this volume "With the rise of new technologies and the quest for higher operating efficiencies at ever higher temperatures, the engineer looks to the ceramist to supply new, high-performance materials where metals or traditional ceramics fall short" This collection of papers, like its predecessor, *Special Ceramics*, published some three years ago, includes a number by authors of international repute There are 25 papers in all, an increase of 20 per cent compared with the previous volume, and there was also a similar increase in the attendance at the symposium, reflecting the growing interest in these new and challenging fields

The topics fall broadly into the following groups preparation and properties of refractory non-oxides and of beryllium oxide, pyrolytic deposits, measurements; techniques; applications The materials covered are mostly non-oxides, but a few oxides, in particular those of beryllium, aluminium and titanium, are included Measurements include rapid methods of determining thermal expansion and thermal conductivity Among the techniques are powder compaction, reaction sintering, hot pressing, as well as pyrolysis Applications include high-temperature nuclear fuels, moderators, etc, parts for rocket motors, protective coatings for refractories, oriented graphite susceptors for use up to 3,400° C, particle accelerators, high-power microwave absorbing elements, super-refractories for magneto-hydrodynamic power generators, and ceramic-to-metal seals for atomic energy applications

The book is excellently produced and the text is well illustrated The wealth of new material presented will commend it primarily to the specialist However, due in great measure to the care with which the contributions to the symposium have been transcribed and edited, most of the text will be found very readable, even by the non-specialist, who could well draw inspiration from the ingenuity brought to bear on some of the problems encountered. For example, the use of (very rapid) 'plunge sintering' in order to utilize the heat of an exothermic reaction for sintering uranium monocarbide, and again, the elegant method used to avoid contamination during the nitriding of aluminium, by levitating the latter in a radiofrequency field There are, of course, numerous points of fundamental significance, to name but two—rate-determining processes in sintering, and the filamentary, electrically conducting paths observed in rutile, apparently related to the time-dependence of conductivity in the unique crystal direction B E WAYE

COMETS

The Nature of Comets

By Dr Nikolaus B Richter. Translated and revised edition by Dr Arthur Beer. With an Introduction and Additional Contributions by Dr R. A Lyttleton Pp. xli + 221 (London. Methuen and Co , Ltd., 1963) 63*s* net.

IN 1954, J. A Bath of Leipzig published *Statistik und Physik der Kometen* by Dr N B. Richter. The monograph summarized the main observational and theoretical features of the nature and behaviour of comets and did much to stimulate interest in a rather neglected field of enquiry. Since then cometary investigations have acquired greater importance, due in part to the rapid development of space research and also to the interest aroused by the appearance of Comet 1957 III Arend–Roland and Comet 1957 V Mrkos

In this, the first English edition of Richter's monograph, the original text has been brought up to date by extensive revision and the addition of new material Further, it contains a challenging introduction by Dr R A Lyttleton, well known in cometary astronomy for his accretion theory of the structure and origin of comets In this, Lyttleton presents his personal views and criticisms of the present state of cometary theory He makes it clear that he does not agree with all the conclusions of Richter, Oort and Whipple According to Richter, almost the entire mass of a comet resides in its comparatively small nucleus, which consists of solid material ranging from dust particles to chunks of meteoritic size As the nucleus approaches close to the Sun, solar radiation causes it to emit gases These gases form the coma, while radiation pressure and corpuscular radiation drive out the cometary tail. Lyttleton, however, remarks that once the existence of large chunks of rock is postulated it becomes necessary to explain why none of them ever get into meteor streams. During a rich meteor shower, millions of small meteors are injected into the Earth's atmosphere, but so far without a single large object of meteoritic proportion coming in at the same time on the same path He also considers that Richter's analysis amounts to convincing proof of the inadequacy of solar heating to account for the great longevity of certain large tail-producing comets

According to Oort the Sun is encompassed by a very distant spherically distributed swarm of comets On the very rare occasions when stars pass sufficiently close to the shell they perturb a comet so that it leaves the shell, falls towards the Sun, and becomes visible from the Earth. So rarely does this happen to a single individual comet that to meet the observed actual supply some 10^{11} comets are assumed to reside in the shell. Lyttleton considers that the hypothesis of the cometary shell is valueless It cannot (as yet) be verified by observation; does not serve to correlate observations, in no way explains the origin of comets, and thus provides no clues whatever as to their structure Whipple's theory has similar limitations It postulates a cometary nucleus in the form of a conglomerate of ices and meteoritic particles Under the action of the Sun's heat volatile compounds in the ices are evaporated and sufficiently irradiated to exhibit the emission lines observed in cometary spectra Lyttleton claims that both theories are merely speculative descriptive schemes which have no foundation for their hypotheses in either established astronomical fact or attested theory The way in which he arrives at this conclusion and then resolves certain objections levelled against the accretion theory shows a nice appreciation of the value and importance of the principles of scientific method

After giving a short account of the historical development of cometary research, Richter proceeds to describe statistical results regarding orbital forms, inclinations and aphelion distances, the perturbations produced by Jupiter, the 'capture hypothesis', comets with planetary orbits and comet groups Section 3 deals with the structure of comets, that is, with the progress made in determining the dimensions, masses, densities and nature of cometary nuclei, heads and tails Section 4 is of particular interest since it gives a preliminary summary of the work done on the two bright comets of 1957 Both objects were subjected to extensive photometric, polarimetric and spectrographic observations Richter's discussion of the available material helps towards clarifying the problems opened up in the last four sections, namely comets as processes of cosmic decay, the origin and formation of comets, cometary problems for future research work; a typical model of a comet A number of valuable cometary tables, a generous bibliography and adequate name and subject indexes complete the monograph Dr A Beer has made an excellent job of the translation The text reads well and is embellished with a well-chosen set of reproductions of photographs of comets A book so interesting and informative deserves the widest possible readership It may not achieve this, but it will, at least be read with pleasure and profit by all astronomers, professional and amateur alike H C KING

THEORY OF ELECTRICAL MACHINES

Electric Machine Analysis using Matrices

By Dr W J Gibbs Pp vii + 70 (London Sir Isaac Pitman and Sons, Ltd , 1962) 17*s* 6*d* net.

METHODS of analysis for the investigation of the behaviour of electrical machines began to be developed in the 1880's For alternating current machines the technique of the vector diagram, the equivalent circuit and the utilization of complex numbers in circuit analysis achieved universal application in the first two decades of the present century. Each type of machine was analysed individually.

What is now termed the 'generalized theory of electrical machines' has been developed during the past thirty years Expressed in terms of the basic equations of an idealized machine and utilizing matrix or tensor methods for their manipulation, it is possible to establish a general theory of electrical machines which will comprehend the behaviour of practically all types of machine under all conditions of operation This unified theory was propounded and developed by Gabriel Kron It is with this subject that Dr Gibbs's book deals

The book aims to present the mathematical basis of the theory as simply and clearly as possible A short introductory chapter describes the purpose of the generalized analysis, its limitations and its method of approach Chapters 2 and 3, entitled, respectively, "The Fundamental Machine" and "Two-Axis Theory", comprise the setting up of the machine equations in terms of the basic two-phase system and the development of the transformations for two-axis representation In these two chapters, in the course of developing and manipulating the equations, the notation of matrices and certain matrix operations are introduced

The next two chapters deal with transient and sub-transient conditions and with the setting up of the torque equations and, in the latter connexion, the rules of matrix multiplication are more fully exemplified It is at this point that the analysis of machines of different types is taken up, direct current machines, induction motors and general commutator machines forming the subjects of individual chapters The treatment is completed by a chapter dealing with small oscillations

The book concludes with a short chapter entitled "Tensors" in which the author describes the relationship of electrical machine theory to differential geometry, the absolute differential calculus and to tensor calculus

No prior knowledge of matrix theory is assumed The requisite rules of operation are developed as required and their utility and validity demonstrated by appropriate examples The most important fundamental concept of the "connection matrix" is introduced in the simple example of the d c series machine and is then more fully developed in relation to a c machines The author states at the outset that, "The true concepts behind the two-axis theory are mathematical and for that reason the mathematical treatment in this book is less complicated and more satisfying than the usual physical approach" This claim can, in large measure, be sustained The theory is developed in a clear, logical and systematic fashion and constitutes an excellent introduction to the subject. None the less, physical conditions viewed along with mathematical development can illuminate the subject and, by adding realism, carry conviction The basic concepts which underlie two-axis theory could with advantage have been illustrated from the physical point of view

Dr Gibbs has arranged his presentation of the subject so as to lead into the important topics with a minimum of digression, and he has succeeded in developing with remarkable economy and clarity the principles of the subject to a point at which the reader can enter the more general literature of the field To a reader with a background knowledge of the conventional theory of electrical machines the treatment is admirably suited In particular the book will serve well the needs of teachers of undergraduate courses dealing with electrical machines, and of students in the final year of such courses J Greig

A RUSSIAN VIEW OF EARTH HISTORY

Basic Problems in Geotectonics

By V V Beloussov Edited by John C Maxwell Translated by Paul T Broneer (International Series in the Earth Sciences) Pp xvi+809 (New York McGraw-Hill Book Company, Inc , London McGraw-Hill Publishing Company, Ltd , 1962) 108*s* 6*d*

Basic Problems in Geotectonics is a monumental synthesis of Earth structure in which Dr Beloussov presents what he believes to be the main outcome of geotectonic research in the U S S R since the Revolution The subject has long been a controversial one, and some of the facts and theories presented in this book are likely to be disputed by many workers in this field

After a preliminary summary of the history of development of geotectonics there follow an outline of the internal structure of the Earth and a discussion of its origin The classification of structural forms observed in rocks exposed at the surface is both novel and stimulating The following section on present and past vertical oscillatory movements is an important one, as these observations form the basis of his main thesis The maps and sections which illustrate the variations in facies and thicknesses of Palæozoic and Mesozoic formations in Central and Western Russia should prove to be of particular interest to workers outside the U S S R , as they give a clear summary of detailed work not always easily accessible in this form The next section discusses the theories of formation of folds and faults, and the last two parts present Dr Beloussov's views on the processes which have led to the arrangement of the larger structural units

Dr Beloussov is very critical of the idea that orogenic belts are developed in regions of large horizontal displacement, although he does find it difficult to see how vertical movements alone can lead to the development of mountain chains where the rocks have been tightly folded and apparently strongly compressed His belief in the prime importance of vertical movements leads him to assume that our knowledge of linear fold zones is totally inadequate and probably fundamentally incorrect He suggests that the folding in the Appalachian Mountain chain has been incorrectly interpreted, and that the greater part of the nappe structure of the European Alpine chain is geometric speculation and, to use one of his favourite and frequent criticisms, "formalistically unsound" The notion that all fold belts are developed on the plan of the Alpine model could certainly be criticized, but Dr Beloussov's denial of the accepted structural geometry of the Alps seems scientifically irresponsible For example, no Alpine geologist would dispute that the Prealpine nappes have been displaced horizontally at least 60 km, not, as Beloussov suggests, a mere 11 km He explains the formation of linear fold belts by vertical movements on long and deep fundamental flaws in the crust Vertical movements on these faults lead to the stretching of the sedimentary cover, and a later reversed movement of the fault-block causes the previously stretched beds to become shortened and folded as they settle back into their original position

Dr Beloussov believes that the deep ocean basins represent regions of subsidence formed as the result of a process of basification or "basaltization" of a relatively homogeneous granitic crust in comparatively recent geological time

This is a book which all interested in Earth structure should read, for in it the evidence supporting the theory of oscillatory vertical movements of the crust is fairly fully documented It should stimulate the reader to re-examine many of the generally accepted facts and theories, although Dr Beloussov's partisan approach to Earth science might be found to be somewhat exasperating J G Ramsay

THE HALLEY BAY EXPEDITION OF THE ROYAL SOCIETY

The Royal Society International Geophysical Year Antarctic Expedition

Halley Bay, Coats Land, Falkland Islands Dependencies, 1955–59 General Editor Sir David Brunt Vol. 2 Radio Astronomy, Ionospheric Physics Pp xv+289+14 plates Vol 3 Seismology, Meteorology Pp. xvii+382+5 plates (London The Royal Society, 1962) 140*s* , 23 dollars per volume

Right from the inception of its International Geophysical Year Antarctic Expedition it has been one of the aims of the Royal Society that the results should be available promptly, particularly in view of the pace of present-day developments in geophysics. It is a considerable achievement that three volumes, containing extensive tables of the essential data obtained, as well as careful analyses of the material available, should have been published so soon after the close of the International Geophysical Year (see *Nature*, 192, 1004 (1961) for review of Volume 1). The present review of Volumes 2 and 3 can give no more than a bare outline of the considerable results achieved.

Volume 2 deals with radio astronomy and ionospheric physics. The report on radio astronomy is presented in two sections. The first section, by P. M. Brenan, deals with observations of radio-star scintillation, using a radio-source in Centaurus, a careful study has been made *inter alia* of the height of the diffracting screen to which the scintillations are attributable, the drift velocities of the ionospheric irregularities causing scintillation, the seasonal and diurnal variations, and the size and shape of the ionospheric irregularities. The overall conclusions are that the height of the diffracting screen is below 1,100 km and probably below 500 km, and that the irregularities, which are steeply inclined to the horizontal, are of width 2.4 km and of length greater than 120 km, the irregular screen at Halley Bay presumably forms a broad band between the *E* and *F* regions at altitudes between approximately 100 km and 200 km. The second section of the radio-astronomy report, by D. P. Harrison, describes radio-echo observations of auroræ and meteors. The difficulties of operating the equipment at Halley Bay were successfully overcome, and, in fact, Halley Bay proved to be a particularly suitable site for observation. A critical discussion is given of the results of the radio-echo data, and tables of indices and counts, arranged chronologically, describe the levels of auroral and meteor activity.

The report in Volume 2 on ionospheric physics, by W. H. Bellchambers, L. W. Barclay, D. L. M. Cansfield and W. R. Piggott, describes the Halley Bay equipment for radio-wave measurements of the ionosphere, and gives a detailed discussion of the experiments, these were planned to provide: (*a*) ionograms showing the relations between the frequency of the radio-waves and the equivalent heights from which they were reflected; (*b*) measures of the absorption suffered in the ionosphere by radio-waves of frequency 2.2 and 4.0 Mc/s, (*c*) measures of the horizontal drift velocity of local irregularities in the reflecting layers of the *E* and *F* regions. Extensive tables are given of the values of the parameters found by analysis of the records. This report, like the preceding one, is well illustrated by plates and diagrams.

Volume 3 is concerned with seismology and meteorology. The seismological report, by J. MacDowall and Mrs. E. M. Lee, is based on the records of a three-component Willmore seismograph installation, built on a floating ice-shelf, this might be thought to be a most unsuitable location for a seismological observatory, but the decision was amply justified by the results obtained. The Synchronome clock of the observatory was rated by vernier time-signals from Canberra, the identification of phases on the records was greatly facilitated by use of the preliminary epicentres, furnished by courtesy of the U.S. Coast and Geodetic Survey from Washington, D.C., via McMurdo Sound, Mawson and Vestfold Hills. The report contains two bulletins of readings of earthquake phases. The first bulletin contains the readings made by MacDowall at Halley Bay and telegraphed monthly to the Royal Society for transmission to World Data Centres, after the return of the expedition, these readings were checked and supplemented where necessary by Mrs. Lee. A new set of readings from the seismographs was then made independently of any epicentral information, the additional phases thus disclosed have been listed in a separate bulletin.

The greater part of Volume 3 constitutes the meteorological report. As Sir Graham Sutton remarks in the preface, "In many ways, Antarctica is the ideal laboratory for geophysical investigations and offers to the meteorologist unique opportunities to study an atmosphere which is completely free from man-made influences". Of the great mass of meteorological information secured, it must suffice to mention here the general headings, such as the "total ozone observations" and the determinations of ozone concentration near the surface of the ice-shelf made by J. MacDowall. In addition, ozone soundings by ozone-sonde were made by J. MacDowall and J. A. Smith, 19 ascents were carried out, of which 16 were successful, and graphs have been constructed showing the variation of ozone concentration with height up to the limits reached (generally about 20 km). This ozone programme is a valuable addition to our store of information.

Observations of short-wave radiation of the Sun and sky were carried out by J. MacDowall and D. T. Tribble, using two solarimeters, and the radiation balance was recorded by a ventilated radiometer. Tables are given of the total solar radiation, the diffuse solar radiation and the net flux of radiation for each day. A section on upper-air meteorological observations, by J. MacDowall and J. M. C. Burton, describes the results of radio-sonde and radar methods of determining upper-air temperature, pressure, humidity and wind velocity. A twice-daily programme of ascents (nearly 1,000 ascents in all) was carried through, and the data for each of the separate ascents have been listed for surface level, standard levels, significant levels, tropopauses and bursts. The analysis of this large body of observations was effected by transferring all the data to 'Hollerith' cards at Halley Bay and afterwards sorting these cards on 'Hollerith' machines at the Meteorological Office.

In these two volumes the high standards of printing and arrangement of Volume 1 have been maintained. An excellent feature is the reprinting, at the beginning of both volumes, of Sir David Brunt's narrative account of the history of the expedition as given in Volume 1. The exposition of the theory, the observations and their analysis is so well balanced for each of the disciplines involved that the volumes should become standard works of reference rather than mere catalogues of observations. The Royal Society and the authors are to be congratulated on this considerable addition to geophysical knowledge.

R. STONELEY

A GREAT SCIENTIFIC ENTERPRISE

Discovery II in the Antarctic

The Story of British Research in the Southern Seas. By John Coleman-Cooke. Pp. 255 + 50 photographs. (London: Odhams Press, Ltd., 1963.) 25*s*.

THE once remote and uninhabited Antarctic is now within a week's air travel from Europe and has many permanent scientific stations, at least one of them the size of a small town. This dramatic change has come about since the end of the Second World War, and is due to the territorial, economic and scientific ambitions of many nations. Prior to the First World War, knowledge of the Antarctic was obtained from the results of various exploring and research expeditions. Between the Wars some similar expeditions were sent out, but a new phase of Antarctic investigations began in 1923 when the *Discovery* Committee started the continuous series of researches that are still in progress. The foundation of this unparalleled programme of investigations was laid by the late Dr. Stanley Kemp, a brilliant scientist and charming character, whose memory is held in high affection by all who had the privilege of serving under him.

The *Discovery* investigations were intended to carry out research on the biology of whales so that accurate knowledge could be brought to the framing of legislation for the protection of the very valuable whaling industry in which over-exploitation threatened ruin similar to that which had overtaken the Arctic whale fishery in the previous century The knowledge was obtained and international agreements were made—but all was in vain, for bureaucrats under pressure from big business are ever weak, and the regulations they made were weak The Antarctic whales are reduced to a vestige of their former abundance and the industry is dying, nearly all the great whaling enterprises have left the business, which is now carried on mainly by Russia and Japan, the newcomers to the Antarctic

In planning the *Discovery* research programmes, Kemp saw that any useful investigation into the biology of whales required a comprehensive examination of their ecology and environment The *Discovery Reports*, which have now reached their thirty-third volume, show the wide fields covered as a result of his foresight, ranging from oceanography and geology to whales, plankton, fishes, seals and birds Although the *Discovery* investigations have not saved the whaling industry, they have provided such an enormous mass of scientific information that as much, probably more, is now known about the circumpolar Antarctic Ocean as about any of the seas

The *Discovery* investigations have been carried out at the marine research station set up on the Island of South Georgia, and with four ships, the *Discovery*, a wooden barque-rigged sailing ship (1924–27), the smaller *William Scoresby* (1926–51), the *Discovery II* (1929–60), later transferred to the National Institute of Oceanography, and the *Discovery* (1963→), the Institute's splendid new research ship

This book gives a general account and history of the *Discovery* investigations, the men who undertook them, the work they did, and the adventures that befell them It also gives some account of the scientific results, presented in a non-technical way for the layman, but it is essentially the story of the field work of the investigations from their beginning to the present, gathered from official and other reports, and especially from conversation with those who took part in them It is difficult for a former member of the *Discovery* Expedition staff to review this book objectively, to a young man the impact of the Antarctic, and the day-to-day experiences of an expedition such as the *Discovery's* in the early days of the investigations nearly forty years ago, were so great as to have formed a delightful background for the rest of his life Putting emotion on one side, however, I can say that the author has successfully caught the spirit of the *Discovery*, and gives a very interesting narrative of the expeditions and their results The book is well illustrated with photographs

L HARRISON MATTHEWS

THE BRACHIOPODA

On the Morphology and Classification of the Brachiopod Suborder Chonetoidea

By Dr Helen M Muir-Wood Pp viii+132+16 plates (London British Museum (Natural History), 1962) 110*s* net

PALÆONTOLOGISTS, who are concerned about the future of systematics, have recently been taking a more critical look at the supra-generic contents of the taxonomic hierarchies that interest them This re-appraisal has undoubtedly derived a great deal of impetus from the present preparation of treatises on palæontology in France, the U S S R and the United States But it also probably reflects an uneasy awareness that classical taxonomic procedures, which have served palæontologists so well in the past, are now being tested by modern biological concepts of speciation and increasing access to computors with the concomitant promise or threat, as the reaction may be, of numerically contrived classifications In effect, the taxonomic pyramids that have been so elaborately built up over the years are now being re-surveyed in order to find out what differences these new methods of construction are likely to have, and although total demolition may ultimately prove to be necessary, this critical examination must go on Such surveys inevitably vary greatly in the quality of their findings, but the best are indisputably those that weigh all evidence against the healthy scepticism of experience and Dr Muir-Wood's study of the morphology and classification of the chonetoid brachiopods is an admirable example of this balanced approach

As an introduction to her monograph, Dr Muir-Wood gives a general account of chonetacean morphology and explores the anatomical, evolutionary and ecological implications of those features peculiar to the group These sections are as comprehensive as one would wish, but different interpretations do spring to mind Has she, for example, paid enough attention to the generalized nature of chonetacean morphology ? Ignoring the hinge-spines, the most striking aspect of the earliest chonetaceans, like *Strophochonetes* and *Protochonetes*, is their unspecialized internal morphology, which is so reminiscent of many young strophomenacean and plectambonitacean shells that the development of the cardinal process, rather than the arrangement of the muscle fields or the absence of dental lamellæ, may ultimately prove to be more diagnostic of group affinity It is also controversial to infer that productoid spines were "open at the distal end" and were, therefore, unlike the chonetacean hinge-spines Apart from differences in their distribution, no detail of shell structure suggests that they were anything but identical in origin, development and function

The systematic descriptions of the genera have been compiled with all the authority that Dr Muir-Wood's experience commands They make the monograph one of the most complete reviews of a major group of the Brachiopoda ever attempted, with a nicely balanced diagnosis for each genus together with a list of all the species it embraces, its geological range and geographical distribution, and it is not surprising to find that her carefully considered judgments have led to the recognition of seven new genera Indeed, the only doubts that linger about the systematic treatment generally are the desirability of so many sub-families Thus the distinctions drawn among the four sub-families comprising the Daviesiellidae do not seem to be more important than the variation tolerated within, for example, the Anopliinae But such differences of opinion simply illustrate the way in which personal taste still pervades palæontological practices, and one can only summarize the impact of the entire study by declaring that students of the brachiopod phylum will be indebted to Dr Muir-Wood for a long time to come for preparing such an invaluable monograph

ALWYN WILLIAMS

FIBRE CHEMISTRY

Textile Chemistry

Vol. 1 The Chemistry of Fibres By Prof R H Peters Pp xiii+477 (Amsterdam, London and New York Elsevier Publishing Company, 1963) 75*s*

PROF PETERS has attempted a most comprehensive work He has concentrated almost the whole science of fibre structure into a treatise of some 450 pages After a general introduction in which he classifies polymers, he proceeds to a detailed investigation of the chemistry and physics of the man-made fibrous polymers Then follow chapters on condensation polymers, addition polymers and copolymers Here one notices the variability of the level of treatment of the subject, but later, when dealing with the natural materials, this variation becomes very obvious If one considers his work on the kinetics of polymerization, the mathematical derivations of many of the equations used in the physical studies of chain-length, distribution and the kinetics of polymerization are set out in most cases as a mathematical exercise It might well be that this could have formed a more mathematical monograph on these known aspects of polymer structure In this case the derivation of the formulæ could have been curtailed in the present work, and the physical significance of the results to the understanding of polymer structure given greater emphasis It should, however, be pointed out that the references in this field are good The chapter on molecular weight determination has two pages devoted to chemical methods and about twenty to a detailed description of physical methods

Chapter 6, Chapter 10 and Chapter 11 give comparatively simple descriptions of cellulose, proteins (mainly wool) and silk fibres respectively Other chapters then deal with the physics and chemistry of these materials, and this treatment of the subjects shows wide variations in levels of presentation This is particularly so on p 194 *et seq* , where an advanced treatment of the reactions of the hydroxyl groups in cellulose is given I feel that, in this work, accessibility of the glucose residue with special reference to crystalline packing should be considered in any theory applicable to cellulose fibres Chapter 9 describes the work which has been done on synthetic polypeptides, but for students it might have been a help to correlate the natural and synthetic α- and β-structures even if the treatment had been critical of some of the theories advanced

Chapter 12 is devoted to the crystalline structure of polymers, and there is no doubt that a re-editing of this chapter should be done It must be pointed out that many of the X-ray diagrams are incorrectly aligned (this can be readily corrected by an arrow giving the fibre axis) and the text of pp 321 *et seq* has been confused Fig. 77*a* is wrongly oriented but correctly labelled, Fig 77*b* is not ramie but most likely polyvinyl alcohol or a similar vinyl fibre Figs 77*c* and 77*d* are incorrectly oriented Fig 77*e* is most likely a polyacrylic fibre with a TiO_2 dilustrant included, and Fig 77*f* is an incorrectly oriented picture of ramie taken on a circular camera and contains no TiO_2

Chapters 13 and 14 are good generally, the former on melting and crystallization of man-made polymers, and the latter on accessibility and crystallinity The chapters on fibre extrusion should probably have included the equations of extrusion This would then have clarified the statement on p 432, which suggests that yarn diameter (presumably denier) on extrusion is a function of the diameter of the jet holes Probably the main criticism of this work is that Prof Peters has attempted to cover too wide a field and in doing so has, maybe of necessity, had to vary the standard of treatment of his material. The scope, however, is good, and there is nothing in the book that could not be rectified by a good re-editing

F. Happey

QUANTITATIVE ORGANIC ANALYSIS

Quantitative Organic Analysis via Functional Groups

By Dr Sidney Siggia Third edition Pp xii + 697. (New York and London: John Wiley and Sons, Inc , 1963) 145*s*

THE third edition of Dr. Siggia's book is described on the dust-jacket as being about double the size of the previous edition The price of the book has been almost quadrupled Neither increase would have been entirely necessary had the author been a little more selective in his choice of material The book consists largely of reprints or partial reprints of papers and articles from other books and journals The sources are quite clearly indicated and the extracts are variously described as being, reprinted with permission, reprinted in part, reprinted, adapted or adopted or are simply referred to Some of the papers are Dr Siggia's own, but his main role has been that of a collector and he has drawn heavily on the American Chemical Society publication, *Analytical Chemistry* Simple reference to the original journals would have sufficed for much of the original discussion, results, tables and diagrams Only the essential analytical procedure need have been retained in full Applied throughout the book this would have reduced the size, lowered the price and in all probability increased the sales

The book is so planned as to devote one chapter to the methods of analysis for each functional group considered These include hydroxyl and carbonyl groups, carboxylic acids and derivatives, alkoxyl and epoxide groups, peroxides, unsaturation, acetate, ketals, amino and imino groups, hydrazides, diazonium salts, quaternary ammonium compounds, isocyanates and isothiocyanates, sulphides, sulphoxides and sulphonic acids and derivatives

Each section is preceded by a discussion of the particular chemical reactions involved in the determination and this is followed by the methods derived from them Methods are laid out in standard fashion indicating apparatus and reagent requirements followed by procedure and calculation All the methods chosen have been tried in the author's laboratory

At the end of the book there are chapters dealing with the development of new analytical methods, the role of quantitative functional group determination in the identification of organic compounds, the use of differential rate of reaction to analyse mixtures and the weighing of volatile and corrosive liquids The chapter on the role of quantitative functional analysis might well have come first rather than almost last, while that on weighing corrosive liquids, although useful, would have been better had it been enlarged to deal with sampling and sample-handling techniques in general The analyses of mixtures by differential rate of reaction is illustrated by reference to mixtures of alcohols, amines, esters, etc , good results being obtained in some instances

The section on physical methods of analysis has been excluded from this edition In view of the importance of these methods in modern organic analysis, not only should it have been retained, but also it should have been enlarged

This book is not likely to recommend itself to students because of its high cost It may be useful, however, to those laboratories engaged in general quantitative organic chemistry where library facilities are limited

P J Cooper

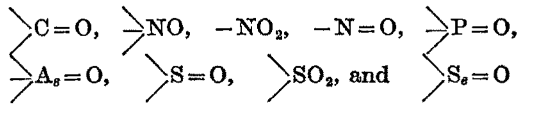
OXO-COMPOUNDS

Inorganic Adduct Molecules of Oxo-Compounds

By Ingvar Lindqvist (Anorganische und Allgemeine Chemie in Einzeldarstellungen, Band 4) Pp vi + 129. (Berlin Springer-Verlag, 1963) 20 D M

AT first sight this book appears to be something midway between a review article and a section of Gmelin Resemblance to the latter owes much to the style of printing, which makes for extremely tedious reading

The book is concerned with adducts formed by Lewis bases in which the donor atom is oxygen, but it is limited to a discussion of so-called 'oxo' donors in which the oxygen atom is linked to only one other atom These donors include molecules containing the following groupings



Donors such as ethers are not included, however

The text opens with a brief discussion of the conditions for adduct formation, followed by a short review of the characteristic co-ordination numbers This review is surprising in that under the heading "Transition Elements" it includes only the elements manganese, iron, cobalt, and nickel, the other elements, which (to British chemists at least) are commonly considered as transition elements, are grouped with the characteristic elements which show the same maximum oxidation state (for example, titanium and zirconium are grouped with germanium and tin) This review is not up to date, since it says nothing of the recent work on higher co-ordination numbers of a number of these elements It is well known, for example, that zirconium can exhibit a co-ordination number of eight, and recently X-ray work has confirmed a similar state of affairs for titanium in its diarsine complex.

Quite a long section follows in which the various known adducts are listed together with original references This is done systematically for each of the donor types. Although this section is mainly just a list of compounds, several useful observations are made at various points, and workers in the general field of co-ordination chemistry will find the references to be almost complete It is disappointing, however, to find that the majority of the 1962 references refer to unpublished information, and this suggests that there has been quite a long delay between the preparation of the manuscript and the appearance of the book.

Various topics are then dealt with. These include a discussion of the physical methods used for the study of molecular adducts, a review of the available structural data, and the catalytic activity of acceptor molecules

The final section of the book discusses "A Theory For Donor-acceptor Interaction with Oxo-compounds", and here the author deliberately sets out to give a very elementary account of the bonding in adducts He is evidently very unhappy about most of the recent developments in theoretical chemistry, since he refers to quantitative theories being "no more than empirical or at best semi-empirical rationalizations couched in a jargon which hides our actual ignorance" Accordingly, he discusses theoretical aspects of the bonding in adducts in a very elementary manner I found this section very tedious and uninspired, and in places naive The discussion on the amount of π-bonding in $M=O$ bonds (p 92) is particularly unfortunate, it is a pity that the author does not quote Van Wazer's recent work in the $P=O$ field, for example

Summing up, it can be said that this book has the appearance of an expanded review article, and that it has suffered by the expansion It is written in a reasonable style, however, and it is only in one or two cases that misuse of English leads to confusion I found very few misprints The book will be of some use to research workers in the field of molecular adducts, but it is unlikely to be of interest to general readers

G W A FOWLES

INTRODUCING ORGANIC CHEMISTRY

Organic Chemistry

By Dr D J Waddington Pp x+442 (London Mills and Boon, Ltd, 1962) 22s 6d

DR WADDINGTON'S book aims to cover requirements for the Advanced Level Examination for the General Certificate of Education, first M B and university entrance scholarships Small print is used to denote parts which the less-advanced candidates may omit Despite this I feel that most students making a start on organic chemistry would be better served by a smaller introductory book Afterwards this would be excellent, the brightest ones might start with it The type, line diagrams and formulæ are very clear and the price reasonable for the size of book

Two short chapters deal with the nature of organic chemistry and compounds, and with general manipulative technique Forty-three chapters follow In most cases each deals with one homologous series or with a small group of related series, but special chapters are included to cover atomic structure, valency, and electronic theory as used in examining the structure of organic molecules and the mechanism of their reactions Another gives an introduction to aromatic chemistry These special chapters form a sandwich between the earlier ones on the aliphatic compounds, beginning with the paraffins, and the succeeding ones on aromatic compounds Then follow chapters on oil, coal, the chemicals derived from them, and polymers They give very useful accounts of many modern developments on the industrial side of the subject A penultimate chapter on analysis is followed by a short, but very useful, one in which the more important types of organic synthetic reactions are conveniently grouped together Chapters on the homologous series give a clear summary of the general nature of the compounds Important individual members are given separate treatment which includes preparative details Towards the end of each chapter, additional preparative work and simple test-tube reactions are outlined which use up little material or time A selection of questions from papers of various examining boards and university scholarships concludes each chapter and a further hundred appear in an appendix Fifteen pages of a general index are followed by short indexes covering named reactions, important terms and inorganic reagents

H DUNNICLIFF

MICROANALYTICAL TECHNIQUES

Quantitative Chemical Micro-Analysis

By Prof C J Van Nieuwenburg and J W L Van Ligten Translated by C G Verver (Elsevier Monographs Chemistry Section) Pp viii+181. (Amsterdam and London Elsevier Publishing Company, 1963) 35s.

QUANTITATIVE Chemical Micro-Analysis is an English version of the original Dutch *Kwantitatieve Chemische Microanalyse*, which describes those microanalytical techniques which have been exhaustively studied and systematized at the Technical University, Delft The authors, Emeritus Prof Van Nieuwenburg and his successor, Dr Van Ligten, are both investigators of eminence in the microchemical field, and their views on the didactic and practical applications of this science will command much attention both in teaching institutions and in the industrial laboratories

They predict that "microanalysis will become the standard form of chemical analysis" and emphasize the saving in cost, the application to minute quantities of starting material and the general neatness and elegance of the method used The development of single-pan fully automatic balances is rightly regarded as a major advance in technique, and the normal practice of permitting objects to reach equilibrium with the vapour of the ambient atmosphere before weighing is strongly advocated, as is the expedient of removing electrostatic charges from the balance housing by means of ionizing radiation The present edition is pocket-sized and contains 181 pages Fifty-three pages of the book are devoted to very detailed descriptions of single gravimetric determinations of numerous elements and acid radicals at concentrations approximating to 1 mg per ml, for example, bismuth with triethylenediamino-cobaltic iodide, silicate as pyramidone silicomolybdate, etc Apparatus for micro-separations is fully described

Twenty-two pages deal with microgravimetric separations of mixtures containing two simple metallic elements, for example, lead and thallium, the following 38 pages being devoted to microvolumetric analysis

The Strohlein or Metrohm plunger-type burettes with 'Teflon' pistons and a capacity of 2 ml are recommended for N/50 titrants Gorbach burettes are described, but the presence of a meniscus causes after-flow The British 'Agla' syringe can be used both as a pipette and as a burette

Much attention is directed to ethylenediamine tetraacetic acid microtitrations, and there are numerous practical examples of direct replacement and of back titrations using 0 02 M solutions.

The volume ends with a most useful chapter on quantitative elementary organic analysis Carbon and hydrogen are determined by the method of Belcher and Godbert, the halogens by the ter Meulen-Sloof technique or alternatively by the Schoniger oxygen-flask Nitrogen is determined by ter Meulen hydrogenation using Ni-ThO_2 catalyst or by the modified Dumas method Didactic considerations have led Van Nieuwenburg and Van Ligten to exclude descriptions of well-known methods such as those of Unterzaucher for oxygen or Zimmermann for sulphur

A useful description is, however, given of molecular weight determination on a micro-scale utilizing the 'isothermal distillation' technique as suggested by Barger, developed by Wright and modified by Van Nieuwenburg and Van Ligten

This publication has a wholesome practical bias and will have an undoubted value and appeal to teachers of microanalysis in academic institutions It has also much to offer the practising analyst who may be called on to work with rather restricted quantities of a parent substance or who wishes to economize on his financial outlay

D T LEWIS

FOREIGN CHEMICALS IN FOODS

Residue Reviews

Residues of Pesticides and other Foreign Chemicals in Foods and Feeds Edited by Francis A. Gunther Vol 1 Pp iv+162 Vol 2 Pp iv+156 Vol 3 Pp iv+170 (Berlin Springer-Verlag, 1963) 22 D M each volume

THE incorporation, often inadvertently, of foreign substances in foodstuffs has caused increasing concern in recent years Attention has been directed especially to residues of chemicals which are needed to control pests, diseases and weeds in crops in the field, to combat parasites of animals used for food, or to protect foodstuffs in transit or storage Much work has been devoted to finding how the crop protection and related chemicals may be safely used A new and specialized field of residue analysis has emerged allowing the levels of contamination in harvested crops to be determined, and the behaviour and ultimate fate of the chemicals have been extensively investigated Some are lost from crops by weathering, some are degraded into harmless derivatives by the plant or animal, and others are eliminated during food processing Some chemicals, however, persist on crops and in soil, tend to accumulate in animal food chains, and may endanger public health in due course Associated with the chemical examinations are toxicological investigations to determine the significance of the residues that may be found Statutory and other measures adopted to protect the consumer include the institution of permissible limits of contamination, maximum recommended rates of use of potentially harmful chemicals, and the observance of adequate periods for weathering

The object of *Residue Reviews* is to bring together the many aspects of the problem of foreign chemicals in foodstuffs The use of substances to improve flavour, appearance, etc , and contamination incurred during manufacture, packaging, etc , are also considered to be suitable subjects for discussion Contributions are made by invitation, and an advisory board of specialists from many countries indicates the international scope of the publication

The three volumes that have so far appeared deal largely with problems arising from the use of chemicals for crop protection. General articles describe the nature of plant surfaces in relation to the use of spray chemicals, the absorption, translocation and metabolism of chemicals in plants, and the processes involved in deposition, degradation, persistence and effectiveness. A valuable series of papers on analytical methods show the advantages conferred on residue analysis by modern techniques of chromatography, polarography and spectrophotometry A few contributions discuss the levels of residues that are found in practice, one deals with the principles underlying the establishment of residue tolerances, and another examines the difficulties and interpretation of tests for carcinogenicity Each article gives summaries in English, French and German and useful references

Work on residues in foodstuffs is expanding so rapidly, and information is scattered in so many journals, that undoubtedly there is ample scope for a publication of this kind To be fully effective, its professed aim of international coverage should be attained, at present emphasis rests on work and reports from the United States, a position that no doubt will change when the series becomes established Many of the contributions give valuable information, but some scarcely reach the standard that may reasonably be expected A considerable amount of tidying-up is needed before the presentation will satisfy critical appraisal· proprietary and coined names of chemicals are used indiscriminately, inconsistencies occur in chemical names, and the subject index is quite inadequate. This last deficiency detracts much from the merits of an otherwise commendable, if expensive, publication

J T MARTIN

ANALYSING FOOD CONTAMINANTS AT LENGTH

Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives

Edited by Gunter Zweig Vol 1. Principles, Methods, and General Applications Pp xiv+637 (New York Academic Press, Inc ; London Academic Press, Inc (London), Ltd , 1963) 171*s* 6*d*

PARKINSON'S Law states that work will always expand to fill the time available for its execution The same principle surely applies to publishers with an infinite supply of paper, editors with an inexhaustible number of contributors and libraries with a seemingly unlimited provision of dollars The present book is priced at £8 11*s* 6*d* Its purpose is to serve only as an introductory review of the principles and general applications of the analytical methods which are to be described in Volumes 2, 3, 4 and 5 that have yet to be published The fortunate possessor of the entire compilation, who will, if he reads all the 3,185 pages which it will doubtless comprise, know all there is to know about the analysis of crops and food for traces of pesticides, plant growth regulators and food additives, will therefore have paid £42 17*s* 6*d* for the privilege

A number of the authors of individual chapters discuss what they call the "clean-up procedures" by which they concentrate the trace residues in which they are interested and free them from the bulk of the sample If an analogous process could have been applied to the book itself, everyone would have been the gainer because there is much in it of value W Thornburg, for example, contributes an admirable, short, clear and practical chapter on the various methods by which trace contaminants of different classes can be separated from the materials with which an analyst may be confronted The chapter by A Bevenue on the application of gas chromatography to the analysis of traces of fumigants, insecticides, herbicides and a list of miscellaneous compounds including phosphorus halides, chlorobenzenes, insect repellents, fungicides, mould inhibitors and antioxidants is a model of how theoretical principles and practical details can be succinctly combined The table of relative retention times on differently packed columns of a long list of pesticides will alone put many analysts in his debt P McKinley's article on paper chromatography is equally satisfactory, the combination of paper chromatography with fly bioassay and cholinesterase-acetylcholine reactions may be novel to workers in other fields There is an interesting, sensible chapter by R Moffitt on residue analysis in the dairy industry, and a useful and informative one by Z F Krzeminski and W A Landmann on methods for pesticide analysis in meat products which may surprise more old-fashioned workers by the number of antibiotics, hormones, arsenical compounds, tranquillizers, antioxidants and pesticides that there may be in a carcass in this modern age

But the speed at which science is advancing is brought home most forcibly by two quite different contributions One is by R Fisher it describes methods for assaying the potency of "microbial pesticides" A "microbial pesticide" is a living micro-organism designed to infect one or other of the insects which constitutes an agricultural nuisance There are at the present time four of these preparations on the market in the United States Three contain specific bacteria—*B thuringiensis*, *B popillae* or *B lentimorbus*—and one a virus as active ingredients Here, indeed, is need for a new sort of analysis The second article by R A Schmitt gives a matter-of-fact account of the elegant analytical procedure by which very small trace concentrations of a diverse variety of substances can be determined simply, quickly and often without the need for preliminary treatment of the substance under examination by the use of neutron activation analysis, a technique in which specific radioactivity is induced in the sample by exposing it to neutrons from a nuclear reactor In view of this evidence of the pace at which scientific understanding and its applications are progressing, is it really ungenerous to doubt whether analytical methods will stand still long enough to make it worth while saving up for Volume 5 of this work? MAGNUS PYKE

FOOD PLANT HYGIENE

Food Plant Sanitation

By Milton E Parker and John H Litchfield Pp v+401 (New York Reinhold Publishing Corporation; London. Chapman and Hall, Ltd , 1962) 96*s* net

IT is somewhat surprising, considering the importance of food plant sanitation ('food plant hygiene' in Britain) to food manufacturers, public health officers and others, that so few books have been published on this subject Many that have been published have not been revised to bring them up to date, this despite many recent developments *Food Plant Sanitation* is, therefore, welcome, it is a good book and, for its size, very comprehensive Unfortunately this is a subject which is hedged around with many legal requirements, and this book is written with the U S Food, Drug, and Cosmetic Act particularly in mind Also, although there are more than 500 references, these are predominantly of American origin The user of the book in the U K will have to have beside him the corresponding British regulations and the many excellent publications of H M.S O relating to this subject

To some extent this concentration on American practice will be of value to readers in Britain They may gather how far, if at all, Britain is behind the United States in food hygiene and they should get some idea of the precautions that must be taken if foodstuffs are to be exported to the United States

The first chapter deals with the role of sanitary practice in quality control and the second with sanitary aspects of buildings and equipment The third, fourth and fifth chapters consider insect and rodent pest control, and then follows a chapter on the effective control of micro-organisms This includes details of the new organic chlorine products which are now gradually coming on to the market in the United Kingdom after being in use in the United States for some time Chapter 7 deals with sanitary aspects of water supplies and Chapter 8 with waste disposal and utilization Some of the methods described here are as yet unfamiliar in Britain, such as the anaerobic digestion system for meat packing-plant wastes Effective detergency and cleaning practice is considered in Chapter 9 This is a thoroughly up-to-date account, and modern detergents and the latest types of clean-in-place methods are mentioned Chapter 10 is headed "Establishing Sanitary Practices" and considers the duties of the 'sanitarian' in the food industry This is a 'species' of which there is no exact counterpart in Britain; perhaps there ought to be The following chapter on "Maintaining Sanitary Practices" deals with such things as employee training in sanitary practices. The last and twelfth chapter is a useful one on the sanitary aspects of packaging material and methods An appendix gives excerpts from the U S Food, Drug and Cosmetic Act and amendments

This book should prove of considerable value to managers, chemists and microbiologists in the food industry, and also to public health officers and consultants concerned with food hygiene H. D G ROPER

ADVANCES IN ENZYMOLOGY

Advances in Enzymology and Related Subjects of Biochemistry

Vol 24 Edited by F F Nord. Pp v+572 (New York and London Interscience Publishers, a Division of John Wiley and Sons, 1962) 120*s*

THE synthesis of enzymes in the living cell and the determination of their structure form part of the much wider problem of protein biosynthesis in general, with all its subtle intricacies of amino-acid activation, energy expenditure and genetic control by nucleic acids Some of the recent enzyme research appears to have been undertaken not so much to provide an answer to the question why a given enzyme specifically acts on one but not another substrate, but with the purpose of unravelling the mechanism of protein biosynthesis, whereby determinations of enzymatic activity serve as a convenient indicator of protein function

In Volume 24 of *Advances in Enzymology and Related Subjects of Biochemistry*, Chantrenne deals at length and in depth with the problem of how biochemical tools receive orders from the genes and how they put them into operation He is well qualified by his own researches to write on that subject, and acts at the same time as an able exponent of the Brussels School, which has significantly contributed to the present-day knowledge of the relationship linking nucleic acid with protein biosynthesis

The manner in which the gene-controlled information determines biosynthetic pathways and the structure of cellular proteins is a repetitive theme in several chapters of the volume The practical approach to the problem of cellular localization of enzymes and their synthesis has greatly benefited from the techniques of centrifugal fractionation developed in another Belgian laboratory, namely, that of de Duve at Louvain It is, therefore, most welcome to have an authoritative, as well as informative, chapter by de Duve, Wattiaux and Baudhuin, on the distribution of enzymes between the sub-cellular fractions in animal tissues Far from being a mere catalogue of results, this chapter can be used as a guide in the application of centrifugal fractionation to the investigation of a variety of biochemical processes.

Among the few enzymes of which the amino-acid sequence has been well established is the ribonuclease, which at present receives a great deal of attention from protein chemists as well as enzymologists The structure and function of ribonuclease form the main topic in the chapter by Scheraga and Rupley, who provide a clear and concise survey of the subject But ribonuclease enters into discussion also in several other chapters, notably that concerned with mechanisms of enzyme catalysis by Westheimer, and the one by Sri Ram, Bier and Maurer, who deal with chemical modifications of proteins and their impact on enzymology, immunochemistry and related subjects. Ribonuclease again figures prominently in the chapter on peptide structures by Šorm, whose analytical results on proteins form the basis of his evolutionary concept whereby the chemical structure of proteins is seen as a reflexion of the evolution of life on Earth, a record, as it were, of the laws of phylogeny and ontogeny of the distant past

Of the three remaining chapters, that by Augenstine offers a detailed and very useful account of the six existing models of enzyme radiosensitivity, in the chapter on sperm metabolism, Salisbury and Lodge expound their views and experiments on the subject of the relationship between sperm survival, motility and metabolism, and the response of spermatozoa to a variety of extraneous agents, lastly, there is a review on molecular properties and transformations of glycogen phosphorylase in animal tissues, by Edwin Krebs and Edmond Fischer, dealing with the first step of glycogenolysis, a process significantly more complex than originally visualized, as it now appears to involve no less than four different enzymes, three nucleotides, a vitamin, and several hormones and metal ions T Mann

PHYSICS IN BIOLOGY AND MEDICINE

Advances in Biological and Medical Physics

Vol VIII Edited by Cornelius A Tobias and John H Lawrence Pp ix+457 (New York and London· Academic Press, 1962) 107*s* 6*d*.

THREE of the eight topics presented in the latest volume in this series are concerned with techniques for the measurement of trace elements, X-ray spectroscopy by J W Gofman, neutron activation analysis by Kwan Hsu and γ-ray spectroscopy by L D Marinelli, C E Miller, H A May and J E Rose, the latter restricted to the external measurement of radioactive elements located within the human body These chapters provide useful descriptions of the physical techniques involved and of the relative sensitivity for different elements Various medical and biological applications of these techniques are described, but with little indication as to their usefulness in comparison with other techniques However, this may be due to the fact that they have not as yet been applied very widely

A chapter by Tor Brustad gives an excellent account of the experimental set-up of the heavy ion linear accelerator (*Hilac*) at the University of California Applications in the field of radiobiology are presented which illustrate well the usefulness of this machine, and also the complexity of interpretation of the experimental results

A lengthy chapter on the interrelations between the hypothalamus and the thyroid is provided by P Blanquet in collaboration with J Faure The detailed descriptions of experimental observations by many workers make it difficult, for those new to the subject, to ascertain what the authors consider to be the most likely mechanisms operating between these two organs However, those working in this field should find the chapter most useful

With the all-embracing title "The Origin of Life on Earth and Elsewhere", Melvin Calvin describes the properties that are required of molecules so that they can give rise to primitive forms of life He describes how simple polyamides and nucleic acids may have interacted and evolved to the great complexity of the biochemical processes that are found in even the simplest organisms to-day

"The Physics of Space Radiation", by Roger Wallace, deals with the various sources of ionizing irradiation found in space and concludes that only that resulting from sporadic solar flares is of real danger in future distant space flights It is suggested that a few minutes' warning of these flares can be obtained so that spacemen may shelter in some well-shielded part of the space vehicle

The final chapter, by Niels Arly and Reidar Eker, is entitled "Mechanisms of Carcinogenesis" After an interesting presentation of the complexity of the carcinogenic process, various mathematical models are considered and the need for a stochastic model is argued Such a model that has been constructed by the authors, is shown to be able to fit satisfactorily a number of results relating different experimental parameters The absence of any information concerning the biological mechanisms involved leaves one with the impression, however, that no real advance has been made in this field N M Blackett

NUCLEOPHILIC ALIPHATIC SUBSTITUTION : MECHANISM AND KINETICS

Nucleophilic Substitution at a Saturated Carbon Atom

By Dr C. A Bunton (Reaction Mechanisms in Organic Chemistry, Vol 1) Pp ix+172 (Amsterdam, London, New York Elsevier Publishing Company, 1963) 40*s*

THIS is the first volume in a series edited by (the late) Prof E D Hughes, and sets an excellent standard for the series as a whole The monograph also occupies a proper position in the series, as the general principles, which are adumbrated in Chapter 1, are useful in other areas of physical organic chemistry, the subjects of following volumes While ideally it might have been better to treat nucleophilic substitution as a saturated carbon atom in the same volume as elimination reactions, there are good practical reasons for the procedure adopted of limiting this volume to substitution only

In places this work leans heavily (but rightly so) on parts of Sir Christopher Ingold's magistral volume, now some ten years old, *Structure and Mechanism in Organic Chemistry* However, in the controversial parts of the subject the author, himself a distinguished member of the University College, London, school, has shown an admirably balanced and fair attitude, especially to the work of American protagonists in the controversies The book is also noteworthy for numerous critical and illuminating footnotes (unfortunately printed in very small type) for many well-chosen references, situated at the end of each chapter, but easily found, because their location is given on every alternate page of the chapter in question

After an opening chapter in which mechanistic and kinetic principles are discussed, with special reference to 'duality of mechanism' (the definitions of the S_N1 and S_N2 mechanisms might have been rather more rigidly set out), the author proceeds to the two most substantial parts of his book—a thorough survey of structural effects on the rate of substitution, and a penetrating account of the stereochemistry of the substitution process in relation to mechanism, which is still one of the most satisfying areas of rationalization in physical organic chemistry

The next two chapters are devoted to solvent and salt effects these are matters of considerable subtlety where the subject is still growing, and raise issues of great theoretical interest, and in the first case of great practical importance There are a brief final chapter on electrophilic catalysis and an addendum dealing with important matters which have arisen since 1961, the date to which the literature has been covered in the main text

All students of physical organic chemistry will wish to study carefully the volume as a whole, and the greater part of it will be valuable to organic chemists generally, especially to third-year honours and first-year Ph D students

N B CHAPMAN

CELLULAR ORGANIZATION

Funktionelle und Morphologische Organisation der Zelle

Wissenschaftliche Konferenz der Gesellschaft Deutscher Naturforscher und Arzte in Rottach-Egern 1962 (9 Beitrage in Deutscher und 6 Beitrage in Englischer Sprache) Pp iv+253 (Berlin, Gottingen and Heidelberg: Springer-Verlag, 1963) 36 D M.

FOR a long time the great issues of cellular organization have been argued in terms of the nucleus The problem has been that of the relations of its physical structure, its chemical activity and its genetic permanence or continuity Now, with the help of electron-microscopy, autoradiography and other techniques, the problem has, as we may say, boiled over into the rest of the cell A wide range of results of this boiling over is displayed in the present symposium A general statement of its purpose and its problems is, however, lacking

It was the continuity and activity carried together by the nucleus which first struck the imagination of cytologists, German cytologists, in the nineteenth century. Clearly any structure in the cell which can achieve this difficult combination deserves to be given the status of an organella Any body which can fulfil its short-term duties within the cell while meeting the long-term needs of propagation and distribution to different cells will thus qualify for this status Presumably, therefore, any of the cell structures in which DNA is now beginning to be recognized will also qualify How far will this property extend ?

The answer to this question has usually been derived in the past from the evidence of experiment, but experiment of the most varied kinds with the most varied organisms The breeding of plants has shown us the self-propagation of the plastid The embryology of animals has demonstrated the same property in the centrosome The development of Protista has revealed the genetical continuity of their versatile fibre-forming organellæ The evidence of immunology has left little doubt of a similar propagation in the higher animals Here, and in the plasmagenes to be inferred from the heredity and development of the higher plants and animals, we do not know what particles or structures provide the basis of continuity But in this symposium both the electron microscope and chemical analysis introduce us to a number of possible candidates for these duties

No such central idea is, however, advanced to give unity to the present discussion Nor is there indeed any central idea in biochemistry which might offer itself for such a purpose The most nearly central problem is that which comes from the work of Mechelke and Beermann with the polytene chromosomes of flies The connexion between heredity and development, and between DNA and RNA, revealed by these studies of development and differentiation, gives us a glimpse of what has long been suspected the hierarchy of structures and functions which lie between the genetic code and its expression It is a hierarchy which will require us more and more to study the cell as a system and to use all the available degrees of magnification in seeing how it has been built and organized. In order to succeed in this task we shall have to exploit our resources in more variety than is done in this symposium Older and newer techniques will have to be more fully combined Plants, omitted from this discussion, will not have to be left out For it is already easy to see that, after the nucleus, their plastids will play the most important part in knitting our ideas together

C D DARLINGTON

THE SENSES OF INSECTS

The Physiology of Insect Senses

By Prof V. G Dethier (Methuen's Monographs on Biological Subjects) Pp ix+266 (London Methuen and Co , Ltd , New York John Wiley and Sons, Inc , 1963) 42*s* net

THIRTY years have elapsed since Eltringham's monograph on *The Senses of Insects* was published in this series At that time, the greater part of the modern work on insect sense organs had been done in Germany This work received little reference in Eltringham's book, which presented the knowledge and ideas of an earlier epoch J. D Carthy dealt with the subject well in his *Introduction to the Behaviour of Invertebrates* some five years ago, but the new monograph by Prof V G Dethier, although it covers again much of the same ground, is very welcome, for it is written by an author who has been personally involved in some of the most significant advances in our understanding of the chemical senses of insects

The author deals at some length with those subjects which are most controversial at the present time the nature of the action of chemostimulants at the receptive site, and the basis of their specificity, the interpretation of the electroretinogram, the varying spectral sensitivity in different insects, and the chemical nature of the photoreceptive substances, the mechanism in the insect eye which enables it to perceive the plane of polarized light, the physiological basis of the great differences which exist between different tactile hairs, depending on the purpose for which such receptors are used, that is, their diverse properties with respect to adaptation, and in the effect of direction of movement on the nervous discharges that result In short, the emphasis throughout is on how insect sense organs work

Advances in the past ten years, supported by electronic methods of neurophysiology and by structural investigations with the electron microscope, have been spectacular From the studies of Slifer, in particular, we know that the thin cuticle which covers the chemoreceptive sensilla is perforated like a sieve and multiple fine dendrites in these perforations have their endings freely exposed to the atmosphere We know, particularly from the work of Dethier and his school, that a single chemoreceptive hair on the tongue of the blowfly is receptive only at the extreme tip, the fly responds positively to stimulation of the hair by sugar, and by water, negatively to electrolytes, and to the mechanical displacement of the hair Each of these perceptions is mediated by a separate dendrite and sense cell From the work of Roeder and Treat we know that although the abdominal ears of the moth have only two neurones apiece, they can recognize both the character and the direction of the cries of a hunting bat

Work done in all countries is well covered and a select list of about 700 titles is included The extensive German work is well reviewed, including that dealing with insect vision Our present lack of understanding in many parts of this subject is reflected in the obscurity of some of the

German writing about it This obscurity comes across in the English version One of the difficulties is how to reconcile the separate innervation of the seven or eight elements that compose the retinula of the ommatidium, with the mosaic theory of vision by the compound eye The recent contributions by Burtt and Catton and by N Yagi were not published in time to receive critical consideration in Dethier's account

This monograph can be strongly recommended, but it is a technical monograph which makes few concessions to the possible limitations of the reader

V B WIGGLESWORTH

PHOTOGRAPHIC DIORAMA OF WILDLIFE

The Wonders of Wildlife in Europe

By Franz A Roedelberger and Vera I Groschoff Pp 232 (280 pictures) (London Constable and Co, Ltd, New York Viking Press, Inc, Toronto· Longmans Green, 1963) 42*s*

THE rise and rapid advance of colour photography in recent years have placed in the hands of the book illustrator a wonderful medium for the illustration of ornithological works

The Wonders of Wildlife in Europe is a fine example of what can be done in this respect It is a picture book, a collection of 280 remarkable photographs, the striking illustrations being accompanied by descriptive paragraphs telling the reader some facts concerning the subject, or rather subjects, shown in the pictures These cover a wide field, from marine life and insects to reptiles, mammals and birds The leading natural history photographers of Europe have contributed to the gallery

By their grace and beauty, birds afford the artist wonderful subjects, and here are shown some of the exquisite results that can be achieved by those using what we may term the 'new medium'

Comparisons are ever invidious, yet they must inevitably arise in a case such as this when we think of the great illustrated bird books of the past, the work of artists who never dreamed of a time when the camera would come into being and take over much of their work Personally I do not think comparison possible The beauty of a plate from Gould's *Humming Birds* cannot be compared with the study of an elephant hawk moth shortly after emerging from the pupa, on p 162 of *The Wonders of Wildlife in Europe*, or the portrait of a little Egyptian vulture on p 41 Each in its way is a thing of beauty, and if the ways are different ways the contrast enhances them

In this volume the colour pictures are, of course, the most eye-catching and impressive of the illustrations, but there are also a large number of monochrome reproductions, including some remarkable snapshots such as that of two young foxes having a dispute and fighting heartily Seemingly the trouble arose over a white hen Another fine shot shows a female white-toothed shrew, *Crocidura leucodon*, followed by her family The first young one is grasping the mother by the fur of her rump, while the rest of the litter are in line astern, holding on to the hindquarters of the one ahead, there are six in all

For the depiction of sheer joy and delight in life a fox-cub springing high in the air (p 196) is hard to beat, it is such a fine photograph and the young animal is in such a joyous pose Another remarkable photograph is of a herd of wild reindeer in extended formation, described as a view over Hardanger Vidda, Norway

There is a foreword by Peter Scott stressing the need to protect wild creatures and laying stress on the problems of the conservation of animal life in general, now and in the future

FRANCES PITT

PRODUCTIVE CYCLES IN THE OCEAN

Plankton and Productivity in the Oceans

By Prof John E G Raymont (International Series of Monographs on Pure and Applied Biology Division Zoology, Vol 18) Pp viii+660 (London and New York Pergamon Press, 1963) 110*s* net

THE first four chapters in this book on oceanic production give a brief account of physical oceanography as it affects life in the sea The distributions of temperature, salinity, oxygen and carbon dioxide are described Pictures of the oceanic current systems are reproduced from Sewell[1] This is part of an attempt to "approach the study of oceanography through biological science". Much of the physics and chemistry relevant to the study of productive cycles is to be found in later chapters

The subject of production is introduced with a chapter on the phytoplankton. The structure of diatoms, dinoflagellates and μ-flagellates is well described The illustrations are clear and well reproduced The decrease in size of diatoms, division rates, formation of auxospores, heterotrophy and flotation are physiological subjects brought into the discussion, which are of particular interest to production biologists Prof Raymont defines nanoplankton as plant cells "not normally retained by the finest silk nets" and they belong to "a variety of plant groups, Chrysophyceae, Chlorophyceae and Cryptophyceae", Yentsch and Ryther[2] are quoted as showing that "the major part (of nanoplankton) is made up of small diatoms rather than microflagellates" Prof Raymont says that the quantitative estimation of nanoplankton is extremely difficult It is quite true that μ-flagellates cannot be counted, but small diatoms and dinoflagellates can be counted quite well with Utermohl's sedimentation technique[3], which is not mentioned

Algal production is described in five chapters An introductory one deals with photosynthesis, compensation depth and compensation point, the inhibition of photosynthesis at high-light intensities near the surface and the adaptation of photosynthesis (measured as mg carbon/mg chlorophyll/h) to different conditions The next four chapters treat of nutrients, light, micronutrients and grazing respectively Since the time of Lohmann[4], who counted all the plankton and who made a simple model of plankton predation, it has been clear that any productive-cycle is a complex sequence involving all these factors all the time The division into chapters treating the factors separately is a little artificial Indeed, one might say that the study of production is not fully described until the chapter on grazing, where the models of productive cycles are dealt with

In the chapter on nutrients, the seasonal changes in phosphorus, nitrogen and silicon and their distributions in the oceans, both vertically and horizontally, are well described It is said that silica is rapidly regenerated, I believe this is so, but the evidence is not given, nor is any authority quoted In the subsequent chapter (on light) a diagram of the nutrient-cycle is given, showing that the decline in nitrogen and phosphorus starts in late April well after the peak of the algal outburst at the end of March This is what happens at Plymouth, comparing Fig 7 29 showing seasonal nutrient distributions with Fig 8 1 giving the seasonal algal distribution In contrast, Fig 7 2 (showing seasonal distributions of phosphorus, nitrogen and chlorophyll in Long Island Sound, from Riley and Conover[5]) demonstrates that the chlorophyll peak occurred exactly when the very low values of nitrate were reached It is this sort of contrast that suggests that the effect of nutrient lack on algal production is not a simple one In discussing models, Prof Raymont points to the need for good physiological estimates Here is one badly needed—the effect of various nutrients on algal division rates A little has been done, but not enough

Seasonal cycles in algal production are described in the next chapter on light and primary production A good account is given of the initiation of productive cycles and of the penetration of light into the sea Another chapter describes the part, if any, played by micronutrients in productive cycles Here again are needed experiments establishing the effect of nutrient concentration on algal division rate

The effect of grazing on algal production is well described, the work of Harvey, Cooper, Lebour and Russell[6] and the work of Fleming[7] being given in some detail. This leads naturally to an account of the model techniques used by Riley and others to describe the course of algal production as a whole Here Prof Raymont makes a good summary of the part played by the various factors, nutrients, light, vertical turbulence and grazing He is perhaps a little chary of setting out any model in its mathematical terms, preferring to state it in words

A systematic account is given of the zooplankton The pictures showing the structure of these animals have been well reproduced In a chapter on geographical distribution, the work on plankton indicators is reviewed lightly, mainly to show where different animals and communities live, rather than to show whether ocean currents can be charted by indicators The seasonal cycles of plankton animals are described briefly giving generation times From this clear review, it appears very strikingly that from all the work on seasonal cycles of zooplankton, there are very few good estimates of reproductive rate, growth rate or mortality rate

A full description of the vertical distribution of plankton animals is given, including their diurnal migrations. It should be emphasized that bathypelagic decapods, euphausiids, sagittæ and fish larvæ dodge slowly hauled plankton nets so some vertical migrations quoted are perhaps not migrations at all In describing the mechanisms that plankton animals might use, Prof Raymont discusses geotaxes and phototaxes in rather loose terms Phototaxis should be defined in terms of the two-light experiment[8], it is difficult to see how the conditions of this experiment reproduce those found in the sea, particularly at dusk and dawn when the important parts of migration occur. Harris's work on the various mechanisms of behaviour that plankton animals can use is not described[9,10], this is an omission

Prof Raymont's own work is concerned with the feeding and respiration of zooplankton His account of this subject is extensive, ranging over the types of food, the quantities eaten and how they are linked to the respiratory rate He does not define the term "filtering rate", which is a volume swept clear of food particles per unit time By an experimental convention, the value is derived from a total mortality rate of algal cells per unit time If the algae are reproducing, the true grazing mortality rate can be higher than the apparent total mortality rate. Because this second step is sometimes not taken, many experimental values of "volume swept clear" are too low Again, Marshall and Orr's method[11] of using phosphorus-32 gives the quantity of food assimilated, but it is not derived from the grazing mortality of the algae A large number of measurements are discussed as if they all meant the same thing Each of the three methods (from total mortality rate of the algae from grazing mortality rate of the algae, and from quantity of food assimilated) is valid in its own right, but should be distinguished in the discussion Prof Raymont goes on to discuss how the zooplankton survive and how the various model techniques in use so far describe the acts of grazing and feeding He concludes that "better results can be achieved only with a greater accumulation of precise knowledge of the physiology of zooplankton" It is astonishing how slowly this precise knowledge is accumulating

The book is intended for workers beginning investigation in the marine field It will find a wider circulation than that There are good and complete accounts of the various parts of the productive cycle in the ocean The history of each development is well described, for example, the progressive search for a nutrient which limits production or for a model which fits the data

The literature cited is almost all in English, excluding much good work in Russian and German, for example, Beklemishev[12] has shown that *Calanus finmarchicus* Gunner is armed with silica teeth D CUSHING

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[4] Lohmann, H , *Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton*, 129 (Wiss Meeresuntersuch, Abt , Kiel, 1908)

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[6] Harvey, H W , Cooper, L H N , Lebour, M V , and Russell, F. S., *J Mar Biol Assoc* , 20, 407 (1935)

[7] Fleming, R H , *J Cons Intern Explor Mer* , 210 (1939)

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[9] Harris, J E , and Mason, P , *Proc Roy Soc* , B, 145, 919, 280 (1956)

[10] Harris, J E , and Wolfe, U K , *Proc Roy Soc* , B, 144, 916, 329 (1955)

[11] Marshall, S M , and Orr, A P , *J Mar Biol Assoc* , 34, 495 (1955)

[12] Beklemishev, K. V , *Dokl Acad Sci* , 97, 3 (1954)

MATERIALS AND METHODS FOR MASTERING MENDELISM

Teaching Genetics in School and University
Edited by Prof C D Darlington and A D Bradshaw
Pp x+117+4 plates (Edinburgh and London Oliver and Boyd, Ltd., 1963) 21*s*

GENETICS is not easy to teach, since to understand the subject-matter the student requires a knowledge of certain aspects of several disciplines including biology, chemistry and mathematics What makes matters worse is that there seems to be an alarming negative correlation between biology and a knowledge of mathematics, anyhow in university students

Because of difficulties in teaching genetics, a number of people with practical experience in it participated in a symposium in order to try to assist themselves and others to improve their methods The meeting, at which I was present, aroused considerable interest in the audience and provided some very helpful suggestions *Teaching Genetics in School and University* is an account of the contributions of each of the participants, together with additional notes on genetics in schools, on certain demonstrations and on sources of material for teaching

The opening contribution by Prof J M Thoday on the organization of genetics teaching in universities gives a brief account of the contribution genetics can make to teaching in those other disciplines between which it forms a bridge In view of this connecting quality, Prof Thoday suggests that a department of genetics should provide a service to other departments, anyhow at an elementary level of teaching, and that special departments covering particular aspects of genetics such as biochemical genetics or human genetics should be viewed with disfavour Prof Thoday's article is stimulating and should be read by all interested in the teaching of the 'life sciences'.

The remainder of the book is devoted to special topics, with particular emphasis on methods of giving practical instruction in genetics The teaching of microbial genetics using bacteria, bacteriophages, fungi and yeast is discussed at considerable length by workers distinguished in this field of genetics There are also contributions on biometrical genetics, developmental genetics and cytogenetics by equally distinguished workers Particular mention must also be made of the article by Prof C D Darlington on the organization of a genetic garden, a most useful adjunct to any department of genetics At the end of the book there is also a section on "Genetics in Schools", brief notes on organisms, materials and experiments that can be used for particularly illuminating practical work and demonstrations, as well as a most useful list of sources of material for teaching genetics.

The coverage of the various aspects of genetics is extremely uneven, and the contents of the various contributions show marked evidence of being conceived quite independently of one another. Consequently, the editors have seen fit simply to group the contributions by broad subject The methods of teaching microbial genetics and cytology are well covered Evolutionary genetics is less-adequately dealt with, although there are some excellent suggestions by Dr. A D Bradshaw and J G Pusey for showing the results of natural selection with respect to the production of hydrogen cyanide in white clover and the evolution of heavy-metal tolerance in several plants Quantitative genetics is perhaps the most difficult of all the sub-divisions of genetics to teach to undergraduates, particularly without practical work Unfortunately the present symposium does little to remedy the difficulty Dr J L Jinks describes an excellent course in biometrical genetics for those few with the ability, but, as he himself admits, many of the undergraduates fall by the wayside because of a lack of the necessary mathematical knowledge Prof Thoday describes a very useful practical exercise in quantitative genetics, but it would have been helpful if several other similar exercises could have been included in the book

The reader hoping to find a course of genetics designed for him will be disappointed by this book, as indeed he must be There is little help to be found by those who hope for a list of topics suitable for a lecture course, other than that given by Dr Jinks and some suggestions for teaching cytology by Dr H Rees The ideas for practical work are not extensive enough to be useful for a complete practical course The reference to genetics in schools in the title seems to have been added as an after-thought to attract a wider public A few of the suggestions, particularly those by D S Falconer on the use of mice, those specifically for schools by Dr Bradshaw, some of the exercises in cytology described by K L Lewis and Dr B John, as well as those by A P Wylie and some of the brief notes, will be of use to a number of school teachers The majority of methods and suggestions require the student (and the teacher) to have an extensive background knowledge and will only be suitable for specialized scholarship work in the sixth form However, the book is going to be of very great use to all those university teachers of genetics who want some suggestions for practical work in fields in which they have not themselves worked, and for this purpose the book can be highly recommended

P. M SHEPPARD

TECHNICAL AND BUSINESS ASPECTS OF FARMING

Farming

General Editor Prof A N Duckham Vol 1 Pp xxiii + 543 + 16 plates Edited by C A Jewell Vol 2 Pp xv + 640 + 16 plates Edited by S Fox Vol 3. Pp xv + 585 + 15 plates Edited by J A C Gibb Vol 4 Pp xv + 501 + 14 plates Edited by Dr J Pearce (London, New York and Melbourne The Caxton Publishing Co, Ltd, 1963) 345s per set of four volumes

THIS book of four volumes on British farming appears at a time when doubts are being raised as to whether the science and up-to-date practices of farming can be treated adequately in one work, its contents give an indication of some of the difficulties Preparation of the four volumes must have taken a number of years, even though shared by the general editor, his four joint editors and eighty-one contributors, and that same period has seen a number of changes in farming, when proved and established practices have been challenged by alternatives largely directed to the saving of manpower and the reduction of costs The task, then, of the many contributors was far from easy in that they were writing of modern and changing techniques and with an eye to likely developments A little of what was written, say, three years ago is out of date, but the authors are to be congratulated on the forward-looking views they have expressed, and in the overall presentation of an interesting review of the practice and science of farming

Farming is written as a practical book for practical men and certainly fulfils that objective for those with an elementary knowledge of the natural sciences It is a useful supplement in helping the farmer to understand the whys and wherefores of what he is doing and how to plan ahead It is also of direct interest to those in industries associated with agriculture

Volume 1 (plants, soils, crops and grass) opens with an interesting account of soil formation and the structure of plants, and then leads on to describe the various ways of improving soils and controlling weeds The tillage crops are each dealt with in turn under three broad sections of field cultivations, harvesting and general information There seems no serious objection to this arrangement if the book is being used for reference For anyone investigating a particular crop, there is merit in bringing together all the information on that crop This arrangement would also help to eliminate the repetition that one detects here and there

The final section of this volume deals with the grass crop It gives an interesting account of types of natural grassland, grass breeding and the value of improved strains Modern methods of managing grass are described, including the newer techniques, which recently have directed attention to the high value of this crop in British agriculture

Volume 2 (livestock), under the title "The Framework of Production", discusses inheritance, the evolution of breeds, the control of growth and food requirements The information is well laid out and is a useful introduction to the more practical aspects of animal management outlined in the later sections of the volume Beef and sheep farming, two very important farming enterprises, would seem to merit rather more attention than the respective 20 and 19 pages allotted to them Milk production in its many aspects, on the other hand, is treated generously with 100 pages The concluding section, "Starting and Maintaining an Animal Enterprise", contains many useful hints on small but important points of management that at times can easily be overlooked

Volume 3 (buildings, roads and machinery) breaks rather new ground in reviewing modern ideas as to the construction of farm buildings The emphasis is placed rather on new buildings, newer materials and the lay-out of farm-steadings, though structural repairs and the maintenance of old buildings also receive attention

The choice of farm power and field machinery and their operation are discussed in relation to the many jobs on the farm Advice is given on their efficiency under varying conditions and how far their use results in the saving of manpower The section forms a good introduction to the financial aspects of farm power and machinery examined in Volume 4

Volume 4 (management and a general index), as a study of farm management, could stand as a book by itself An interesting description of British farming is followed by a study of management under such headings as "Getting Started in Farming", "Farm Capital", "Farm Labour", "Management for Profit", and "Management Controls and Farm Planning". Many examples are given to show how a combination of technical knowledge and managerial ability is necessary for successful farming. The sections on management controls and farm planning in particular should be of great value to the practising farmer, large or small, in the conduct of his business.

The index to the four volumes runs to 126 pages (a double column of references on each page) On test, I found a minimum of trouble in turning up any particular reference This is a valuable feature, for at the price of 345s. the book may be in use as a reference in libraries as often as it is taken down from private book-shelves for reading and for study. It is a text-book as well as a reference book, written in good style and easy to read

It is not possible to cater for the specialist farmer or grower, even in a work of this size For example, only one chapter is set aside for fruit and vegetable growing, but readers will find a number of references to other publications in which fuller information may be obtained and useful notes as to how to use the book

There are 1,350 illustrations in all. The plates in particular are excellent in quality The many diagrams are clear and helpful, but a number of the smaller reproduced photographs are less well chosen, are indistinct and might have been omitted J A McMILLAN

DAIRY BACTERIOLOGY

Bacteriology for Dairy Students

By Dr Alan Seaman Pp vii+202 (London Cleaver-Hume Press, Ltd, 1963) 25s

COMPARED with their counterparts in North America, students of agriculture and dairying in Britain are poorly served with suitable text-books in the important subject of microbiology The publication of Dr Seaman's small text-book is, therefore, a welcome addition Dr Seaman is a younger recruit to the ranks of lecturers in microbiology, and it would be easy, and discouraging, to be over-critical of this first edition The subject which he has attempted to cover in 194 pages is large and obviously many aspects could receive only brief mention There is, however, a lack of balance in the treatment given in different chapters

In the opening chapters the author discusses the morphology, isolation and nutrition of micro-organisms It is unfortunate that the writer has chosen to illustrate various aspects of microbial morphology by somewhat crude drawings There are available to-day numerous excellent electron photomicrographs (for which, presumably, permission to reproduce could have been obtained), illustrating such features as the bacterial cell-wall, cytoplasmic membrane, spores, flagella, etc Use of such photomicrographs would have considerably enhanced this section

In the chapter on bacterial nutrition the author has made a commendable attempt to simplify a complex subject, but he has been unable to avoid using a terminology which may be beyond the grasp of the reader for whom the book is designed

Before proceeding to a discussion of the microbiology of milk and milk products, Dr Seaman has included four chapters on bacterial destruction, immunology, identification and variation, systematics and a chapter on historical development The chapters on bacterial destruction and systematic bacteriology are good, but immunology should have received rather fuller treatment than six paragraphs, in view of its importance in disease control in the dairy herd and in bacterial classification While the chapter on historical development reflects Dr Seaman's wide reading on his subject, it is doubtful if, in a short elementary text-book of this type, so much space should have been devoted to this aspect

It is regrettable that the development of the advisory services in England and Wales since the Second World War has divorced the teaching departments of colleges and universities, from intimate contact with problems of the agricultural and dairy industries As a result the chapters on the microbiology of milk and milk products give an unfortunate impression of a theoretical or academic approach to the subject and little attempt has been made to emphasize the more important aspects The work concludes with a short chapter on the microbiology of water and sewage

The book is written in a simple direct style, but occasionally the language is somewhat ambiguous. Each chapter concludes with references for further reading, but in a number of instances more useful references might have been chosen Possibly a fuller bibliographical appendix would have served the purpose better. There are a number of errors which will no doubt be eliminated in later editions The book is described on the cover as a useful student revision text for examination purposes I myself hope that in future more detailed treatment can be given to the section on milk and milk products whereby the book could become a standard text-book for students of dairying

Despite these criticisms, the book has the great merit of providing in compact form a serviceable text in bacteriology for students of dairying and for workers in other food industries and can be recommended to such students It is hoped that the demand will be such as to encourage Dr Seaman to plan a more comprehensive second edition

D A McKENZIE

MEDICINAL CHEMISTRY

Pharmaceutical Chemistry

Plenary Lectures presented at the International Symposium on Pharmaceutical Chemistry held in Florence, 17–19 September 1962. (International Union of Pure and Applied Chemistry in conjunction with the Italian Society of Pharmaceutical Sciences) Pp v+207–492 (London Butterworth and Co (Publishers), Ltd, 1963) 60s

RESEARCH workers in the field of medicinal chemistry should not be deterred by the title, which might reasonably be understood to relate to the art of tablet preparation In fact it contains sixteen up-to-date (mid-1962) reviews, most of which will be found of value to those interested in the mode of action and discovery of drugs Dr Ing has made a most thoughtful contribution with his paper on the interaction of drugs and receptors He stresses the need to avoid acceptance of facile single explanations of drug action This cannot be over-emphasized Dr Ing nevertheless almost falls into the trap himself when he concludes that he is forced to think in terms of an exact fit between the drug and the receptor

Near miracles in the synthesis of medium-sized polypeptides are becoming almost a commonplace when a sufficient number of analogues of a polypeptide hormone can be synthesized to allow a study of structure-activity relationships to point approximately to the active centre Dr. Schwyzer's review of polypeptide hormones, for example, ACTH, oxytocin, angiotensin and kallidin, is masterly, but he, too, sounds a note of caution He appears to reject any suggestion that secondary or tertiary conformations, stable in the solid-state or in solution, are relevant to the biological activity of a polypeptide Dr Hofmann deals in greater detail with two of these hormones, ACTH and the melanocyte-expanding hormone, α-MSH He has established, from the studies of synthetic fragments and their analogues, that full activity resides in the first 20 of the 39 residues of the ACTH molecule and that the Lys Lys Arg Arg unit at positions 15–18, the free terminal α-amino group and all or some of the ε-amino groups of the lysine residues are important

The *cyclo*peptides of the phalloidin group obtained from poisonous mushrooms are substances from the unreal world of D-amino-acids, γ-hydroxy-α-amino-acids and *allo*-hydroxyproline and in which alanine alone is respectably normal Prof Wieland writes a fascinating account of these compounds Not least interesting are his discussion of the additional element of asymmetry introduced by their bicyclic structure and his disclosure that of the two forms of phalloidin, which are thus capable of existence, only one is biologically active

Prof Bovet points to the difficulties in the classification of psychotropic drugs Nevertheless, he provides a useful survey on the basis of the commonly accepted arrangement of the drugs into their various categories With many of the drugs, both stimulant and depressant, their clinical effects, pharmacological properties and, to some extent, molecular structures are in good accord An exception is imipramine, a drug of value in the treatment of endogenous depressions, but similar in structure and in several pharmacological properties to the phenothiazine tranquillizers

From a careful review of the properties of noradrenaline and its *N*-alkyl derivatives, Prof Pratesi asserts that the nature of the "cationic head" decides whether the biological effect be α- or β-adrenergic in character One instinctively feels that this is an over-simplification

Of the remaining contributions to this volume, some of which are purely chemical in nature and might therefore be felt to be rather out of place, specially worthy of mention is that by Prof Prelog on the siderochromes, a remarkable series of iron-containing naturally occurring compounds comprising both antibiotics and growth factors

Although the report of a widely based symposium must inevitably have something of a magazine flavour, this is nevertheless a valuable publication The reproduction of chemical formulæ is of a high standard, and it is with diffidence that the omission of a chlorine atom from each of the structures and prochlorperazine and chlorprothixene is noted. The photographic reproductions are of poor quality.

A F Crowther

A PIONEER IN STATE MEDICINE

Sir John Simon 1816–1904 and English Social Administration.

By Dr Royston Lambert Pp 669 (London Macgibbon and Kee, Ltd , 1963) 63*s* net

SIR JOHN SIMON, who well and truly laid the foundations of State Medicine in England and Wales and successfully combined this work with arduous duties as surgeon on the staff of St Thomas's Hospital, London, forms the subject of this authoritative biography to which Dr. Royston Lambert, Fellow of King's College, Cambridge, has devoted several years of painstaking research and investigation

John Simon, of French ancestry, was born in London on October 10, 1816 His father was a well-to-do stockbroker, and the son was brought up at Blackheath and educated at Dr C P Burney's school at Greenwich After a year's study of German and French in Rhenish Prussia he was apprenticed to J H Green, professor of surgery at the new King's College in London He learned surgery also at St Thomas's Hospital, then in the Borough Simon became a Member of the Royal College of Surgeons in 1838 and a Fellow in 1844 In 1840 he was made senior assistant-surgeon to the new King's College Hospital For a time he hesitated as to his vocation and studied Oriental languages, especially Persian, in the British Museum In 1842 he began physiological and anatomical research work on the thymus and thyroid glands which gained for him the Fellowship of the Royal Society in 1845 In 1847 he became the first lecturer in pathology at St Thomas's Hospital with surgical charge of forty beds By 1853 he was full surgeon and lectured, taught and operated at the hospital until 1876 He was president of the Royal College of Surgeons from 1878 until 1879 His distinction as a leading London surgeon is overshadowed by his achievements in public health

In 1848 he was appointed the first Medical Officer of Health of the City of London In that year he married Jane O'Meara, who was related to Barry E O'Meara, Napoleon's doctor at St Helena In that year also the first Public Health Act was passed, mainly as a result of the work of the medical pioneers, Southwood Smith, Arnott and Kay-Shuttleworth, set forth in Edwin Chadwick's famous *Report on the Sanitary Condition of the Labouring Population, 1842*, and the General Board of Health was established There was pressing need to deal with cholera and smallpox This Board was a triumvirate comprising the First Commissioner of Works, Chadwick and Lord Ashley (afterwards the Earl of Shaftesbury) It did useful work, but in 1854 it was reconstituted with Sir Benjamin Hall, M P , as president and a medical advisory committee, of which Simon was a member The autocratic Chadwick retired on a pension In 1855 Simon became the first medical officer of the Central Health Authority In that year medical officers of health had been appointed for all London districts They included Dr John Burdon Sanderson, Dr George Buchanan, Dr Odling, Dr Parry, Dr. Robert Barnes and Dr T Stevenson, all of whom advanced organized public health Three years later the General Board of Health was abolished Its medical duties, including vaccination, were transferred to the Privy Council and its Poor Law administration to the Home Office In spite of the important work Simon had done for public health, it was proposed to abolish the post of medical officer, and only through the private intervention of the Prince Consort was this retrograde step altered Thus the Privy Council obtained in its medical officer a man of great abilities and wisdom, burning with zeal beneath a calm demeanour to benefit mankind

John Simon was medical officer to the Privy Council from 1858 until 1871, and held a similar appointment under the new Local Government Board from 1871 until 1876. He was Crown Nominee of the General Medical Council, 1876–95, created C B on his retirement and advanced to K C B in 1887' He died from acute bronchitis in 1904

Simon's *Reports on the Sanitary Condition of the City of London* and his annual reports to the Privy Council and the Local Government Board are vivid pictures of social and sanitary evils, and describe the results of important medical researches by the scientists, Burdon Sanderson, Klein, J W Thudichum and others, together with comprehensive investigations into disease and its prevention He broadened the outlook on public health and the prevention of disease, demonstrated that they were an essential aspect of medicine, and was the director and disseminator of new and fundamental knowledge on these subjects

Admirably and fully, Dr Lambert describes Simon's conflicts with the poor law administrators, his frustrations, triumphs, honours and successes He gives an account of the joys and sorrows of his private life

Simon took an unduly pessimistic view of his great life-work, and one minor criticism of the biography is that Dr Lambert might have appreciated more fully the progress made in public health and epidemiology by the medical staff of the Local Government Board after Simon's retirement No less than four of Simon's successors in the post of medical officer had served under him as medical inspectors—Seaton, Buchanan, Thorne and Power They maintained the high traditions set forth by Simon, and preached the hygienic gospel with the fervour of disciples Simon himself stated, on p 414 of his *English Sanitary Institutions* (a historical and philosophical exposition of the history of English hygiene (1890)), that "the very conspicuous ability of the Department under Dr Buchanan as shown in the works and in the administrative advice which the Department supplies, is something of which English Medical Science has every reason to be proud" Afterwards, with the advice and energetic direction of another medical officer of the Board, Sir Arthur Newsholme, the first State social health services were founded

This is a valuable and excellent biography of one of England's greatest benefactors ARTHUR MACNALTY

TREATMENT OF CANCER

Biotherapy of Malignant Tumours

By N G Klyuyeva and G. I Roskin Translated from the Russian by J J Oliver Translation edited by Dr W J P Neish Pp ix+315+132 plates (London and New York, Pergamon Press, 1963) 80*s*

SINCE the early work of Coley at the end of the nineteenth century, reports have frequently appeared suggesting that the growth and development of malignant disease may be modified significantly by certain intercurrent infections or by various microbial products In recent times numerous low molecular weight products of microbiological origin have been isolated, chemically characterized and shown to have carcinostatic properties in animals and in man These relatively simple substances are, however, only part of a much broader spectrum of natural products to the therapeutic use of which the authors of this Russian work apply the general term "Biotherapy" Within this definition they include the use of such diverse agents as viable micro-organisms pathogenic or otherwise, microbial toxins, lysates and filtrates, oncolytic viruses, bacteriophage, yeast and fungal products and related materials of greater complexity and less well defined than the simple carcinostatic antibiotics The preliminary chapters of the book are devoted to brief reviews of these topics, reviews which in many instances are out-dated at the present time due to the six-year delay that has occurred between the publication of the original Russian text and the appearance of its translation

A primary object of this book is to present the experience that the authors and their clinical colleagues accrued over the period 1946–57 in the use of *Trypanosoma cruzi* extracts (cruzin) in the treatment of human cancers, particularly of the lip and breast This they have done by way of detailed case reports supplemented in the English translation by a selection of the results obtained more recently by French investigators utilizing similar *Trypanosomal* products The authors claim that complete regression can be induced in a proportion of human malignancies of various types and localization by prolonged administration of the extracts and that the treatment can be effective against primary, secondary and recurrent growths with no detectable pathological effect on the function or structure of normal tissues In cases of advanced inoperable disease, a transition to an operable condition may occasionally arise and likewise, where possible, surgical intervention during the course of treatment may accelerate the process of oncolysis

In subsequent chapters the authors describe the histological and cytological changes induced in malignant tissues of both human and animal origin under the influence of *T cruzi* extracts They describe a complex series of changes in the growth characteristics of tumour tissues in which the cells undergo progessive diminution in nuclear and nucleolar sizes, in mitotic activity, in ribonucleic acid content and protein synthesizing ability They believe that this process of 'normalization' and 'loss of aggressiveness' leads to the establishment of a new relationship between erstwhile malignant tissue and the normal body defence mechanisms Subject to the degree to which this process can be established, there follows a subsequent reaction in which the tumour region is invaded by cells of the reticulo-endothelial system leading to the destruction of tumour cells and their replacement by connective tissue as the final act of the carcinolytic process where this goes to completion

To the experimentalist, it is perhaps unfortunate that much of the earlier work leading to the present clinical investigation was published in a volume under the same title by the authors in 1946 and will not be readily available to Western investigators Nevertheless, the present observations are of interest to oncologists generally, particularly to those interested in the immunological aspects of cancer C L LEESE

TRENDS IN CANCER RESEARCH

Advances in Cancer Research

Vol 7. Edited by Prof Alexander Haddow and Sidney Weinhouse Pp ix+599 (New York Academic Press, Inc , London Academic Press, Inc. (London), Ltd , 1963) 128*s* 6*d*.

Canadian Cancer Conference

Proceedings of the Fifth Canadian Cancer Research Conference, Honey Harbour, Ontario, June 10–14, 1962 Edited by R W Begg, C P Leblond, R L. Noble, R J. Rossiter, R M Taylor and A C Wallace Pp xii+479 (New York Academic Press, Inc , London Academic Press Inc (London), Ltd , 1963) 100*s*.

THE present volume of *Advances in Cancer Research* contains seven chapters Beard discusses at considerable length our present knowledge of avian virus tumours, while Negroni, in a shorter chapter, presents information about rabbit and mouse tumour viruses as well as chicken tumour viruses and 'passenger' viruses, such as Riley's Agent In a long chapter, Brockmann deals with the mechanisms of resistance to anti-cancer agents, and questions of cross-resistance and collateral sensitivity in cancer chemotherapy are considered by Hutchinson. These chapters should be of considerable interest to workers directly concerned with virus tumours and chemotherapy

Of more general interest perhaps is an analysis by Kotin and Falk of the contribution of atmospheric factors in the pathogenesis of cancer of the lung Farber has surveyed the carcinogenic properties of ethionine and concludes that its activity may be due to its ability to produce altered DNA and/or RNA within the cell At last, according to Farber, there is some factual support for hypotheses that altered chromosomal or extrachromosomal nucleic acids play a key part in cancer production In a short chapter, Court Brown and Tough present the interesting work of their group in Edinburgh and of a group of workers in Philadelphia on the discovery of an abnormal chromosome Ph^1 in the marrow cells of patients with chronic myeloid leukæmia This specific chromosomal abnormality seems to be closely connected with the primary cause of the disease

Volume 5 of the *Canadian Cancer Conference* presents sets of papers devoted to cellular organization, cell interaction, immunology and chemotherapy The scope of this work is more general than that of the present volume of *Advances* and it will probably appeal to a wider audience of cancer workers

In the section on cellular organization, consideration is given to the mode of formation of polypeptide chains and to the comparative molecular biology of virus and cellular nucleic acids Autoradiographic investigations which deal with the sites of ribonucleic acid and protein synthesis in the cell are presented, and Ochoa discusses the nature of the genetic code Interaction of viral ribonucleic acid with mammalian cells is described, and Leslie deals with the control of gene expression which may be influenced by histones In an interesting discussion of chromosomes and carcinogenesis Stich concludes that a genetic concept of cancer must be given consideration, but the question still remains as to whether chromosomal changes in early neoplasia are cause or consequence of cancer formation

Ford and Clarke present evidence of clonal proliferation in primary reticular neoplasms (compare chapter by Court Brown and Tough in *Advances*)

Another topic of present interest in cancer research is that of cellular interactions This section deals with cellular interactions *in vitro*, describes an attempt to determine the time of mitosis in relation to ribonucleic acid and deoxyribonucleic synthesis and contains a discussion of a subcellular agent which produces ascites tumours in mice Daoust has contributed a chapter on pre-neoplastic liver parenchyma in which the idea again emerges that changes in nucleic acids may represent essential steps in carcinogenesis The section is concluded with a discussion of cell interactions by Weiss

Immunological aspects of cancer research are considered in seven chapters which deal with acquired immunological tolerance, auto-antibody formation, immunity to virus-induced tumours, etc , and, in the final section, chemotherapy, mechanisms of resistance to antimetabolites are described as well as cross-resistance In the final chapter Burchenal surveys the problems in clinical resistance

These two volumes from Academic Press are valuable additions to their respective series They seem to point the way to proliferation of research in the region of chromosomal and nucleic acid abnormalities which may prove to be at the very root of the carcinogenic process

W. J P Neish

DETECTING BLOOD DISORDERS

Atlas de Thrombodynamographie

Par G Marchal, M E Leroux et M Samama Pp 186 (Paris. Service de Propagande, Édition, Information, 1962) 60 N F

THE authors have produced a comprehensive atlas to illustrate the various changes they can measure in certain blood disorders using the thrombo-elastograph as designed by Dr Hartert

This ingenious piece of apparatus records on film the oscillations of a light beam reflected from a mirror mounted on a torsion wire On this wire is suspended a plunger which is immersed in whole blood or plasma contained in a stainless steel cuvette The latter oscillates regularly 5° on either side of the midline, and as the blood or plasma clots naturally or after the addition of calcium, the fibrin formed grips the plunger and so transmits the movements of the cuvette to the film The trace on the film records the time it takes for fibrin to appear first, the rate of fibrin formation and the gripping force of the fibrin formed

The details of the apparatus and the technique of operating are described in detail together with a useful description of artefacts that may be encountered The sequence of blood coagulation and the role of each plasma factor and platelet function are discussed and illustrated Tracings to show the different patterns obtained in certain disorders are also reproduced, namely, thrombocytopenia, abnormalities of platelet function, fibrinogen deficiency, plasma coagulation factor deficiencies, circulating anti-coagulants, prothrombin deficiency, fibrinolysis, hyper-coagulable states, and anticoagulant-induced defects. The authors illustrate and describe adaptation of the thrombo-elastograph technique to define specific coagulation defects and also the use of this machine in defining the changes in the blood following open-heart surgery

There is a very comprehensive text accompanying the illustrations, and the publishers are to be congratulated on printing this in French, English and German It is a pity that more care has not been taken with the English text, which is quaint and in parts frankly misleading due to the translation being too literal Further, the authors can be criticized for trying to convert this into an incomplete text-book on hæmorrhagic diseases

A shorter text confined specifically to the problems and value of thrombo-elastography would have been more acceptable An unusual feature is the mixture of advertisements with the bibliography, which is incomplete Authors' names in the text are not designated by the dates of their articles and some authorities quoted in the text are not mentioned in the list of references

Although there are these defects, this book should be read by those who have, or contemplate having, this instrument to assist in the problems of routine diagnosis or for research purposes A A Sharp

SOUTHERN NIGERIAN ANTHROPOMETRY

The Physical Anthropology of Southern Nigeria

A Biometric Study in Statistical Method By the late P. A Talbot and H Mulhall (Occasional publications of the Cambridge University Museum of Archæology and Ethnology) Pp xvi + 127 (Cambridge The University Press, 1962) 32*s* 6*d*

THIS monograph was made possible by the late Dr P Amaury Talbot, former resident, Civil Administration, Southern Nigeria, who for thirty years collected anthropometric and anthroposcopic data in this region which he administered After his death, Mrs Talbot put the data at the disposal of the Duckworth Laboratory of Physical Anthropology, Cambridge, the director of which, Dr J C Trevor, further sought the statistical help of Dr Mulhall It has been the latter author's task to reduce the mass of undigested data to the meticulous and precise analysis seen in this published work

The first chapter is understandably devoted to previous regional classification of Southern Nigeria and the relevant population numbers (although, incidentally, the sample sizes utilized in the analysis are not relative to the population totals for the regional groups) This is followed by a diversion into statistical procedures, Chapters 2–7, which are meant as a general introduction to anthropometric statistics, as well as to the final detailed analysis Mulhall is to be congratulated on the way in which he carefully takes us through the statistical methods, problems of sampling, probability and theoretical distribution models, statistical relationships, and problems of group divergence

The original contribution of this book is in its analysis of the measurements of nearly 7,000 inhabitants from more than 50 groups Although there is some debate as to the relative merits of an exhaustive analysis of a few dimensions (12 in this case), as opposed to the less-detailed analysis of more measurements, one is left wishing that a few more had been available

This analysis of the Nigerian data forms the last two chapters First, the body measurements are considered, culminating in the computation of D^2 values for all the regional groups This is followed by a consideration of skin colour (alas, Talbot was recording in the days before colorimetric scales and the portable spectrophotometer were in use) This pigmentary information is a useful addition to the study, and in part corroborates and in part challenges the conclusions drawn from the metrical investigation Finally, there is a reclassification of the groups analysed

As Trevor and Mulhall intended, this is a survey of statistical methods in some aspects of physical anthropology, combined with a detailed analysis of data from one area of West Africa—a somewhat unique combination

A final chapter reviewing the results of this analysis in relation to other human biological findings from Nigeria (serological, dermatoglyphic, etc) would have been a very useful addition Also, perhaps some mention could have been made of possible environmental differences between the groups (climatic and especially nutritional) As certain abnormal hæmoglobin deficiency traits, relatively common in Nigeria, can also affect body dimensions, it would have been interesting to have these brought into a general discussion as well.

In view of the fact that photographs were taken by Talbot (presumably in standard positions), it is a pity that a representative sample was not included Some may consider the lack of an index a serious deficiency, although the monograph is not so long that this becomes a cumbersome problem D. R Brothwell

AMATEUR SOCIOLOGIZING

Conference Across a Continent

(An account of H R H the Duke of Edinburgh's Second Commonwealth Study Conference on the Human Consequences of the Changing Industrial Environment in the Commonwealth and Empire, Canada, May 13–June 6, 1962 Pp. 521 + 104 photographs (Toronto Macmillan of Canada, London Macmillan and Co , Ltd , 1963) 40*s* net

In 1956 a study conference, inspired by H R H the Duke of Edinburgh, was held at Oxford on the human problems of industrial communities within the Commonwealth and Empire This proved to be so successful, especially to the Canadians who attended it, that a second study conference was organized in Canada during May 13–June 6, 1962 Both conferences have taken the same general form, documents and papers were circulated beforehand, lectures were given to the assembled participants, discussion groups were formed to consider the material presented, study tours were arranged to different kinds of industrial communities in the country where the conference was held, and the conference reassembled eventually to discuss experiences and the reports of the several study groups There can be scarcely any doubt but that on both occasions the participants were worked hard, enjoyed themselves enormously, and returned home feeling that the experience had broadened their outlook and that they had learned something of importance. It is open to question, however, whether anything beyond the emotional and intellectual stimulation of the participants is achieved by programmes of this nature

From the point of view of one who attended neither conference and has only the published reports of the proceedings to judge them by, the Canadian Study Conference must be reckoned inferior to that held in Britain Whereas in 1956 twenty-four 'background' papers have been reprinted[1], many of them of excellent quality, only six appear to have been circulated on this later occasion in *Conference Across a Continent*, and none of these is outstanding Of course, Canada is a very large country, and the conference was regularly on the move, from the opening in Montreal to the closure in Vancouver, so that apparently more time was devoted to the study tours than was the case previously, but surely this is not a good reason for failing to circulate and to reprint investigations into the human consequences of the changing industrial environment of Canada which could make a contribution to our knowledge of these matters

The sociologist, indeed, might well complain that the emphasis on touring 'study groups' is misplaced What real knowledge of a very complex situation can such groups obtain in so short a time? The report of the Canadian study tours claims that the groups were capable of generating a "dynamic" that was "almost overwhelming" Conceivably, many of those who were caught up in this "dynamic" underwent a change in their manner of looking at the social environment But there is always a serious danger in such a circumstance that the individual concerned will mistake this experience for understanding As the report itself acknowledges, "the social process known as group dynamics can lead to a construction of worth-while design or to a pooling of ignorance" (p 69) Only if the group is trained beforehand in the techniques of social enquiry is it likely that it will make reasonably precise and objective discoveries There is no evidence on this occasion that the enthusiasm of the amateur was guided away from hasty generalization through the employment of the expert methodology of the professional

Consider, for example, a question listed in the discussion sheets which were provided as part of the study-tour programme as a guide to what might be 'usefully asked' of local informants—"Do many in the community think that some families prefer to draw unemployment insurance rather than work?" (p 478) This is apparently a quantitative question, involving at least two numerical variables, for which in principle there are precise answers. Were the conference members told about how such answers are obtained in a modern social survey? Were they made aware that a sampling procedure, utilizing statistics and at least a rudimentary notion of the mathematical theory of probability, is used, and is to be preferred, for good logical reasons, to the casual guesses of the man-in-the-street who believes he knows what his friends and neighbours think? Were they given any instruction at all which could help them to distinguish between the prejudices of one informant and the empirically based knowledge of another? Apparently not, for the list of questions themselves were regarded by some of the conference members as a strait jacket As one of them put it, "if these questionnaires had not been prepared, the group reports might have had more individuality and thrown light on subjects which may have been omitted" (p 76) No one seems to have stopped to ask him the crucial question—"But what sort of light?"

It cannot be too often reiterated that the study of the human consequences and social implications of technical and industrial change is beset by logical pitfalls and methodological difficulties of considerable complexity A survey of research in this area, presented to a conference sponsored by the International Social Science Council in 1959[2] pointed out that far too often expert social historians, economists, sociologists, social psychologists and social anthropologists leap to conclusions which are, as a matter of fact, not supported by the data in their investigations If this is so of the professional, how much more likely it is that the untrained amateur will be led astray by his enthusiastic desire for quick results! This should not be taken to imply that there is no place for the amateur in contemporary social science Of course there is, just as there is still some place for the amateur in astronomy, and even to some extent in nuclear physics But the part to be played by such people is clearly dwindling, and they can make no contribution except in so far as their enquiries can be fitted into the framework of established theory in these subjects

Nor should it be assumed that conferences such as these, bringing managers and trade unionists together from all parts of the Commonwealth and Empire, are valueless A few weeks of extensive travel and intensive intellectual and emotional stimulation make an impact on the participants which is not quickly eradicated Useful contacts and firm friendships are established, and the published reports serve not only to awaken recollections of a collective experience but also as a directory for future communications Such conferences also act to freshen psychologically people who have become a little dull in their pursuit of the everyday, industrial routine, and if they learn in the process that people accustomed to very different ways of life can nevertheless get along well together, so much is to the good The pity is that one of the aims of such conferences—the Duke of Edinburgh's idea, "to give them a chance to take a broad view of their responsibilities during the process of industrialization or during the further development of existing industrial communities" (p 21)—should have become clouded by failure to teach them the lesson of contemporary social science, namely, that the painstakingly acquired tools of social investigation are better utilized for this purpose than the traditional rule of thumb.

J. A Banks

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[1] *H R H the Duke of Edinburgh's Study Conference on the Human Problems of Industrial Communities within the Commonwealth and Empire*, 2 (London Oxford University Press, 1957)

[2] *Social Implications of Technological Change* (Paris International Social Science Council, 1962)

AFTER THE SCIENTIFIC REVOLUTION

Inventing the Future

By Professor Dennis Gabor Pp 231 (London Secker and Warburg, Ltd , 1963) 21*s* net

WE should have realized a long time ago that it is really P G Wodehouse's Bertie Wooster who is riding the wave of the future, and that barring accidents the widely acclaimed disappearance of the Leisure Class looks like being followed, after only a very few years, by an overwhelming comeback The Age of Plenty is already dawning and, although at its first appearance, we are tempted to greet it as cheerfully as people who have spent a long cold night out of doors see the first rays of the Sun, we are already beginning to realize that by mid-day it's going to be a real scorcher No need to work, no diseases, no need to read, travel from any part of the globe to any other within a few hours—how in the world are we going to fill our time ?

Of course, in those old best-sellers whenever the sheer vacuity of Bertie Wooster's perennial leisure had led him into some particularly disturbing piece of mischief, he turned for advice to his ever-capable and far-sighted manservant Jeeves Dr Gabor has set himself nothing less than the task of playing Jeeves—and this time one who has first-rate scientific expertise at his fingertips—to the whole leisured world of the future An extraordinarily good job he makes of it This is a short book, only 190 pages of text and about another 30 of notes There are very few scientists, and practically no reasonably educated non-scientists, who would not get great profit from devoting to its reading the few hours which is all it demands On almost every page there is something which, if not always entirely novel, is put into a way which is fresh and thought-provoking and often witty

A great number of these points, of course, call aloud for discussion The book is so compressed that it is impossible to summarize all the numerous arguments it contains, or to indicate particular points of agreement or doubt In the broadest possible outline the main framework is made by the three major problems which mankind now faces of nuclear annihilation, of being swamped by a flood of babies, and of being bored to death About the first two Dr Gabor shows a robust, and reasonably convincing, optimism The equilibrium in the balance of nuclear power is, as he points out, a remarkably stable one as between the United States and the U S S R , and probably strong enough to absorb the potential threat of China long enough for that country's crusading ardour to die down to a manageable level The population explosion is perhaps more challenging, but technological advance in the production of foodstuffs is, or could be, rapid enough to give us a certain breathing space From a moderately long-term point of view there are clearly several ways in which the human birth-rate could be controlled, many of them involving social influences on individual behaviour which we, our generation, might find difficult to stomach but which our successors may easily take in their stride There is, after all, an extraordinary capacity for homœostasis in almost all biological systems, and if *Homo sapiens* succeeded in breeding itself out of a livelihood this is something almost no other species has been able to accomplish.

It is when he turns to the problems of an age of leisure and plenty that Dr Gabor becomes most stimulating I can perhaps best indicate the character of the book by a few quotations "Between 1947 and the end of 1961 the U S work force had expanded from 60 million to 71 million, but the total number of production line workers had decreased by 7 per cent . They have a good conscience, working so hard at their telephones and typewriters or rushing from one place to the other for urgent conferences This is just the wonderful thing in this modern system of waste that it makes people believe that they are useful" (pp 109, 110) " as C E M Joad said 'Work is the only occupation yet invented which mankind has been able to endure in any but the smallest possible doses' The truth is rather that in a country in which the economic game is played passionately, this threatens to produce a dull conformity, the very opposite of freedom" (p 135) "Stagnation, at whatever level remains an ugly word to intellectuals who have grown up in an epoch of material progress" (p 135) "In the first years of childhood probably not much harshness will be needed to educate citizens for the age of leisure but there can be little doubt that hardship will be a necessary component in the education of the adolescent" (p 138) "Western object-mindedness and work addiction is one extreme, Eastern quietism and introversion is another . . We must not give up hope that we shall be able to assimilate something from the East which will be better for the restless Western mind than alcohol or opium" (pp 140, 141) "There is no limitation to the number of those who can enjoy science other than their own ability to comprehend it My idea of the amateur scientist is that he shall be a *knower* not a *doer*" (p 144) "Certainly, the masculine valuation of strife and progress has created our civilization, it will just as certainly destroy it if it goes on much longer unmitigated by feminine principle" (p 149)

This last quotation brings me to the one point of criticism I should like to make of this book It is, in some rather indefinable way, a peculiarly impersonal work, rather like an engineer's rough drawings trying out a series of ideas for some major design I find it difficult to pin down exactly from where the impression arises; possibly from the fact that Dr Gabor scarcely mentions religion or any activities related to it, possibly again from the fact that although he values something that he refers to as a "feminine principle", he does not mention sexual activity and indeed scarcely refers to inter-personal relations, although surely religion, sex and personal relations have always in the past filled the greater part of the lives of those few individuals who have enjoyed ample leisure However, few people will—and I am sure the author would not expect them to—read this book in order to be told exactly how to solve the problems of the future What they will find in it is a most provocative list of problems with which mankind is likely to be confronted, and a large number of positive and buoyant suggestions for some of the things we might do about them

C H Waddington

THE FLOODTIDE OF MANKIND

Our Crowded Planet

Essays on the Pressures of Population Edited by Fairfield Osborn (Sponsored by The Conservation Foundation.) Pp 192 (London George Allen and Unwin, Ltd , 1963) 21*s* net

THIS volume, sponsored by the Conservation Foundation, comprises a series of twenty-one specially commissioned essays from authors of international fame long associated in their writings with varied aspects of the population problem In a brief introduction by Fairfield Osborn, we are reminded that the "inordinately rapid increase of populations . . faces everybody everywhere" The essays are all short, ranging from a little more than a thousand words to about five thousand, and one could scarcely expect anything new to be said in so restricted a space. There is, obviously unavoidably, a great deal of repetition most essays give figures for world population and rates of increase which vary considerably, and all are out of date according to the present issue of the *Demographic Yearbook of United Nations* Internal evidence suggests that some of the essays were written several years ago when the world's population was, according to p. 183, 2 75 'billion' [U S billion], increasing by nearly 50 million a year at more than 1 5 per cent That the present-day figures are 3 2 billion, 63 million a year and more than 1 9 per cent only serves to emphasize how we are, in fact, being overtaken by events, and gives point to the comment of Sir Charles Darwin (there is no indication of his death) that we are living "in what is a fantastically abnormal period of history and one which must inevitably end soon . the end to such things as affluent societies and welfare states, and our descendants will look back to the nineteenth and twentieth centuries as a golden age of easy life"

The essays are arranged in five groups Five in the first deal with population pressures on man's natural environment, and it is not difficult to guess the lines taken by Marston Bates, Darwin, Paul Sears, Frederick Osborn and Fraser Darling Marston Bates, with others later in the book, reminds us that the human animal evolved as part of the world biological system, he has become an "ecological dominant", and there must inevitably emerge some "wise principle of co-existence between man and nature" to end "his present cancer-like spree of reproduction" We are constantly reminded in these essays, indirectly rather than of purpose, of the isolation of the United States where the 'modern commoner thinks nothing' of an escort of a thousand slaves (that is, a motor-car developing 100 horse-power) (Sears), but where "many social workers to-day would lose their jobs if they were caught giving contraceptive advice" (Osborn) It is Osborn who brings out the advantages of "assortative mating" consequent on greater mobility, otherwise the inevitable trend of mankind using modern medical skills is the reverse of selective breeding among animals Osborn concludes that "public health authorities will some day accept responsibility for genetic defect as they now accept responsibility for controlling contagious disease"

Part 2 has seven essays dealing with population pressures on economic and political trends by Eugene Black, Earle Rauber, Lord Boyd Orr, Sir Solly Zuckerman, H S Commager, Grenville Clark and Arnold Toynbee In each case the author puts forward views already associated with him. Eugene Black tends to highlight the differences between the economist's and the scientist's basic thinking, though he too concludes, "unless population growth can be restrained we may have to abandon for this generation our hopes of economic progress in the crowded lands of Asia and the Middle East" At the same time he is not convinced that population growth will eventually outrun the development of the world's resources Lord Boyd Orr has been converted to the optimistic belief that "the present explosion of population is merely the birth throes of a new age of stabilized population in a world of peace and plenty" To this Sir Solly Zuckerman adds, "the problem of population pressure becomes simply a problem of politics and leadership", and Arnold Toynbee looks to a new and permanent United Nations Relief and Rehabilitation Agency to achieve world unity for production and distribution of food

Four important essays, making up Part 3, supply a valuable corrective to world generalizations by presenting case studies of India (M C Chagla), Japan (Chikao Honda) and Latin America (E Beltran) with the veteran W C Lowdermilk drawing on his wide experience in the less-developed lands Independent India in 1947 planned for a population in 1961 of 430,000,000, actually it reached 438,000,000—"a dark and dismal future for millions, bitterness and frustration, poverty and unemployment, political discontent and unrest" Voluntary sterilization, described as simple, painless and free, is advocated The miracle of Japan's population change with the 1957 natural increase of 0 887 per cent, the lowest ever recorded in Japan's modern demography, is attributed to conscious planned parenthood, but there is some doubt as to its permanence

The two essays on the population problem and religion are unsatisfactory. Bishop J A Pike of California, as a Protestant, attacks the 'official' Roman Catholic attitude which Father Robert Gannon, former President of Fordham, calls a "monolithic illusion" It is left to Bates to comment that inoculation is as 'unnatural' as contraception Finally Joseph Wood Krutch, André Maurois and Julian Huxley are left to consider population pressures on morals and ethics André Maurois is in no doubt of the terrible retrogression since 1914 basically due to the wild proliferation of men bringing forth unintelligent generations, and calls for a reversion of the tide. The history of mankind records examples of such great reversions. Christianity is one Julian Huxley looks to "the vision of evolutionary humanism, essentially religious", and we should devote ourselves with truly religious devotion to the cause.

A well-selected list of general and technical readings concludes this interesting collection, which, if it contains nothing new, summarizes in convenient and readable form the considered views of twenty-two leading thinkers It is most unfortunate that the publishers should print the names of sixteen of these on the dust cover, unhappily leaving out six authors, all non-British, who in fact have the most valuable contributions to make In the cause of international understanding this dust cover should be withdrawn at once

L DUDLEY STAMP

THE UNITY OF LIFE

The Unseen World

By Prof René Dubos Pp xi+112 (New York The Rockefeller Institute Press, London Oxford University Press, 1962) 35s net

In a foreword to this book, Detlev W Bronk explains that the Rockefeller Institute has decided to initiate an annual series of lectures similar to the famous Christmas Lectures at the Royal Institution This is welcome news, because there can be few establishments in the world with such a distinguished staff of scientists Dr Dubos is a microbiologist of international repute, and so well has he succeeded in presenting his subject that it is bound to fire off the imaginations of many young people, as yet undecided on their future careers The remarkable thing about this beautifully produced book is that it touches on so many interesting aspects of biology in so short a space. If it manages to send a middle-aged and somewhat blasé reviewer scurrying over to the library to find out more about lichens, cheeses, orchid seeds, the colour of tulips and the production of hæmoglobin in root nodules, it will surely capture many a young reader

Throughout the book one senses that Dr Dubos has a deep feeling about the fundamental similarity of different forms of life Many biochemical processes in micro-organisms are identical with those in higher animals and are often easier to investigate The electron microscope is beginning to show that there are also fundamental structural similarities The mitochondria of yeasts are not very different in form from those found in higher plants protozoa and man Even where apparent differences exist we now realize that these are often only modifications of a basic design The buildings may look different, but the bricks are similar. The association of enzyme system with various membranous components of the cell is well established, and it may be that mutations and adaptations in response to unusual environments are accompanied by ultrastructural changes. If so, micro-organisms would seem to be the best experimental material for this type of investigation. One must agree wholeheartedly with Dubos's statement "The biophysical and biochemical unity of life is certainly one of the largest contributions of modern science to philosophical thought".

In so short a book it is inevitable that some topics are discussed inadequately. There is an occasional loose statement, as, for example, in discussing Pasteur's work ". . . he found that one solution deviated polarized light to the right and the other to the left", but one sympathizes with the difficulty of trying to explain polarized light to a general audience. The section on antibiotics is very brief, and the birth dates of Florey and Chain are unfortunately interchanged. It is perhaps a pity that space could not be found for the contributions of John Tyndall, himself closely associated with the Royal Institution. His *Essays on the Floating Matter of the Air* published in 1881 are an excellent example of clear thinking and he must surely rank among the great pioneers of biophysics and microbiology. Not only did he observe the antagonism between some moulds and bacteria but he stressed the importance of species differences. In describing the contamination of bacterial cultures by *Penicillia* he writes "In every case where the mould was thick and coherent the Bacteria died, or became dormant, and fell to the bottom as a sediment . . . The turnip-infusion, after developing in the first instance its myriadfold Bacterial life, frequently contracts mould, which stifles the Bacteria and clears the liquid all the way between the sediment and the scum . . . The Bacteria which manufacture a green pigment appear to be uniformly victorious in their fight with the Penicillium". So near, yet sixty years too far. Dubos's book makes us wonder what great discoveries are being missed to-day.

R. Barer

ESSAYS OF GEORGE SARTON

Sarton on the History of Science

Essays by George Sarton. Selected and edited by Dorothy Stimson. Pp. xvi+383. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1962.) 80s. net.

GEORGE SARTON, first professor of the history of science at Harvard, died in 1956. For many years he had been one of the leading authorities on his subject, and the editor of this collection of his essays claims that for forty years his name was "practically synonymous with the history of science". So far as the United States is concerned, this statement is unlikely to be challenged.

Sarton was a man of great erudition, an exact and exacting scholar, to which accomplishments he coupled immense energy. He is probably best known as the founder of the international journal, *Isis*, devoted to the history of science, which he edited for some forty years, and to which he regularly contributed a valuable critical bibliography on the history of science and related subjects. He was, however, a prolific writer and, apart from his editorial work, was the author of a number of books and of numerous essays and articles on various aspects of his subject. Before his death, Sarton drew up a list of those among the latter that he considered worth re-publishing. All but three of the twenty-three articles included in *Sarton on the History of Science* were included in this list. Thirteen were published originally in *Isis*. The remainder appeared in a variety of publications for the most part not easily accessible. The selection has been made, and the articles edited, at the request of Sarton's literary executors, by Prof. Dorothy Stimson, whose name may be familiar to British readers as the author of *Scientists and Amateurs*, a short history of the Royal Society.

The essays have been arranged in three groups to illustrate the varying aspects of Sarton's work. Those in the first group emphasize his activities as propagandist for the study of the history of science, and among them is the last important article he wrote, an essay on the history of science contributed to the *Encyclopedia Americana* in 1956. Those in the second group illustrate his work as a historian of science proper. They are mainly biographical, and the subjects chosen range from an essay on Avicenna to one on the work of the twentieth-century scientist, H. C. J. Mosely. A long article on Leonardo da Vinci, not hitherto published in full, is included. The third group has been selected to illustrate Sarton's methods and procedure as a writer and editor. In this section there are articles on such diverse subjects as English as an international language, notes on reviewing learned books, the study of early scientific text-books, and the history of twentieth-century science.

Apart from the value of some of these essays as source material, for example, that on the sixteenth-century Belgian scientist Simon Stevin, this book forms a readable introduction to Sarton's work as a whole. As such it ought to be welcomed by British students of the history of science to whom his contributions to the subject are possibly not as well or, at least, as fully known as they deserve to be.

HOMAGE TO GEORGE PÓLYA

Studies in Mathematical Analysis and Related Topics

Essays in Honor of George Pólya. (Stanford Studies in Mathematics and Statistics, No. 4.) Edited by Gabor Szego, Charles Loewner, Stefan Bergman, Menahem Max Schiffer, Jerzy Neyman, David Gilbarg, Herbert Solomon. Pp. xxi+447. (London: Oxford University Press, 1962.) 80s.

PAPERS from sixty notable mathematicians testify to the respect and, more, to the affection in which Prof. Pólya is held by the contemporary mathematical world. His high professional competence is shown by an output of more than 200 research memoirs, the lucidity of his exposition is known to his readers as well as to those fortunate enough to have heard him lecture. But this is not all; every teacher of mathematics, every young research worker in the field of analysis, can find inspiration and assistance in those of his books in which he has dealt, explicitly or implicitly, with the modes of mathematical discovery and creation. In the middle 1920's, Pólya (in collaboration with Szego), in the two volumes of *Aufgaben und Lehrsatze aus der Analysis*, offered the young analyst a remarkable opportunity of sharpening the tools of his trade by studying the grouped sets of problems (with solutions) on most topics in analysis from the fundamentals of limit theory to complex variable and the analytic theory of numbers; here the novice could see how general theorems could be applied and sharpened in applications to special problems.

After the Second World War, Pólya began writing on the motivation and presentation of mathematical discovery. He emphasized the part played by intuition (perhaps better described as controlled guess-work), a part as important as that played by strict logical inference. Great mathematicians such as Gauss have delighted in publishing their discoveries in concise synthetic form; in recent years, research memoirs have been compressed to extremes in response to editorial demands for economy of space. The young student may marvel at the results, but he will not easily see how they were arrived at. In his books *How to Solve It* (Princeton, 1946) and *Induction and Analogy in Mathematics* (Oxford, 1954), Pólya shows by an opulent variety of examples how a mixture of inductive and analogical argument can lead not only to the conjectural theorem but also, often enough, to the lines on which a deductive proof may be founded. Take, as an example, Carleman's theorem, that if each a_n is positive

$$\Sigma(a_1a_2 \ldots a_n)^{1/n} < e\,\Sigma a_n$$

where, on the right, e is the best possible constant. Pólya's proof of this in the *Proc. London Math. Soc.* (24; 1926) may strike the casual reader as a *tour de force*, but the author's own dissection of his proof shows how naturally the argument flows from a plausible line of approach. Much of the work is within the limits of a good school course, and teachers who make use of his ideas need no longer fear that reluctant intellectual assent ('You've proved it, but I still don't believe it'), which is so often their meagre reward. Pólya has shown us how to convince our students that mathematics is as exciting, as imaginative, as æsthetically satisfying as any other field of intelligent enquiry. Britain needs more mathematicians; it will not get them by stressing utilitarian values, but it may get them by presenting Pólya's view of mathematics as a superb intellectual adventure.

MATHEMATICS IN THE U.S.S.R.

Recent Soviet Contributions to Mathematics

Edited by Dr. J. P. LaSalle and Dr. S. Lefschetz. Pp. viii + 324. (New York and London: The Macmillan Company, a Division of the Crowell-Collier Publishing Company, 1962.) 8.75 dollars, 66*s*.

THOSE who cannot make a direct study of Russian mathematics, because of linguistic deficiency, may find difficulty in assessing the true worth of its immense productivity. To help them, a panel of well-known American mathematicians has devoted a year to an intensive survey of Russian mathematical output, principally for the period 1957–60, and has now reported on some major topics in pure mathematics. The main headings are algebra, control and stability, functional analysis, numerical analysis, ordinary and partial differential equations, probability and information theory, and topology.

The editors have themselves added to the special reports a 'general appraisal'. The conclusions they draw are well worth consideration. While they regard the United States and the U.S.S.R. as world leaders of about equal strength, they believe that the U.S.S.R. can move more rapidly than the United States in the practical applications of mathematical theory, and may possibly surpass the United States in such matters as control theory, numerical analysis, and differential equations, perhaps because of the intense drive by the U.S.S.R. towards automation in industrial production. They emphasize that while the gap in the United States between mathematics and its applications is widening, mathematicians in the U.S.S.R. are not neatly segregated into the 'pure' and 'applied' categories; there, mathematicians, scientists and engineers work closely together, so that, for example, there are many engineers who understand and exploit the most recent of advanced mathematical techniques. Mathematicians themselves enjoy a high prestige and status. Excellent books are available at very low prices, the large number of mathematical periodicals means that room can be found for long expository articles as well as for the highly condensed specialist-to-specialist research memoirs. It is this breadth of view, coupled with motivated specialization, which accounts for the fact that in control theory the quality of work is as good as that in the United States while the quantity and number of participants is much greater, and that in the field of differential equations the lead is now indisputably with U.S.S.R. On the other hand, this may explain why certain topics are under-developed; Lefschetz, in his own report on topology, suggests that the reason why there has been no significant contribution to modern algebraic geometry in the U.S.S.R. is that the subject is self-contained and has no direct influence on applications to applied problems.

This appraisal gives the impression that the Russians have solved the problem of combining breadth of view with sharpness of specialist penetration in a mode which is no longer common in the United States or in Britain. How has this been done? Is Britain's examination system partly to blame? The old Tripos, with its many defects, at least knew no distinction between pure and applied mathematics. J. J.'s first paper was on the theory of numbers, A. R. Forsyth's on hydrodynamics. One thing seems certain: it is not enough for academics, industrialists or politicians in Britain to talk glibly about the need for more mathematicians, it is necessary to look to the kind of mathematicians that are wanted. Britain must have expert pure mathematicians who nevertheless do not despise the stimulus of application, and applied mathematicians who can exploit the most abstract and general theories of the pure mathematician.

T. A. BROADBENT

ATTITUDES IN INDUSTRY

Industrial Participation

Theory and Practice—a Case Study. By J. A. Banks. (Social Research Series.) Pp. 150. (Liverpool: The University Press, 1963.) 25*s*. net.

THE problem of getting employees at all levels in a business organization to participate in its smooth running is one which is frequently posed and seldom solved. In a case study of a factory employing some 500 male manual workers, J. A. Banks has attempted to discover the forces which influence participation in different departments of the factory, among employees with varying periods of service in the particular company, and on different shifts. As a measure of participation he uses the desire to seek promotion to the rank of supervisor, the willingness to seek responsibility by serving as a shop steward, and the extent to which employees were not only prepared but also actually participated in the company's formal joint consultation machinery.

The conclusions are not unexpected and confirm earlier studies of a similar nature. What matters most in influencing the degree to which employees are prepared to contribute to an organization's well-being is not the mechanics of joint consultation, not its technical efficiency, and not necessarily its social groupings. It is, quite simply, the inter-personal factors which continue to make an employee feel he is or is not wanted.

This is the essence of a study which takes a long time in the telling and which, throughout, bears an air of unreality. This is mainly because the statistical analyses are too small to command anything like the general import which Banks indicates and because of his excessive preoccupation with them. The author's lack of awareness of what is happening in industry is indicated in a reference to promotion. "Although there are signs," he writes, "that at the management level recruitment from outside is gradually replacing promotion from within, at the supervisory grade we still largely expect to recruit by promotion from the shop floor". This reads more like a lament from a charge-hand than an observation by a skilled industrial investigator. Does it need Lord Robbins, or the report of the Committee on Higher Education of which he was chairman, to persuade the author that the graduate recruit differs only from the secondary modern recruit in that his period of formal education lasts longer and that, until they start work, both must be considered as outsiders? Moreover, that, increasingly, supervisory jobs are being filled by graduates and their equivalents as part of their development on the way up?

T. H. HAWKINS

SHORT REVIEWS

Logic, Computing Machines, and Automation

By Alice Mary Hilton. Pp. xxi+427. (Washington, D.C.: Spartan Books; London: Cleaver-Hume Press, Ltd., 1963.) 72*s*.

MATHEMATICAL logic is an essential component in the basic theory of computors and automatic controllers. However, to-day these subjects are in their early stages and the most erudite specialist would find it impossible to write a book explaining adequately all the connexions between logic and these applications. It is, therefore, a very difficult undertaking to give some notion of these connexions to the general reader, which is what *Logic, Computing Machines, and Automation* sets out to do. The book is a succession of summaries of mathematical logic, some parts of mathematics, the operation and programming of computers, and a small section on automation. Parts of these summaries are quite good, but the real problem is to link them together so as to give the reader a coherent picture. Little attempt is made at this linking; for example, the reader has two pages in which to struggle with the ideas of material implication, conditional implication, and strict implication; if he succeeds in mastering them he then finds that they lead nowhere. A good exposition for the general reader remains to be written; when it comes it will be much less ambitious, and much more attuned to the way that people absorb difficult technical material.

J. J. FLORENTIN

Electrical Instruments and Measurements

By W. Alexander. Second edition. (Cleaver-Hume Electrical Series, No. 5.) Pp. 350. (London: Cleaver-Hume Press, Ltd., 1962.) 25*s*. net.

THIS book was first published in 1951 and has now been revised for the second edition. It is primarily intended for the use of technicians, and the small size of the pages makes it easy to carry about in one's pocket. The only obvious disadvantage of the small page size is that some of the detail of the diagrams and photographs is difficult to see clearly.

The book opens with a very readable chapter on fundamental principles and this is followed with simple descriptions of ammeters, voltmeters, wattmeters, power-factor meters, frequency meters, quantity meters and energy meters. The nature of the quantities measured is described with a minimum of mathematics and plentiful diagrams, and there are useful chapters on the testing of instruments and circuits. A few test questions and numerical answers are included. The material is generally well chosen, but the measurement of three-phase volt-amperes and power factor is inadequately covered and the possibility of obtaining widely different results according to the definition of volt-amperes does not appear to be mentioned.

Instrument transformers are discussed as a means of extending the range of voltage and current measurement, but the testing of these devices is not described. The only method of compensating errors given is "turns compensation" for ratio, and on p. 87 the author denies the existence of methods for compensating phase error, which is astonishing.

Apart from these minor blemishes the book is excellent and should prove very popular.

A. H. M. ARNOLD

Electronic Properties of Diamonds

By Prof. F. C. Champion. Pp. vii+132. (London: Butterworth and Co. (Publishers), Ltd., 1963.) 25*s*.

THIS scholarly small monograph on the present state of knowledge about the electronic properties of the diamond crystal is most welcome. There are two important research schools concerned with the electro-optical properties of diamonds, that of Prof. Ditchburn at Reading, and that of Prof. Champion himself at London, and as might be expected this account is authoritative. Diamond, despite it chemical simplicity, is a crystal which seems to exhibit an enormous range of physical structural complexities, and from the bewildering mass of published data the author has produced a readable summary which will be invaluable to all scientists (and there are many) engaged on the investigation of the physical properties of diamond.

The book is divided into two roughly equal parts. The first part consists of eight brief chapters which review a formidable amount of already reported experimental material concerning: (*a*) optical properties of theoretically perfect and of real diamonds, (*b*) electrical and photo-conductivity, (*c*) diamonds as scintillation and as conduction counters for radiations, (*d*) radiation damage to diamond; (*e*) semi-conducting diamonds, (*f*) electro-luminescence.

The second part is a novel contribution for a monograph of this size, since it is in effect an original attempt to explain a considerable number of the physical properties of diamond in terms of structural defects and energy-levels. Prof. Champion achieves a fair measure of success with his theory and accounts for numerous specific properties in some detail. Undoubtedly a stimulating section, much of which seems very sound to me.

A very useful brief monograph indeed.

S. TOLANSKY

The Application of Mathematical Statistics to Chemical Analysis

By V. V. Nalimov. Translated by Prasenjit Basu. Pp. ix+294. (Oxford, London, Paris and Frankfurt: Pergamon Press, 1963.) 84*s*.

THE title of this book will immediately catch the eye of any chemist seeking instruction in the use of statistics in his work. The newcomer to statistical methods will, however, find the book heavy going and would do well to obtain his primary background in the subject from some simpler English text-book. On the other hand, those chemists who already possess some knowledge of statistical methods will appreciate the value of a book applying statistics to their particular problems.

Our old friends, the Normal, Binomial and Poisson distributions, the '*t*' test and the χ^2 test, are all described with reference to chemical analysis. The analysis of variance is applied to the problem of variance between and within laboratories. The statistics of regression are applied to the standard graphs widely used in modern analysis and, in this connexion, the attention given to the correlation between the parameters of the regression equation is interesting as this correlation is not usually discussed in our elementary monographs. Another technique which may be fresh ground to some chemists is that of sequential analysis which is described here, oddly enough without reference to *Sequential Analysis* by Abraham Wald

There are various minor errors of either translation or printing which might confuse the uninstructed beginner, since the sense can only be recovered by a critical examination of the text A notable omission is that of the levels of significance from Table 6, pp 264–267

In discussing analysis of variance it is unfortunate that the author has chosen to describe what is clearly variation 'within columns' as variation 'between rows', a term which bears a quite different meaning in English text-books

I found the English somewhat stilted and prolix, but perhaps this is a common feature of translations Nevertheless, these various blemishes need not deter the prospective purchaser and the book will be an instructive addition to the chemist's bookshelf provided, as suggested here, it is not the only statistical text-book there

O F NEWMAN

Analytical Chemistry 1962

The Proceedings of the International Symposium held at Birmingham University (U K), April, 1962, in Honour of Fritz Feigl to Commemorate his 70th Birthday Edited by Philip W West, A M G MacDonald and T S West Pp x+411 (Amsterdam and London Elsevier Publishing Company, 1963) 90*s*

THIS book is a compilation of sixty-five lectures presented at the Feigl Symposium and the resulting discussions To fashion such a miscellany of papers on chemical analysis into book form, and at the same time preserve a certain continuity, presents difficulties, but the editors have surmounted these with considerable success.

After two introductory papers, including a biographical sketch of Dr Feigl, the first section of twelve papers deals with spot-testing and qualitative analysis It is in this section that most original work is reported on Three papers on semiquantitative spot-test methods and then fifteen papers on organic reagents and their applications to chemical analysis follow These latter groups of papers are mostly in the form of reviews

The second half of the book is devoted, apart from three miscellaneous papers at the end, to physico-chemical analysis comprising thirty contributions on electrochemical, radiochemical and optical methods, separation processes and titrimetric analysis Again, these papers are mainly reviews, most with a sizable and useful list of references One criticism of this section is possibly its lack of balance with six papers on polarographic techniques and four on atomic absorption This leads to a certain repetition especially within the contributions on the latter topic In contrast only one paper on gas chromatography appears

The book is well produced and remarkably free from typographical errors although C instead of c appears as the abbreviation for curie (pp. 23, 87 and 105) It can be recommended to analysts as providing an up-to-date account of most topics within the sphere of modern analytical chemistry

R WOOD

How Chemical Reactions Occur

An Introduction to Chemical Kinetics and Reaction Mechanisms By Edward L King (General Chemistry Monograph Series) Pp xi+148 (New York and Amsterdam W A Benjamin, Inc , 1963) 2 15 dollars paper-back, 4 35 dollars cloth back.

ABLE pupils in the sixth form, or students in their first year, will gain a great deal from *How Chemical Reactions Occur* It makes no attempt to introduce a large number of reactions, the few chosen are well known and are used to the full First the meaning of 'reaction mechanism' is established and attractive diagrams add to the clarity The rate laws are next introduced, and adequate space is given to defining the terms involved· rate, concentration and velocity constant Some methods of examining rates of reaction are given, and this subject is taken up again in a later chapter when very fast reactions are discussed The treatment of molecular collision frequency will appeal to many students, and the discussion of activation energy which follows is equally well done The remainder of the book is given to using the ideas so carefully introduced The reactions chosen serve ideally There are interesting chapters on catalysis and on the use of radioisotopes in the examination of mechanisms

Each chapter ends with a number of problems, some of them well worn, many quite novel There is an interesting glossary and a very detailed reading list Some of the books suggested demand a great deal more mathematical ability than the one under discussion requires Here is a book which will win its way into a great many personal libraries.

D C FIRTH

The Structure of Molecules

An Introduction to Molecular Spectroscopy By Gordon M Barrow (The General Chemistry Monograph Series) Pp xi+156 (New York and Amsterdam W. A Benjamin, Inc , 1963) 2 15 dollars paper back, 4 35 dollars cloth back

DR BARROW has written this account of molecular spectroscopy with a simplicity and clarity possible only from a born teacher, but since he is also one of the subject's chief practitioners, he makes no apologies for the difficulties involved in analysing spectra of all but the simplest molecules The monograph will be welcomed by first- and second-year students at universities and technical colleges

The book covers rotational, vibrational, vibrational-rotational and electronic spectra, and explains how bond-lengths, bond-angles and force constants can be determined to a high degree of accuracy The energy considerations of vibration and rotation are discussed in classical terms and the quantum limitations are imposed without recourse to the Schrodinger equation Sixth-form mathematics is all that is required from the student General practical details are given and each chapter gives an account of the possibilities of future research Each chapter begins with a survey of the material to be presented and concludes with a summary and with numerical calculations from the text

The introductory chapter deals with the nature of radiation the ways energy can be stored within a molecule and how the quantum laws are applied The final chapter on the electronically excited states of molecules will prompt the student to ask questions about the properties manifested by these entities

Dr Barrow retains a humility before his subject, and one marvels at "the way one is led very directly into the world of molecular dimensions", and how much of the geometry and movement within a molecule can be deduced from spectra

D M RUSSELL

Chemical Thermodynamics

By Prof J A V Butler Fifth edition Pp xvii+601 (London Macmillan and Co , Ltd ; New York· St Martin's Press, Inc , 1962) 25*s* net.

PROF BUTLER'S *Chemical Thermodynamics* requires little introduction It is a tribute to its popularity (perhaps more with university teachers than with their students) that the book has retained its position for so long and that its thirty-fifth anniversary should be celebrated with a fifth edition The bulk of the book remains largely unaltered, and the author is unashamed that up-to-date values have not been substituted for some thermochemical quantities because the original data well suit the author's purpose of illustrating his arguments and principles. This reflects the function of the book—

it is a text-book, and its role is to give the student the thermodynamic background required by a degree course and to be an introduction to more advanced treatises Part 1 covers the first two laws of thermodynamics, dilute solutions and electrochemistry, Part 2, with entropy, free energy, the third law, solutions and thermodynamic functions, is designed to be studied as a second course

There are two new chapters. One is an introduction to the thermodynamics of irreversible processes (such as transport across membranes), and the other (by Dr D. J R Laurence) is on the thermodynamics of polymers, and discusses the extensions necessary to account for the properties of polymer solutions, in which entropy considerations are naturally dominant, and the consequences which occur when solvent and solute are of a different order of molecular magnitude D M Russell

Pyridine and its Derivatives

Part 3 Edited by Erwin Klingsberg (The Chemistry of Heterocyclic Compounds a Series of Monographs, Vol 14, Part 3) Pp ix+914 (New York and London Interscience Publishers, a Division of John Wiley and Sons, 1962) 488*s*

THIS, the third and largest, part follows the format of the previous two and comprises four chapters on some functional derivatives of pyridine

A S Tomcufcik and L N Starker contribute to the first chapter (177 pp) on aminopyridines References to infra-red studies relating to the structure of these compounds are quoted up to 1952 Perhaps some note might have been included here to the later contributions made by Katritzky *et al*

The next two chapters are devoted to carboxylic acids The first is by E P Oliveto and includes an excellent account of their preparation reactions and functional derivatives The chapter concludes with a short account of some aspects of the naturally occurring derivatives of this group in vitamins and alkaloids The second chapter on acids, by J C Godfrey, is concerned with the carboxylate group as a side-chain in the pyridine nucleus The subject material is given the same systematic treatment as before

Pyridinols and pyridones, which are important intermediates in the armamentarium of the synthetic organic chemist, are adequately dealt with in the last chapter (382 pp) by H Meislich The aforementioned chapters are remarkably free from errors and each is well supplemented with tables and a comprehensive bibliography This is a most useful contribution to the chemistry of pyridine and maintains the high standard of the series

The high cost precludes its purchase by the vocational organic chemist, and any browsing involves the borrowing for the night or week-end from the works or academic reference library R Hull

Spectrometric Identification of Organic Compounds

By Dr Robert M Silverstein and Dr G Clayton Bassler Pp viii+177 (New York and London John Wiley and Sons, Inc , 1963) 64*s*

THIS is in many ways an unusual, but welcome, scientific publication The strongly didactic character of its presentation will appeal particularly to advanced students who are anxious to improve their knowledge of modern instrumental techniques and of their diverse applications

The book is of quarto size, the text printed legibly on good paper, and the tables and diagrammatic illustrations are all that one could reasonably expect Four earlier chapters are individually devoted to the fairly detailed study of each of four spectrometric methods The older, rugged techniques of infra-red and ultra-violet spectrometry are considered side-by-side with the more complex modern instrumental systems concerned with nuclear magnetic resonance and fragment mass spectroscopy The finer details of instrument design are left to more authoritative text-books, but sufficient information is given to provide the tyro with a clear picture of the functioning of a particular instrument and of the theoretical implications of the recorded data which it provides

In later chapters, the authors illustrate the powerful part which these techniques can play in the unequivocal identification of small (but pure) amounts of various organic compounds Tetramerous analytical exercises have been carried out with these instruments on various organic substances All the experimental data are clearly presented, the physical clues are marshalled and critically examined Conclusions are then drawn with a Holmesian precision and provide a composite molecular and structural diagnosis which could well excite the admiration of sterner and less-gullible observers than the susceptible Dr Watson Quantities of less than one milligram of unknown substance are adequate for all techniques with the exception of nuclear magnetic resonance, and only in the special instance of fragment mass spectroscopy does the original sample under examination become irrecoverable

Tables of numerical data are given which greatly simplify the interpretation of the many quatern spectra which the authors have provided for twenty organic compounds of various types Thus 26 pages are devoted to J. H Benyon's tables for the masses and isotopic abundance ratios for various combinations of carbon, hydrogen, oxygen and nitrogen, that is, values of the 100 per cent parent peak "P" and of $(P + 1)$ and $(P + 2)$ Similarly useful tabular data are provided for the other spectrometric disciplines described in this work The final chapter provides 10 sets of quatern spectra with Beilstein references, a most useful complementary student exercise

This book is reasonably priced and is certain to attract the attention of those who wish to revise their knowledge of the older techniques and to keep abreast of the surging tide of modern physical science D T Lewis

Les Cyclitols

Chimie, Biochimie, Biologie Par Prof Théodore Posternak (Chimie des Substances Naturelles) Pp 492 (Paris Hermann, 1962) 48 N F

THIS volume, one of the admirable series on the chemistry of natural products, deals with the monocyclic polyhydric alcohols in general, but its main concern is with the inositols and derived substances These are, of course, the most important of the natural cyclitols and constitute a large group of compounds which, however, are chemically relatively simple These range from the unsubstituted hexahydroxy*cyclo*hexanes through methylated derivatives to the complex lipids, the phosphoinositides *Meso*(myo)-inositol is a growth factor for many organisms including mammals, although its status as a vitamin, in man, is disputed

Prof. Posternak has devoted much of his research career to the chemistry and biochemistry of these compounds, and has now, fittingly, produced this scholarly work in which every aspect of the subject is discussed in a detailed but readable fashion The book is timely, because much of the chemistry is now well understood as a result of work in the intervening years since the previous major work in this field was published some fifteen years ago On the contrary, however, the biological involvement of these substances is not at all clear and one may expect that the book will give added stimulus to further work

This soft-covered, but sturdy, volume is beautifully produced References include those to work published in 1960 but a number of pages of addenda at the end of the book discuss more recent work It should find its way on to the shelves of all chemical and biochemical libraries D M Brown

Biological Receptor Mechanisms

Edited by J W L Beament (Symposia of the Society for Experimental Biology, No 16) Pp vi+372 (Cambridge At the University Press, 1962 Published for the Company of Biologists on behalf of the Society for Experimental Biology) 50s net

THIS volume can be recommended to all interested in the physiology of sense organs it is well produced and represents excellent value The editor defines biological receptor mechanisms as "mechanisms whereby living organisms transform or transduce the information in their environment" About one-quarter of the book is devoted to botanical problems.

Five articles concern aspects of the physiology of vision, with emphasis on the primary process of light-energy capture in vertebrates and on the optical characteristics of the arthropod compound eye An account of the dislocation theory of smell is followed by a survey of recent work on the chemoreceptive hair of insects The evidence for an electric sense in fish is reviewed Further contributions deal with animal temperature receptors, the ear, the labyrinth, and the electrophysiology of single mammalian mechano-receptors The problem of coding in primary receptor neurones is then discussed

The four botanical contributions are restricted to photosynthesis, non-photosynthetic effects of light on higher plants and on fungi, and 'gravi-perception'

This symposium is a valuable summary of progress made in the past decade The contributions are well illustrated and well documented, and achieve a uniformly high standard Unfortunately, since the zoological material is confined to vertebrates and arthropods, it is left to the imagination of the reader to establish a connexion between the botanical articles and the rest of the book Some animal physiologists may feel that the word 'perception' is abused by the botanists, and that photosynthesis is scarcely a receptor mechanism

D H SPROULL

Infectious Diseases of the Honey-Bee

By Dr Leslie Bailey Pp 176 (London Land Books Limited, 1963) 25s

THIS is the first book in English to be devoted entirely to diseases of the honey-bee, and it is both very comprehensive and interesting The first part deals with the biology of pathogens found in honey-bee colonies followed by the pathology, epidemiology and world distribution of the diseases they cause, in the second part, techniques of diagnosis and control are covered in detail There is also a chapter on non-infectious diseases and diseases of unknown origin

The author's style is for the most part clear and concise, and the photographs and diagrams illustrate the text very satisfactorily The seven graphs and histograms (all recording experiments by the author) are, however, not all presented in a form which can be immediately understood, and this is unfortunate in a book which is intended not only for scientists but also for beekeepers with some biological knowledge Certain other minor criticisms can be made of this otherwise very successful book For example, there are two contradictory statements about the harmfulness to bees of Frow's mixture for acarine disease (p 117), and the control of *Braula coeca* receives only one short sentence despite its relatively widespread occurrence On a few occasions, general statements are made about normal colony development without supporting evidence being provided, but these omissions are outweighed by the 390 references to original work on diseases quoted unobtrusively in the text and listed at the end

Dr Bailey has undoubtedly taken the right course in using his specialist knowledge to produce a book primarily for biologists, and it is to his credit that it will also be welcomed by beekeepers with an active interest in science

M DELIA ALLEN

The Compound Eye of Lepidoptera

Approach from Organic Evolution By Prof. N. Yagi and Prof N Koyama Pp 319. (Tokyo, Japan Maruzen and Co, Ltd, 1963) n p.

THIS is a very complete and fully illustrated account of the morphology of the compound eyes of the Lepidoptera, considered especially from the point of view of the evolution of the group

All features of the eye, both external and internal, are described in considerable detail and are illustrated by many diagrams and photographs The structures are then described in each of thirty-seven families representing most types of the order but rather heavily weighted on the side of butterflies and the larger moths For each group a phylogenetic diagram is constructed which shows relationships so far as these can be deduced from eye-structure alone It is perhaps misleading to refer to phylogenetic relationship when such a narrow range of structure is considered, but it is easy to extract the facts from the speculation The present account is much the fullest one available of the structure of the eyes of Lepidoptera.

Final sections deal with rhythmic migrations of retinular pigment and with the theory of image-formation in the compound eye The authors reach the same conclusion as Burtt and Catton did in 1962, that a number of separate images in numerous ommatidia are integrated and that image-formation does not depend on a pattern formed of lighter and darker dots, each dot corresponding to an ommatidium The volume constitutes an important contribution to our knowledge of the compound eye and of the morphology of the Lepidoptera

O W RICHARDS

California Desert Wildflowers

By Philip A Munz Pp 122 (96 plates) (Berkeley and Los Angeles · University of California Press, London : Cambridge University Press, 1962) 2 95 dollars, 24s

California Mountain Wildflowers

By Philip A Munz Pp 122 (96 plates) (Berkeley and Los Angeles University of California Press, London Cambridge University Press, 1963) -2 95 dollars, 24s

Early Uses of California Plants

By Edward K Balls (California Natural History Guides 10) Pp 103+8 plates (Berkeley and Los Angeles University of California Press, London Cambridge University Press, 1962) 1 75 dollars, 14s

CALIFORNIA Desert Wildflowers and *California Mountain Wildflowers* complete the author's attractively produced, non-technical, three-volume series which can be "placed in the glove box of the car or easily carried on a hike" The two slim volumes are identical in format and include an introductory section concerning the habitat and identification The majority of the species then dealt with are illustrated by clear line drawings and divided into groups according to flower colour Here, and in the comprehensive colour section also, the pages are divided into two columns, illustrations in the outer, and text, giving the common and botanical names, diagnostic features, habitat and distribution of each species, in the inner The selection of species has been well planned to avoid overlap between the volumes, to figure the interesting though not necessarily showy or common, and, in the case of complex groups of species, to illustrate one as an example and mention others closely similar Indexes are provided Together with their companion volume *California Spring Wildflowers* these guides are admirable for their purpose and should be of especial interest to the intending visitor to California who has a love of wild-flowers

Early Uses of California Plants is the tenth in a series of pocket-sized guides "for the student, traveler or sportsman who wants to know more about outdoor California"

About a hundred species are dealt with according to their chief use and grouped as 'food plants', 'drink plants', etc Many are illustrated by line drawings (without scale) and by eight pages of coloured and half-tone photographs of varying quality The booklet ends with an article on present-day uses, a bibliography, and an index of vernacular names A handy little volume for the tourist with an interest in ethnobotany D R HUNT

Selected Botanical Papers

Edited by Prof Irving William Knobloch Pp xiv+311 (Englewood Cliffs, N J., and London Prentice-Hall Inc, 1963) 32*s*

PAPERS mostly of a rather general and philosophical kind, selected to illustrate the purpose and methods of a number of different branches of botanical science, make up this botanical digest for first-year university students Emphasis is American and modern, but not exclusively so; Humboldt, Nehemiah Grew, Fabricius and Aristotle accompanying Merrill, Blakeslee, Erdtman and Went. As they appear in their 'digested' state, the papers are incomplete and I wish that more had been given of some of them instead of the irritatingly naive little catechisms at the end of each

The idea behind this book—that the first-year university course is too often no more than a background for subsequent more advanced courses, irrespective of whether the students will proceed to the latter, and that the first course should, therefore, be more broadly and more philosophically based—is a good one The editor's confidence in the assertion in his preface, that large proportions of first-year students (82 per cent is quoted for botanists) in certain universities in the United States "*never* go further" in their subjects, seems questionable if it is based purely on academic records It is possible to keep a worth-while interest in a subject without proceeding to advanced university courses in it J P M BRENAN

Symposium on the Impact of Man on Humid Tropics Vegetation

Goroka, Territory of Papua and New Guinea, September 1960 Sponsored by the Administration of the Territory of Papua and New Guinea and the Unesco Science Co-operation Office for South East Asia Pp 402 (Canberra A J Arthur, Commonwealth Government Printer, 1963)

THIS publication of the proceedings of the Goroka symposium (see *Nature*, 189, 24, 1961) should interest biologists, educationists and administrators throughout the tropics It deals with the effect of untutored man on his environment About forty papers are grouped under the seven headings of the physical and human background of the Far Eastern humid tropics, wild food, the effects of fire, of grazing, and of shifting cultivation, the secondary vegetation thus produced, and the consequences of these activities on land-management, human plight, and scenery

Many of the papers are illustrated with maps and half-tone plates, and each chapter ends with the verbatim discussion which took place at Goroka The scope is vast, the issues are various, and nowhere is there a clear picture of causation, because the few investigators in this enormous region from Thailand to New Caledonia are still obliged to study by observation and inference

The sponsors are to be congratulated for this permanent record of pioneer conclusions, which J S Womersley has ably edited I hope that the publication, which is the third on the humid tropics from the Unesco Science Co-operation Office in Indonesia, will influence Unesco not to overlook the clear-cut issue of the humid tropics in the re-arrangement of its overseas programme The humid tropics stand for a biological richness which man, as yet, can only deplete E J H CORNER

Pathogenic Organisms in Relation to Pasteurized Cured Meats

By J I Anderton (Scientific and Technical Surveys, No 40) Pp 158 (Leatherhead The British Food Manufacturing Industries Research Association, 1963) 40*s*

HAM is a product which has been produced for centuries past Relatively large-scale production, different methods of marketing and retailing and the apparent demand by (or gradual conditioning of?) the 'average consumer' for less heavily cured products have all played their part in bringing about various changes over the years In turn, the use of weaker brines, lower processing temperatures and the retailer's keenness to display the sliced product (with no guarantee that refrigerated storage conditions will be maintained until time of consumption) have been matters of concern to microbiologists in particular in recent years In *Pathogenic Organisms in Relation to Pasteurized Cured Meats*, Anderton has critically reviewed the published literature relating to this potential hazard

The first main section is entitled "Possible Sources of Contamination" and each of the main types of spoilage organism is discussed Anderton then proceeds to survey each of the main processes involved, such as the effects produced during curing, smoking, boning, trimming, heat processing and storage Other sections relate to the detection of food-poisoning organisms, bacteriological standards for canned hams and the effect of antibiotics Most microbiologists will be aware that contamination is liable to occur at virtually any stage in the manufacturing process This booklet will nevertheless be of considerable interest to them and should assist those of their number employed in industry, who may find it necessary to advise or warn processing staff who wish to make further changes in the manufacturing procedure In fact this survey amounts to a warning for the future rather than a post-mortem after the damage has been done Further, the attention directed to the gaps in the literature should be most useful to research workers in this field

Two pounds may seem to some a high price for a booklet with a thin cover When one considers, however, that the list of references at the end covers some seven pages it will be readily realized that its production represents a comprehensive survey of the literature of a 'delicate' subject D PEARSON

Brompton Hospital Reports

Vol 31, 1962 (A Collection of Papers by Members of the Staffs of the Hospitals for Diseases of the Chest (Brompton Hospital and London Chest Hospital) and of the Institute of Diseases of the Chest) Pp xii+292 (London Lloyd-Luke (Medical Books), Ltd, 1963) 15*s* net

VOLUME 31 of the *Brompton Hospital Reports* is again noteworthy for the collection of articles dealing with various aspects of the diagnosis and treatment of diseases of the chest as well as a number of studies concerned with the embryology, histology and anatomy of the respiratory tract The volume also contains an article by Dr H R Barrett which was delivered as an address to the American Association for Thoracic Surgery at St Louis, Missouri, in April 1962 It is concerned with the publication of scientific journals and shows that responsibility for the production of responsible publications rests as much with the readers as with the editors and publishers Much wise advice is given to would-be authors, while all editors will respect the propriety of the rejection slip sent to a young graduate by a Chinese mandarin It read "We have read your manuscript with boundless delight If we were to publish your paper it would be impossible for us to publish any work of lower standard and, as it is unthinkable that, within the next thousand years, we shall see its equal, we are, to our great regret, compelled to return your divine composition, and to beg you a thousand times to overlook our short sight and timidity" T H HAWKINS

The Solar Corona

Proceedings of International Astronomical Union Symposium No. 16, held at Cloudcroft, New Mexico, U.S.A., 28–30 August, 1961. Edited by John W. Evans. Pp. xi+344. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.) 100s.

THE thirty papers presented at the Cloudcroft symposium, and contained in this volume, provide a comprehensive review of the considerable amount of research on the solar corona which is at present being undertaken. Contributions have been grouped in three sections—"Local Physics of the Corona", "Coronal Phenomena", and "Larger Aspects of the Corona"—each of which is introduced by one or two review articles.

The first section, with the introductory paper by C. W. Allen, discusses such topics as the discrepancy in the coronal temperatures obtained by different methods, the ionization and excitation equilibria and coronal line emission, and possible heating mechanisms. Mention should be made of a paper on "Solar X-Ray Emission" by H. Friedman which gives a survey of some impressive results in this wave-length region, obtained from rocket and satellite work. The second section has two reviews, the fast and the slow phenomena being discussed by J. P. Wild and by M. Waldmeier respectively. The flare-associated phenomena and the relationship of the corona to prominences receive much attention. There are also two or three interesting papers on observational techniques which might have been better grouped under a separate heading.

The section on the "Larger Aspects of the Corona" deals with questions of general structure, and with the origin and nature of corpuscular streams. This is probably the aspect of the subject which is most speculative at present, but which is most likely to benefit in the next few years from rocket observations in interplanetary space. The review for this section was written by S. Chapman, while E. R. Mustel made a substantial contribution on the corpuscular streams and their relationship to regions of solar activity. The volume also contains short reports of nine informal discussions, which are useful in pin-pointing those problems most in need of attention. A further section contains five communicated papers which could not be presented at the symposium for want of time.

During recent years, the study of the solar corona appears to have entered a new phase, with new observational techniques providing a wealth of information from the X-ray region to the radio wave-lengths. These *Proceedings* provide ample evidence of the rapid increase which is occurring in our understanding of the corona as a consequence. D. J. FAULKNER

An Economic Geography of Oil

By Dr. Peter R. Odell. (Bell's Advanced Economic Geographies.) Pp. xii+219. (London: G. Bell and Sons, Ltd., 1963.) 22s. 6d. net.

AVAILABLE literature of the petroleum industry contains more than average references to essential economics. They are chiefly statistical, as they would necessarily be, and as such find a relatively limited appeal in the broader sense of the technology. The trouble with these books and essays is that they tend to date in a matter of a few years after publication because, in the oil world particularly, economic circumstances change so rapidly that the mathematics of supply, demand, refining, transport and distribution throughout the world can never be reduced to static formulæ. This book, however, is in a rather different category in that the stress is on basic geographical analysis of the activities of the world oil industry, not so much on purely present-day statistical data and hypothetical trends in the future. Coming from the pen of a lecturer in geography at the London School of Economics and Political Science, it would be anticipated that the discipline of geographical science applied to the primary commercial factors in the industry would break new and profitable ground. Although the book is designated as an introductory investigation, partly owing to limitations on space, also because of what the author modestly describes as "... my own inability to grasp and to bring out all the relevant factors which contribute to the geographical distribution of the production, refining and consumption of oil", there is little doubt that a more ambitious treatise on the subject would, at this juncture, probably have defeated its object.

The book is divided into four parts—(1) the pattern of world oil supply, (2) the pattern of world oil demand, (3) refining, transport and distribution, (4) conclusion—concerned with the oil industry and economic development. Part 1 embraces consideration of the industry's resources, the world pattern of production and the determining factors thereof. Part 2 discusses the pattern of oil consumption and the factors influencing this demand. Part 3 describes the pattern of refining and the determinants of refinery location, also transport and distribution. Part 4 is in itself quite a masterly exposition of the relationship of the industry to the countries within which its activities are at present located. Here the author quotes R. C. Estall and R. O. Buchanan: "Oil does not in general attract major industries to its source of origin ... major industrial concentrations are not a normal feature of oilfields as they have been of coalfields". The author accepts this point as valid both in the past and to-day, and he advances good reasons why this should be so.

The book is well written, and not overwhelmed with masses of statistics although well supported with outline maps, tables and diagrams. As an attempt to bring an extremely complex and ramified subject into assimilable proportions, it deserves praise. H. B. MILNER

Spirit and Man

An Essay on Being and Value. By Prof. Nathan Rotenstreich. Pp. 257. (The Hague: Martinus Nijhoff, 1963.) 20.75 guilders.

SPIRIT and Man is essentially a study in ethics. The outlook is contemporary, but with a well-balanced historical background. Novelty is scarcely to be expected, but the author makes several interesting observations. Examples are the limitations of calculating machines, the risk of obliterating creativeness and responsibility if systems of Government are too 'paternal', and the dilemma of true freedom in terms of detachment from man's immediate environment. Evidently, some of these questions are of importance to scientists, especially problems relating to team-work in research, and the delicate balance between policies dictated from outside, and the desire of the individual (sometimes) to follow his own inclinations.

The book is divided into three main parts: (1) dimensions, (2) features and (3) significance. In addition, there is an adequate index.

Naturally, the reader will expect a decidedly axiological slant, and in this he will not be disappointed. In fact, the writer sums up by declaring his purpose to be to establish man's worthiness as anchored in the fact that he is a subject, and that the value of his being so is not arbitrary, either anthropocentrically or otherwise.

The text, however, is marred by an excessive number of printer's errors (singulars for plurals, misplaced punctuation, careless spelling, and so forth). The proof-reading must have been much below the standard usually achieved by the publishers. In addition, the writer has the tiresome habit of using the phrase "to be sure" far too often, sometimes even twice on the same page. Nevertheless, it is a useful book in itself, and worth careful study.

F. I. G. RAWLINS

(*Continued from page 960*)

nating between the PGE and PGF types. The main active constituent of Q was concentrated by this means into fractions 10–17 (concentrate S; 20 mg) of a 60-component fractionation (Fig. 4) and chromatographically resembled a PGF. Successive further fractionation with change in pH finally produced an acidic concentrate U ($\sim 0{\cdot}5$ mg) showing intense PGF-type activity corresponding to an equivalent content of 2 mg of PGF$_{1\alpha}$. A similar process applied to concentrate R led to concentrate V ($\sim 0{\cdot}5$ mg) with pronounced PGE-type activity, corresponding to an equivalent content of 0·2 mg of PGE$_1$.

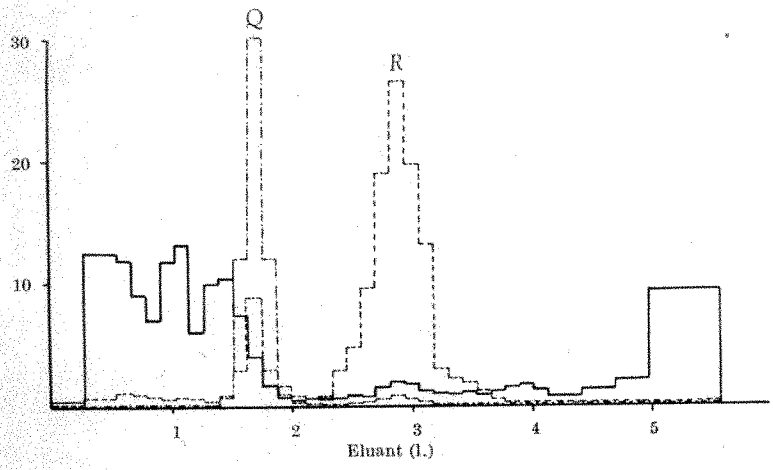

Fig. 2. Reversed-phase partition chromatography of concentrate P. Stationary phase: 70 ml. iso-octanol/chloroform (1 : 1) on 100 g of hydrophobic 'Embacel'. Moving phase: 47·5 per cent aqueous methanol. —— Weight distribution; abscissa units 1 mg/ml.; -·-·- bioassay on rabbit jejunum; abscissa units 6 μg PGF$_{1\alpha}$ equiv./ml.; - - - - bioassay on guinea pig uterus; abscissa units 15 ng PGE$_1$ equiv./ml.

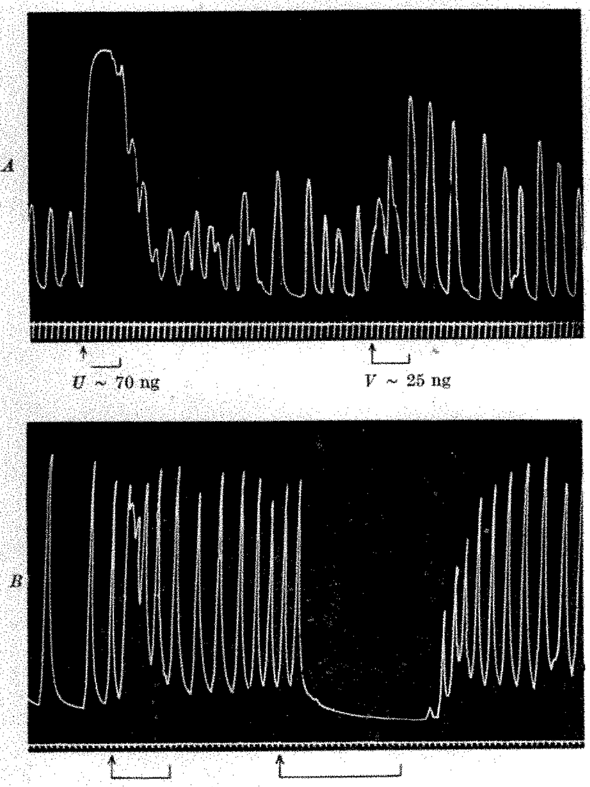

Fig. 3. Effects of menstrual stimulants on human myometrial preparation *in vitro* (bath-volume 4 ml.; time marker, 1 min). A, preparation showing good positive responses; B, preparation showing well-marked inhibitory responses to concentrate V

The next step involved the precise identification of the actual active principles in concentrates U and V. So far the tentative correlation with the two prostaglandin types had rested on the close parallel between the chromatographic behaviour and the type of biological activity. A routine chemical identification by preparation of derivatives was obviously impossible in view of the minute amounts of the concentrates U and V available. Accordingly, thin-layer chromatography comparisons were made between the concentrates U and V and authentic samples of PGE$_1$, PGF$_{1\alpha}$ and PGF$_{1\beta}$ kindly supplied by Prof. Bergström.

Concentrate U showed a single major spot by thin-layer chromatography which was readily detectable by the characteristic red-brown fluorescence in ultra-violet light which developed after its exposure to acid ceric sulphate, followed by moderate heat; this spot contained the physiologically active substance. Both PGF$_{1\alpha}$ and PGF$_{1\beta}$ gave this colour reaction and the former gave a thin-layer chromatography spot close to that given by this menstrual stimulant of concentrate U (Fig. 5a).

Treatment of concentrate U with diazomethane gave a relatively inactive product which most significantly showed the characteristic spot (thin-layer chromatography) at a slightly greater R_F value than that of authentic PGF$_{1\alpha}$ methyl ester (Fig. 5b). Location of this spot by an iodine spray[11] facilitated extraction of the active substance without damage. This product was then exhaustively methylated with diazomethane–boron trifluoride and examined by thin-layer chromatography. The major discrete spot behaved chromatographically in a manner exactly analogous to that shown by PGF$_{1\alpha}$ trimethyl ether methyl ester using the hexane–ether system.

At this stage, both the sample and comparison spots of the completely methylated products were extracted, after location by the iodine technique, and the minute amounts of product (~ 50 μg) examined separately by gas liquid chromatography. As may be seen (Fig. 6), the product derived from concentrate U and that derived from PGF$_{1\alpha}$ behaved very similarly, although not identically. By comparison of the relative retention volumes with those for standard fatty acids on this non-polar column the observed difference strongly suggested that the menstrual stimulant present in concentrate U was in fact PGF$_{2\alpha}$ which contains one more double bond than PGF$_{1\alpha}$ in the carboxylated side-chain.

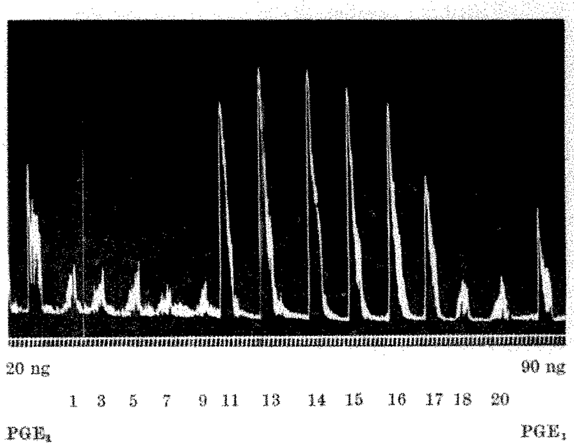

Fig. 4. Rabbit jejunum responses (bath-volume 4 ml.; time marker 1 min) to 1/1,000th aliquot of fractions 1–20 of reversed-phase chromatogram of concentrate Q showing PGF-type in fractions 11–17 (concentrate S)

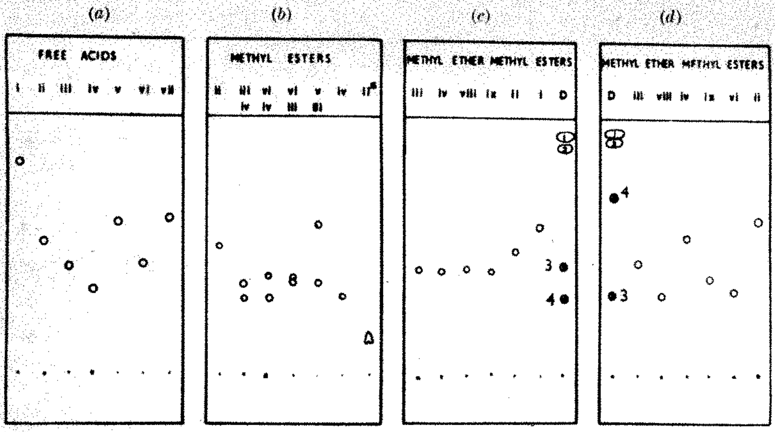

Fig. 5. Characteristic thin layer chromatograms on 0·2 mm layers of 'Kieselgel *G*' of prostaglandins and allied compounds. *a*, Chloroform/methanol/water, 60 : 10 : 0·5 v/v. Layers made up with 0·2 N oxalic acid pre-washed with developing solvent and developed in saturated atmosphere. *b*, As in (*a*) except that layers were made up normally and not pre-washed. Note tailing of phloionolic acid (ii*); *c*, Hexane/ether, 1 : 1; saturated atmosphere development. *d*, AnalaR chloroform in non-saturated atmosphere. Substances: (i) 15-hydroxypentadecanoic acid; (ii) 9,10,18-trihydroxyoctadecanoic (phloionolic acid); (iii) $PGF_{1\alpha}$; (iv) $PGF_{1\beta}$; (v) PGE_1; (vi) concentrate *U*; (vii) concentrate *V*; (viii) least polar borohydride reduction product from concentrate *V*; (ix) most polar borohydride reduction product from concentrate *V*. *D*, Dye marker mixture of (1) azobenzene, (2) sudan red, (3) *p*-hydroxyazobenzene, (4) *p*-aminoazobenzene

This was substantiated by the thin-layer chromatography behaviour of the trimethyl ether methyl ester derivatives of $PGF_{1\alpha}$ and of the menstrual stimulant using silver nitrate complex formation on the plates[12]. The relative change in R_F value on formation of the complex indicated that the menstrual stimulant contained an additional double bond as compared with $PGF_{1\alpha}$ and that this double bond was probably of the *cis* configuration.

Final confirmation of this identity with $PGF_{2\alpha}$ was obtained by mass spectrometric comparison of the trimethyl ether methyl ester derivatives of $PGF_{1\alpha}$ and of the menstrual stimulant.

As only small quantities of each compound were available (~ 10 μg), a special technique was utilized by which the compounds were introduced directly into the ion source. The spectra were obtained by an A.E.I. *M.S.*9 double-focusing mass spectrometer and were run and analysed by Dr. M. Barber and Dr. M. Elliott of A.E.I., Ltd., Manchester, and Dr. R. I. Reed of this Department, who report as follows.

"The spectrum of $PGF_{1\alpha}$ trimethyl ether methyl ester has already been reported[13] and our spectrum agreed closely (Fig. 7*B*). One interesting difference observed by us was the parent molecular ion M/e = 412 which was not previously reported.

"The spectrum of the concentrate *U* derivative was very similar (Fig. 7*A*). Up to M/e = 179 the two spectra are the same whereas above M/e = 249 the corresponding ions have a mass two units smaller in the menstrual derivative. The two compounds are therefore of similar structure, but the concentrate *U* derivative contains one more double bond. The cracking pattern is entirely consistent with the assignment of the structure $PGF_{2\alpha}$ trimethyl ether methyl ester to this concentrate *U* derivative."

Concentrate *V* was similarly examined by thin layer chromatography (Fig. 5*a*) and showed, after destructive spraying and heating, a pale-blue spot under ultra-violet light (a characteristic of the PGE family) at a position closely similar to that simultaneously obtained for an authentic sample of PGE_1; the activity of this spot was also shown to be of the PGE type.

Sodium borohydride reduction of an aliquot of *V* (equivalent to 6 μg PGE_1) and examination of the products by thin-layer chromatography revealed that the pale blue spot moving in the PGE region had been converted into two spots closely comparable in R_F value and colour to $PGF_{1\alpha}$ and $PGF_{1\beta}$. This chromatographic change was paralleled by the corresponding change in the type of physiological activity which was now only present in the PGF region of the plate.

The remainder of the concentrate *V* was similarly reduced and the PGF-type products purified using Bergström's second system. After conversion to the trimethyl ether methyl ester derivatives the two isomers were resolved by thin-layer chromatography on 'Kieselgel *G*' plates by means of chloroform (Fig. 5). In this system the $PGF_{2\beta}$ derivative migrates faster than the corresponding $PGF_{2\alpha}$ compound and, although the conditions are difficult to control, rather remarkable separations are possible. Final purification of each trimethyl ether methyl ester using the more consistent, but less selective, hexane ether system was followed by gas liquid chromatograph analysis of the products. One of the isomers was found to be identical with that obtained from concentrate *U* (that is, $GPF_{2\alpha}$) and the other possessed an R_F value in accordance with that predicted for $PGF_{2\beta}$. Thus the identity of the menstrual stimulant in concentrate *V* was confirmed as PGE_2.

The known occurrence of prostaglandins in human semen[9] made it highly desirable to exclude the possibility that this might be the source of the prostaglandins in our menstrual fluid samples. Accordingly, a number of specimens were examined which were known to be free from this possible adulteration, and which had been collected in menstrual cups in order to avoid any contamination from the usual absorbent materials. They showed physiological, chromatographic and chemical behaviour identical with those exhibited by the main collection.

The identification procedures described in this article were carried out on amounts of material ranging from

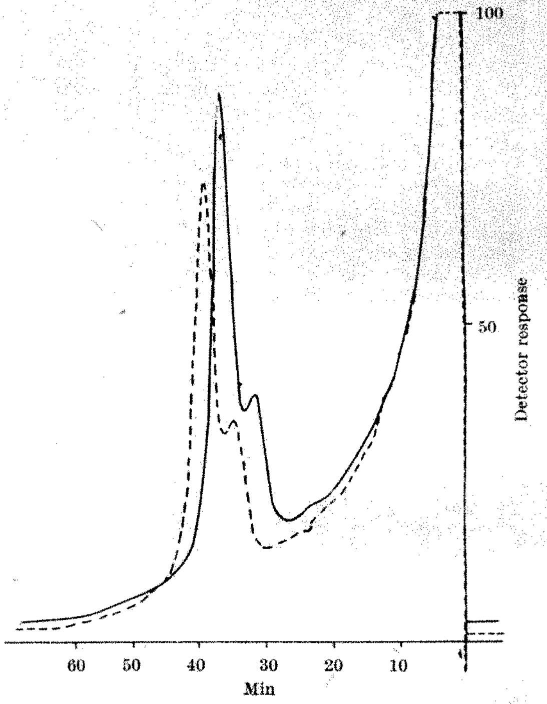

Fig. 6. Gas-liquid chromatograms of the trimethyl ether methyl esters derived from (*a*) $PGF_{2\alpha}$ isolated from menstrual fluid (——) and (*b*) $PGF_1\alpha$ (- - - -). Column, 6 ft. × ¼ in. of 10 per cent 'Apiezon *L*' on siliconized 'Embacel' 80–100 mesh; column temperature 233° C; preheater 240° C; inlet pressure 15 lb./in.^{2}; gas flow 30 ml. of argon per min; detector voltage 1,750; attenuation × 1; load 0·5 μl., of 0·2 per cent solution in chloroform. Retention time for hexacosane 66·5 min and eicosane 8·4 min

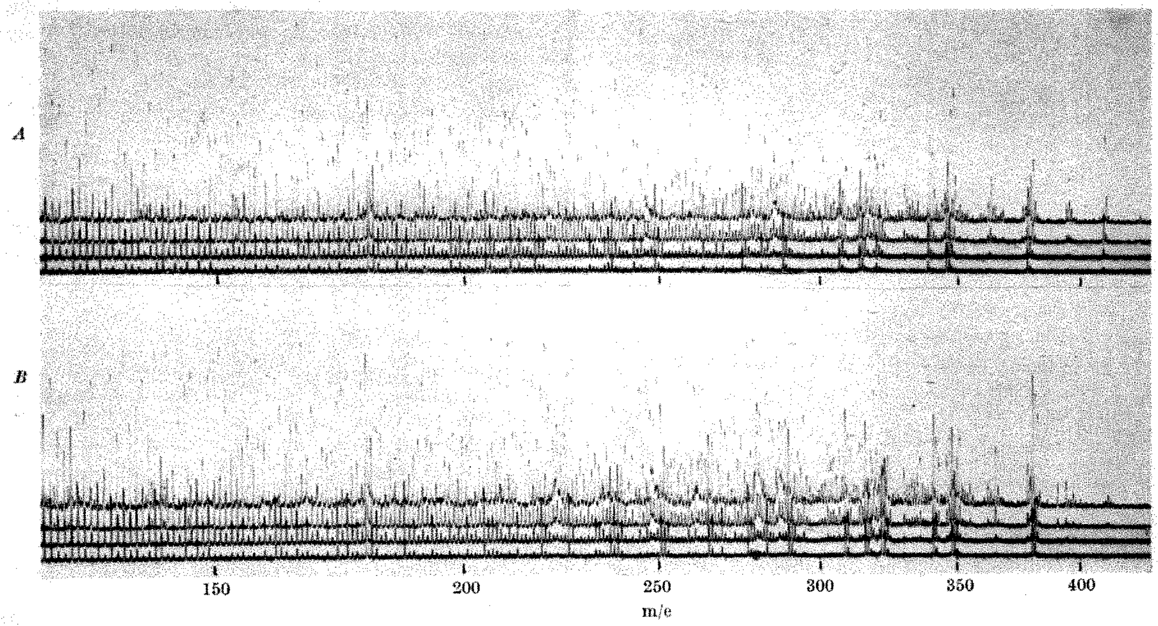

Fig. 7. Mass spectra of the trimethyl ether methyl esters of *A*, $PGF_{2\alpha}$ from concentrate *U*; *B*, $PGF_{1\alpha}$

1 to 50 μg and demonstrate convincingly the power of modern separation and spectroscopic techniques when allied to physiological potency of such a high order.

The isolation of these two prostaglandins from human menstrual fluid adds this source to the several already described[9] for this class of compound. From our present results we cannot estimate their concentration in the secretory endometrium; but the yield of $PGF_{2\alpha}$ from menstrual fluid is comparable with that obtained by Bergström *et al.* from pig lung[14]. The quantity of PGE_2 is, however, much less than that of the PGE substances in human semen[9]. The 'menstrual stimulant' prostaglandins in the female may be thought of as homologous to the seminal prostaglandins in the male; however, the wide distribution of smaller amounts of these highly potent substances suggests that they may have some general type of action. For example, Vogt[15] has suggested that the smooth-muscle contracting long-chain hydroxy-acids may interact with calcium ions, and Clegg, Hopkinson and Pickles[16] have independently suggested a somewhat similar interaction with magnesium ions.

This work was carried out under the auspices of the Medical Research Council and the National Research laevelopment Corporation. We acknowledge with particuDr gratitude the generous provision of reference samples by Prof. Sune Bergström.

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ANOMALY OF ALDOLASE IN PRIMARY LIVER CANCER

By FANNY SCHAPIRA, JEAN-CLAUDE DREYFUS and GEORGES SCHAPIRA
Laboratoire de Recherches de Biochimie Médicale, Hôpital des Enfants-Malades, Paris

RESEARCH in enzymology has been more extensive in experimental cancer than in human cancer, quantitative investigations not always being valid in post-mortem cases and biopsies not always being possible.

The discovery of multiple molecular forms of the enzymes named by Markert and Moller "isozymes"[1] enabled us to re-orient this research on a new basis. In the animal, Angeletti and Moore[2,3] have shown three peaks for glucose-6-phosphate dehydrogenase of mouse rhabdomyosarcoma, by chromatography on substituted celluloses, instead of one peak in the normal muscle; they have obtained analogous results on the acid phosphatase of the rat hepatoma, in which the chromatographic pattern is different from that of normal liver.

In man, Starkweather and Schoch[4], removing cancers of diverse origins a few hours after death, found that the distribution of the five isozymes of lactic-dehydrogenase, very varied in normal tissue, tended to become uniform and to predominate in one peak in cancer tissue.

The mode of action on the two substrates, fructose-1,6-diphosphate and fructose-1-phosphate, of aldolase from different tissues, and more particularly of hepatic aldolase, has given us a new approach for the enzymological examination of hepatomas.

One of us, with Payet[5], had, in 1960, pointed out that hyperaldolasæmia in the course of a primary liver cancer was not of the hepatic type, in the sense that the ratio of the two aldolase activities, fructose-1,6-diphosphoaldolase and fructose-1-phosphoaldolase $\left(\frac{\text{F-1,6-P}}{\text{F-1-P}}\right)$ was in the range of 3, instead of 1 or nearly 1 as in the hepatic type; this ratio of 1 being that found in the normal liver. We had at that time suggested the following interpretation: hyperaldolasæmia comes from the

Table 1. ALDOLASE ACTIVITIES OF NORMAL HUMAN LIVER (IN μMOLE OF SUBSTRATE METABOLIZED PER MIN AT 38°)

	F-1,6-P	F-1-P	Ratio
Normal human adult			
Post mortem	6·6	6·5	1·02
	6·5	6·3	1·03
	5·3	5·0	1·05
	2·95	2·9	1·01
Biopsy	11·2	10·6	1·05
Prematures*			
Fœtal age (months)			
7	1·18	0·48	2·5
6	2·3	1·15	2·0
5·5	2·5	1·2	2·1
5·5	1·17	0·37	3·0
5	1·24	0·33	3·7
4	0·78	0·33	2·4
3	2·25	0·92	2·5

* Some of these prematures had lived several days.

Table 2. ALDOLASE ACTIVITIES OF RAT LIVER

	F-1,6-P	F-1-P	Ratio
Adult normal	11·7	11·7	1·0
Fœtal (eighteenth day)	3·75	1·5	2·5
Intoxicated by CCl_4	5·66	5·6	1·01

Table 3. ALDOLASE ACTIVITIES OF PATHOLOGICAL HUMAN LIVER

	F-1,6-P	F-1-P	Ratio
Fructose intolerance (biopsies of infants)	3·45	0·516	6·7
	1·51	0·283	5·3
	2·0	0·30	6·6
	1·25	0·27	4·6
Benign fructosuria (biopsy of adult)	11·8	11·8	1·0
'Cardiac' liver	1·66	1·60	1·04
Acute hepatitis	3·23	2·75	1·17
Cirrhosis	0·8	0·8	1·0
	2·05	1·60	1·28
	2·50	1·55	1·64
	3·3	2·1	1·6
	3·6	2·0	1·8
	2·1	0·84	2·5
		M:	1·63 ± 0·21

Table 4. ALDOLASE ACTIVITIES OF CANCEROUS LIVER

	F-1,6-P	F-1-P	Ratio
(1) Human primary cancer (hepatomas)*			
Case No. 1	0·9	0·4	2·2
Case No. 2	0·66	0·22	3·0
Case No. 3	2·8	0·68	4·1
Case No. 4	1·0	0·225	4·4
Case No. 5	1·17	0·23	5·0
Case No. 6	6·0	1·0	6·0
Case No. 7	0·5	0·083	6·0
Case No. 8	1·1	0·083	13·0
		M:	5·48 ± 1·17
(2) Rat hepatomas*			
1	0·17	0·007	24
	0·15	0·003	50
	0·14	0·0035	40

* In terms of units per mg of protein of the tumour extract.

tumour itself and it possesses, therefore, a type of aldolase which is different from that found in normal liver. In a preliminary investigation, we have found the ratio of these aldolase activities to be higher in the cancerous region than in the non-cancerous cirrhotic region.

Continuing this work on a new case of human hepatoma, we found a ratio of aldolase activities of 4·1 (ref. 6). We have compared this result with the work on fœtal liver of Hers and Eggermont[7] which we have confirmed.

The present work extends these results to eight cases of human hepatomas and to three experimental hepatomas and seeks to explain their significance.

The experimental tumour is that isolated by Zajdela[8]: an ascites hepatoma, transplanted to adult Wistar rats; the cells are collected seven days after transplant, washed, and homogenized. The samples of human liver are generally removed a few hours after death; some come from biopsy. Animal or human liver is homogenized in 10–20 volumes of water.

The assay for aldolase activity is carried out according to the technique of Sibley and Lehninger[9] which we have adapted (as a micro-method) to the assay for fructose-1-phosphoaldolase activity[10]. These results are expressed in μmole of substrate metabolized per min per g of wet tissue.

Table 1 summarizes aldolase activities of the normal human adult liver and the premature liver; Table 2 summarizes that of rat liver; Table 3 shows the activities of human liver during the course of different pathological states; Table 4, activities during human and experimental primary cancer.

The following facts emerge:

(1) The ratio of aldolase activities is 1, or near 1, in normal adult human or rat liver.

(2) This ratio does not vary *post portem*. It remains equal to, or very near, 1 during benign fructosuria and during the course of viral or toxic hepatitis.

(3) In fœtal liver (human or animal) the ratio of aldolase activities rises to 2 or 3. It rises also, although more slightly, in cirrhosis (M, 1·63 ± 0·21); and much more, as shown by Hers[7,11], in fructose intolerance: we found it to be between 4·6 and 6·7 in four cases.

(4) In human hepatoma, Table 4 shows an anomaly of aldolase, comparable with fructose intolerance because the ratio of the activities is 5·48 ± 1·17 (with the extreme values of 2·2 and 13·0). In the experimental hepatoma of Zajdela, this ratio is between 20 and 50.

The elevation of the ratio of aldolase activities $\frac{\text{F-1,6-P}}{\text{F-1-P}}$ is constant in hepatoma. However, one finds too an increase of this ratio in cirrhosis, in fructose intolerance and in fœtal liver.

The concomitant fibrosis does not explain the rise of this ratio in the course of hepatoma; not only is cirrhosis inconstant, and often moderate in hepatomas, but also in pure cirrhotic liver the ratio rises to an average of 1·6; it is significantly different from the ratio of aldolase activities in hepatoma ($P < 0.02$).

What is the significance of this modification of aldolase activities ?

(1) According to Kaletta-Gmunder, Wolf and Leuthard[12] there exist, in liver, two aldolases, F-1,6-P aldolase and F-1-P aldolase. According to this hypothesis, one would simply find a more rapid disappearance of the second enzyme in cancer. However, one may put forward several arguments against the hypothesis of two enzymes differentiated by their action on two substrates distinct and present in the normal adult liver: a constant ratio of the two activities in several animal species[13] and also a constant ratio during purification and after crystallization[14]; the impossibility, at least at present, of really separating these two activities, either by chromatography[11] or electrophoresis[13].

(2) The comparison with fœtal liver or liver of fructose intolerance brings to mind the ideas of Greenstein[15], for whom the tissular dedifferentiation of a cancer is accompanied by a dedifferentiation of the enzymatic pattern which tends towards a common type. One could suppose that the new enzymatic specificity (proved by the elevation of the ratio of aldolase activities) is expressed by a dedifferentiation at the molecular level. Aldolase would become of the fœtal (or perhaps even embryonic) type in the course of hepatoma: one may notice that in the experimental hepatoma of Zajdela the F-1-P aldolase activity has almost disappeared, and the ratio rises a great deal, meaning, perhaps, a greater dedifferentiation.

Our observations may be compared with those of Starkweather and Schoch[4], who noticed a tendency towards uniformity of the isozymes of the lactic dehydrogenase in cancer of diverse origins.

(3) Hepatic aldolase and muscular aldolase are certainly different; the immunological investigations of Blostein[16] have confirmed this. We can then wonder whether during cancer there appears a new enzymatic specificity, making a molecular disease. However, Nisselbaum and Bodansky[17], examining the properties of the isozymes of lactic dehydrogenase of normal liver and hepatoma, could not show any difference of specificity.

(4) If we accept the hypothesis of Hers that the fœtal aldolase is distinct from the adult aldolase, we

may postulate that in the normal adult this aldolase exists only in trace amounts because its synthesis is repressed by the gene which has become predominant over adult aldolase (in the same way the synthesis of fœtal hæmoglobin is repressed by the gene of the β-chain of adult hæmoglobin).

In cancer the biosynthesis of adult aldolase would be inhibited and also that of the other enzymes very differentiated. The embryonic form of the enzyme would cease to be repressed and could be synthesized in abundant amounts. Such a situation would be comparable with the increase of the biosynthesis of fœtal hæmoglobin in β-thalassæmia. It could be generalized in cancer: all the specialized enzymes of a tissue would disappear, suppressing the repression on the embryonic forms, less differentiated, of the same enzymes.

Further investigations are obviously necessary to appreciate the significance of these facts. In any event, they give arguments for generalizing the concept of the loss of specificity of the enzymatic (or more generally proteic) constituents of tissue in cancer, this loss of specificity being a true molecular lesion.

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AFFINITY OF ANDAMANESE RECENT STONE INDUSTRY

By PRATAP CHANDRA DUTTA

Anthropological Survey of India, Indian Museum, Calcutta, 13

I HAVE recently given detailed accounts of the technology and typology of the stone artefacts recovered from a kitchen-midden site at the Beehive Island in Middle Andaman[1,2]. I have also reported[3,4] the results of my excavation of the kitchen-midden at the village of Chouldari, near Port Blair, in South Andaman. The scope for instituting a study on the affinity of the Andamanese recent stone industry is a rather restricted one, the yield of the artefacts being from a single site. The collection nevertheless provides no less than an important indicator permitting such an attempt. It is now time that an investigation in this line be taken up in view of the present ethnographic interest of the Andamans.

The Andaman Archipelago, comprising some 204 islands and islets, between 10° and 14° N. and 92° and 94° E., is situated in the south-eastern part of the Bay of Bengal. The geographical situation of the Andamans in the mid-waters is highly significant because of its somewhat intermediary position between the mainland of India and that of the Far East Islands including the Malaya Peninsula, both claiming to belong to two main divergent lithi-cultural zones. It would be really interesting to know the probable affinities in the past, if any, of the Andamanese artefacts with either of those two main divergent cultures.

It has already been shown[1,2] that the Andamanese stone artefacts, which have been regarded as belonging to the mesolithic culture, represented a recent flake industry based on blades. The implements are small and were fabricated out of the siliceous rocky elements. While classifying the tool types (Table 1), it emerges that of the major types those constituting the industry were blades, points, scrapers, flakes and plenty of by-products; some true microliths, such as blunted back blade, bladelets, lunates, perforators, also formed part of the collection. The implements (Fig. 1) are mostly primitive showing the crude nature of the workmanship. The ratio of occurrence of finished tools (33 per cent) to by-products (67 per cent) is roughly 1 : 2, which favours the view that the site was possibly a factory site. It is certain that the site was continuously inhabited in the past by the originators of this industry resulting in the formation of the kitchen-midden mound of considerable dimensions.

Table 1. TYPOLOGICAL ANALYSIS OF THE ANDAMANESE ARTEFACTS

	Types	Per cent
Finished	Tools	32·93
By-product	Cores	2·03
	Flakes	65·04
Analysis of finished tools	Blades	29·63
	Points	19·75
	Scrapers	23·46
	Choppers	1·23
	Lunates	2·47
	Trapeze and trapezoids	14·81
	Perforators	8·64

On the basis of the evidence put forward in course of an exhaustive investigation of the industry[2], one cannot escape the conclusion that the Andamanese stone industry represented a flake and blade industry so characteristic of the mesolithic culture; and the circumstances reported on by me[2], coupled with the material excavated, led to the assumption that the economy of the people was based primarily on hunting and collecting. Other concomitants of this industry were mainly the coiled pottery, fractured and split-up animal bones belonging to *Sus andamanensis* and mollusc shells of Gastropoda and Lamellibranchia, the details of which have already been given[4] elsewhere.

From an examination of the artefacts it is apparent that no such affinity of the present series could be drawn with the types represented in the Indian sub-continent due, no doubt, to the diversity of material techniques. It has been claimed by some investigators[5-12] that the development of the Indian microlithic industries is broadly similar, in some regions, to the types represented in the North African Capsian, Oranian and Sebilian, East African Wilton and, in others, to Natufian of Palestine.

The features of the Andamanese recent stone industry, though lacking in confirmatory details, indicate that the industry shows a considerable resemblance in character

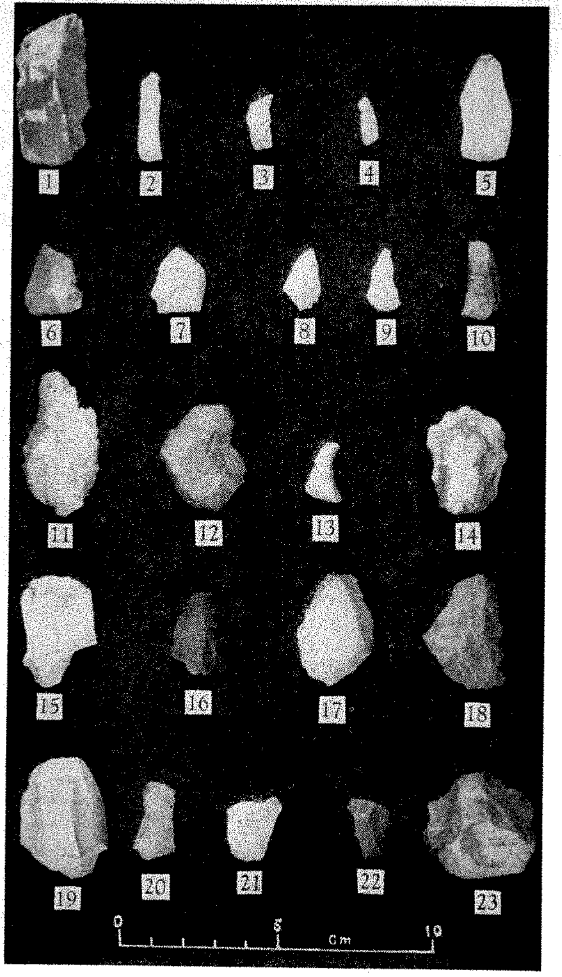

Fig. 1. Nos. 1–5, 10, 13, 20 blades; 6–9, points; 11, 12, 14, 15, 17, 18, scrapers; 21, trapeze; 16, 22, flakes; 19, 23, cores
[*Photo: S. K. Chattopadhyay*]

with the Toalean and allied flake and blade industries of the Far East Islands and South East Asia, claiming, of course, nothing as to their coevality in time.

The flake and blade industry was classically developed in south-western Celebes[13], which included artefacts, such as flakes, blades, knives, arrow-heads and some true microliths. This particular industry is more commonly known as Toalean after the existing Toal Aborigines of Celebes. The flake and blade industry has also been found in the Djambi District[14], on the east coast of Sumatra[15], and on the Bandung Plateau in western Java[16,17]; and van Heekern[18] also traced a related industry of secondary importance, associated with the Sampung Bone culture, in western Java. Evans[19] reported on the occurrence of mesolithic flakes in Borneo, whereas Sarasin[20] recorded the flake industries of the Lesser Sunda Islands, and later, Verhoeven[21] discovered implements belonging to the flake and blade industry in West Flores.

The site at Tanjong Bunga, which is situated at the tip of the Malayan Peninsula, yielded so far the only Malayan artefacts[22] which could possibly be recognized as representative of the flake and blade culture. The obsidian implements from Luzon[23] and the artefacts, such as scrapers, small knives, awls and crude points from Rizal Province[24] of the Philippines, belonging to the mesolithic culture, are comparable with the present series.

Clark[25], like others, also observed "that these industries came to be identified with hunting peoples of Negrito stock . . .". The Aborigines[26–38] of the Andaman Islands are a very dark, very short, brachycephalic people having pepper-corn pattern of hair and infantile characteristics.

In this context, attention should be directed to the ethnic affiliation of the Andamanese. To mention the view of Lapicque[39], who worked intensively on the problem of Negritos of South East Asia including Andamans, that the people of the Andamans bear similarities in physical features with Negritos of South East Asia. Martin[40] and Sullivan[41], both of whom examined the craniological evidence, discussed in detail the racial affinities of Andamanese with the Semang of Philippines and were of opinion that the people of Andamans and Philippines originated from the same racial stock. Nippold[42] also sought a common origin of the people of both groups of the Islands from South East Asian pygmies.

But how and when they came to inhabit these widely separated Islands from the mainland of Asia is still a matter of controversy. Kaudern's[43] observations, however, based primarily on Molengraaff's[44] researches on the rise and fall in sea-level during the prehistoric period, suggested that the migrations of the pygmies occurred directly from Asia to both the Andamans and the Philippines in the Quaternary Age during glacial periods.

It may, therefore, be admitted that the recent stone industry of the Andamans, which represented a flake and blade industry of the mesolithic stage of culture, was broadly similar in character to the Toalean and allied flake and blade industries of the Far East Islands. It seems, however, in all probability, when more material becomes available, that the Andamanese industry may bring to light some genetic relationship with those of the Far East Islands including South East Asia. The geography and the environment of the Andaman Islands are such that they caused the survival of the mesolithic stage of culture and the hunting and collecting economy from the early period down to comparatively recent times.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Active Dark Filaments and Type III Bursts

At the Ondrejov Observatory, simultaneous observations of a spectrohelioscope by L Krivsky and radiometers at 231 and 260 Mc/s by A Tlamicha were made, showing a clear time coincidence between a type of group of bursts, even when the duration is short, and active dark filaments without flare in more than 15 cases during 1960–62 This type of group of bursts referred to as type N_A (and N_B) (ref 1) is a group of short duration bursts without remarkable enhancement of base-level

In order to identify the spectral type of these bursts, the same type of groups of bursts recorded at the Tokyo Astronomical Observatory with a polarimeter at 200 Mc/s has been compared with the spectral data (15–210 Mc/s) obtained at the Dapto Station, Sydney The foregoing type of bursts seems to be the group of type III bursts Almost all the identifications were made by using a list of the spectral observations *Spectral Classification of Solar Activity*, Dapto, Sydney, but some of them were already identified with type III bursts by a direct comparison with the spectral records by the courtesy of the Sydney group, and also these bursts are almost unpolarized as the type III bursts at 200 Mc/s usually are

In order to certify the correlation between the active dark filaments and type III bursts, active prominences and active filaments (without flare) observed in 1960 with a spectrohelioscope has been compared with radio activities observed with an interferometer and a polarimeter at 200 Mc/s at the Tokyo Astronomical Observatory Even though the number of the optical observations is quite small, a good coincidence in position is found in 5 of 6 cases between the active prominences (and active filament) without flare and groups of type III bursts at 200 Mc/s occurring in the periods of the optical activities The number of association of the type III bursts with the optical activities is 8 out of 21 despite the fact that the high-frequency cut-off for type III bursts may sometimes occur below 200 Mc/s and that the radio waves from the extreme limb may not be observed (Table 1)

Table 1

Active prominences	No of events	No of association	Type III group at 200 Mc/s Coincidence in position	No observation in position	No coincidence in position
Prominence	20	7	4	2	1
Filament	1	1	1	0	0
	21	8	5	2	1

During the periods of the active prominences and active filaments already mentioned, 27 out of 33 cases have type III groups given in the list of *Spectral Classification of Solar Activity*, Dapto, Sydney, in some frequency ranges between 210 Mc/s and 15 Mc/s, although chance coincidences may be included and also the sensitivity of the spectrometer is not enough to record weaker type III bursts

These results may support a statistically significant, but small, correlation, which has been shown by G Swarup, P H Stone and A Maxwell[2], between surges without flares and fast-drift bursts observed with spectrometers The correlation is probably better if more weaker type III bursts were able to be recorded C S Warwick[3] has shown a good correlation between large ascending prominences and radio activities at 200 Mc/s J P Wild and H Zirin[4] have reported that, in some cases, limb surges were associated with groups of type I bursts These groups of bursts shown in their paper, however, seem to us to be groups of type III bursts

In order to derive a more definite conclusion, we should like to point out the importance of observations of active dark filaments and active prominences with spectrohelioscope using line shifter, taking a direct contact with radio observations with interferometer, more sensitive spectrometer and polarimeter Both the starting and the location to be observed by the helioscope can be determined by radio observations

We thank Mr Kai for help in the determination of radio positions

A Tlamicha *
T Takakura

Tokyo Astronomical Observatory,
Mitaka,
Tokyo

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* Visitor from the Observatory, Ondrejov, Czechoslovakia

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PHYSICS

Sodium-24 produced by Cosmic Radiation

Cosmic radiation produces several radioactive nuclides by spallation of atmospheric argon Some of them have been detected, first phosphorus-32 in rain-water[1], then sulphur-35 (ref 2) and afterwards phosphorus-33 (ref 3) Sodium-22 was also found[4] in spite of its very low yield caused by the large mass difference compared with argon-40 Furthermore, Winsberg[5] succeeded in finding the short-lived nuclide chlorine-39 (55 min half-life)

The success in detecting sodium-22 and chlorine-39 suggested that it might also be possible to find sodium-24 (15 h half-life) which had not so far been detected It was to be expected that the production rate would be of the same order as that of sodium-22 and that the yield in rain would be sufficient for measurement

Indeed sodium-24 has been detected in several rains for the first time during the work recorded here

A detector had to be designed with an appreciable efficiency and a very low background Nearly 100 per cent of sodium-24 decay by β^--emission ($E_{\max}$ 1 4 MeV) to a 4 12-MeV level of magnesium-24 The de-excitation from this level to the ground-state is associated with a γ-cascade of 2 75 MeV and 1 37 MeV The 2 75-MeV ray was measured by a 4 in × 2 in sodium iodide (Tl)-crystal with a bore-hole of 2 in diameter and 0 5 in depth The 1 37-MeV peak cannot be used because it is obscured by the 1 28-MeV peak and the Compton distribution of the 1 79-MeV sum peak of sodium-22 always present at the same time To reduce the background the β-radiation is measured in coincidence by a flow counter which extends into the bore-hole of the crystal Except for the bottom the detector is surrounded by a guard counter in anticoincidence to eliminate the effects caused by cosmic ray mesons The whole detector unit is screened by 5 5 in lead The counting efficiency of the arrangement is 1 4–1 7 per cent (depending on the β-absorption in the sodium chloride layer of the sample), the background being 0 22 c p h or 0 003 c p m The crystal itself has a counting efficiency of 4 per cent and a background of 72 c p h or 1 2 c p m

Rain-water was collected over an area of 8 6 m^2 and put through an ion-exchange column of 6 cm diameter and 52 cm length filled with 'Dowex' cation exchange resin, 50 × 8, 50–100 mesh The sodium was eluated selectively by 0 67 N hydrochloric acid A first fraction of effluent amounting to 4 0 l was discarded, the subsequent fraction of 2 9 l contained about 98 per cent of the sodium The eluate was evaporated to dryness, dissolved again, and aluminium chloride added Aluminium was precipitated with diluted ammonium hydroxide Na^+ remained in solution whereas other ions, if present, were precipitated almost entirely with the aluminium hydroxide The solution containing the sodium was evaporated to dryness Finally, the ammonium salts were removed by heating The sample was transferred to the counting device as crystallized sodium chloride

The sodium-24 was identified not only by its γ-radiation but also by its life-time The decay was measured for three half-lives The results exclude an essential error in measurement (exceeding the statistical error) caused by possible spurious contributions of thorium *B* and its daughters (2 62-MeV peak)

Three samples were measured on June 7, 1963, from 248 l of rain-water an activity of 0 18 ± 0 02 d p m /l or 235 ± 25 atoms/l , on July 4, 1963, from 203 l of rain-water an activity of 0 35 ± 0 03 d p m /l or 455 ± 40 atoms/l , on October 7, 1963, from 330 l of rain water an activity of 0 19 ± 0 03 d p m /l or 245 ± 40 atoms/l

The fact that the concentration does not vary considerably with season confirms that the stratospheric contribution if any is small

According to Lal and Peters[6] a tropospheric production rate of about 6×10^6 atoms/m^2 year can be estimated for sodium-22 in the geomagnetic latitude of Heidelberg and therefore for sodium-24 also When the concentration values given here are converted to mean annual rainfall at Heidelberg, a deposition of about 2×10^5 atoms/m^2 year is obtained for sodium-24 Because of the short half-life it was to be expected that only a small fraction of the produced activity could be detected at ground-level

I thank Prof O Haxel and Dr G Schumann for facilities for this work and for advice

W RÖDEL

Zweites Physikalisches Institut der Universitat,
Heidelberg, Germany

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Measurement of Surface Tension by Interferometric Means

FOR a sessile drop of liquid of surface tension, density and radius of curvature R_0 at the vertex, the differential equation[1]

$$y'' = (2+\beta y)\,[1+(y')^2]^{3/2} - \frac{y'}{x}[1+(y')^2],\ \beta = g\rho R_0{}^2/\gamma$$

for drop shape (Fig 1) in terms of the co-ordinates measured from the vertex and scaled with respect to R_0, has recently been solved numerically on a digital computer by Staicopolus[2] and the *Fortran II* programme is available The method used is more accurate than the elaborate approximation procedure of Bashforth and Adams[1] and, although not mentioned by Staicopolus, the results are also more accurate than Porter's[3] values of $\gamma/\rho gr$ as a function of h/r The results allow γ and r/R_0 to be related to h and r through a polynomial expression in $z = (r/h - A)/C$

A and C being constants, 1 5922 and 0 5922 respectively

The accurate expressions plus further use of the computed data enable γ to be determined for small drops and bubbles using an interference microscope to obtain r/h from r/R_0, thus avoiding the usual requirement of a direct accurate measurement of h with its attendant difficulty[4]

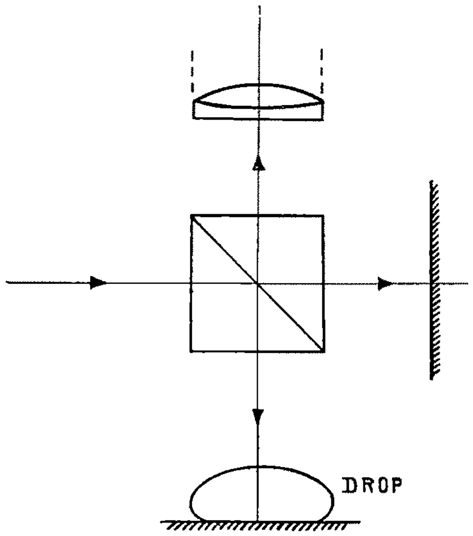

Fig 1

From a measurement of r and R_0 and the digital computer results, h/r can be determined accurately to four decimal places after fitting an expression $z = f_2(1 - r/R_0)$ in the same way as the inverse form[2] $r/R_0 = f_1(z)$ A fourth-order polynomial (Table 1) gives this accuracy for small drops where $r \sim 0(R_0)$ This method improves on h/r determined from r/R_0 assuming the elliptical approximation[5] for the surface above the plane of greatest section, since that approximation does not always hold to the accuracy required

Table 1

Polynomial coefficients

$$z = (r/h - A)/C = \sum_{n=0}^{4} a_n\,(1 - r/R_0)^n$$

a_0	−1 0000
a_1	2 2755
a_2	−0 3056
a_3	0 2620
a_4	0 0571

Having obtained h/r from $z = f_2(1 - r/R_0)$ the value of γ is found by substitution into the polynomial expression $\gamma = g\rho R_0{}^2/B(z)$ given by Staicopolus[2]

An interference microscope (Fig 1), embodying the Michelson principle[6], was used at times 26 in measurements on small samples of glycerol and related liquids The formulæ for the radius of curvature by Newton's rings were used since it is relatively simple to show that for small drops or bubbles ($r \sim$ 0 5 mm) the curved surface over the small region (radius 0 07 mm) at the vertex which is observed in the interference microscope may be taken as spherical to the required degree of approximation, that is, the actual surface and the assumed spherical surface do not differ by more than a small fraction of a wave-length of light

The method of least squares was used to determine R_0 from the experimental observations made using the green 5461 Å line from a mercury 198 lamp The accurate magnification for the reduction of measurements made on photographic plates was obtained by repeated measurements on a 1-mm scale photographed alongside the drop and which was precision ruled into 10 divisions on a metrological ruling engine at the National Standards Laboratory, Sydney

For typical cases of small sessile drops, 1 mm radius, of glycerol, r was measured to ± 0 0001 cm and R_0 to ± 0 00005 cm, allowing γ to be determined as 61 5 ± 1 dynes/cm at 25° C

The method is particularly useful when only small samples are available. It also applies to sessile bubbles and allows the surface tension between a liquid and a gas to be measured conveniently under difficult conditions sometimes encountered.

The experimental observations were made while I was at the Commonwealth Scientific and Industrial Research Organization, Sydney.

B. S. THORNTON

Honeywell Pty. Ltd.,
Sydney, Australia.

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OCEANOGRAPHY

Measurements of Equatorial Currents in the Gilbert Islands Area, July – August 1963

IN a recent article, Montgomery[1] reviewed the observations of the equatorial undercurrent, a current which has been recorded at least seasonally in each ocean. This easterly flow is found near the equator, usually below a westerly surface current; the easterly velocity maximum is located within the thermocline. Measurements in the central Pacific Ocean have shown the undercurrent commonly to be separate from the easterly surface flow of the equatorial countercurrent to the north, to be symmetrical about the equator, to be relatively steady, and to have a maximum velocity of two to three knots. In the western Pacific Ocean, on the other hand, the data indicate that the undercurrent is by comparison less strong and less steady, is commonly not symmetrical about the equator, and is often joined to the equatorial countercurrent by a continuous layer of easterly flow. Montgomery chose the Gilbert Islands, which cross the equator at 174° E., as the natural dividing line between the central and western regions of the equatorial Pacific Ocean. This chain of islands apparently forms the only sizeable obstruction to the flow of the undercurrent between New Guinea and the Galapagos Islands, a distance of some 8,000 miles.

The previous measurements of the current happen to have been made mostly well to the east or west of this dividing line, with few data from the vicinity of the Gilbert Islands and no measurements within the inter-island channels themselves. To provide a first look at this interesting area, a programme of water sampling and current measurements was added to the hydrographic survey carried out in the Gilbert Islands during July–August 1963 by H.M.S. *Cook*, the Royal Navy surveying ship, in the south-west Pacific Ocean.

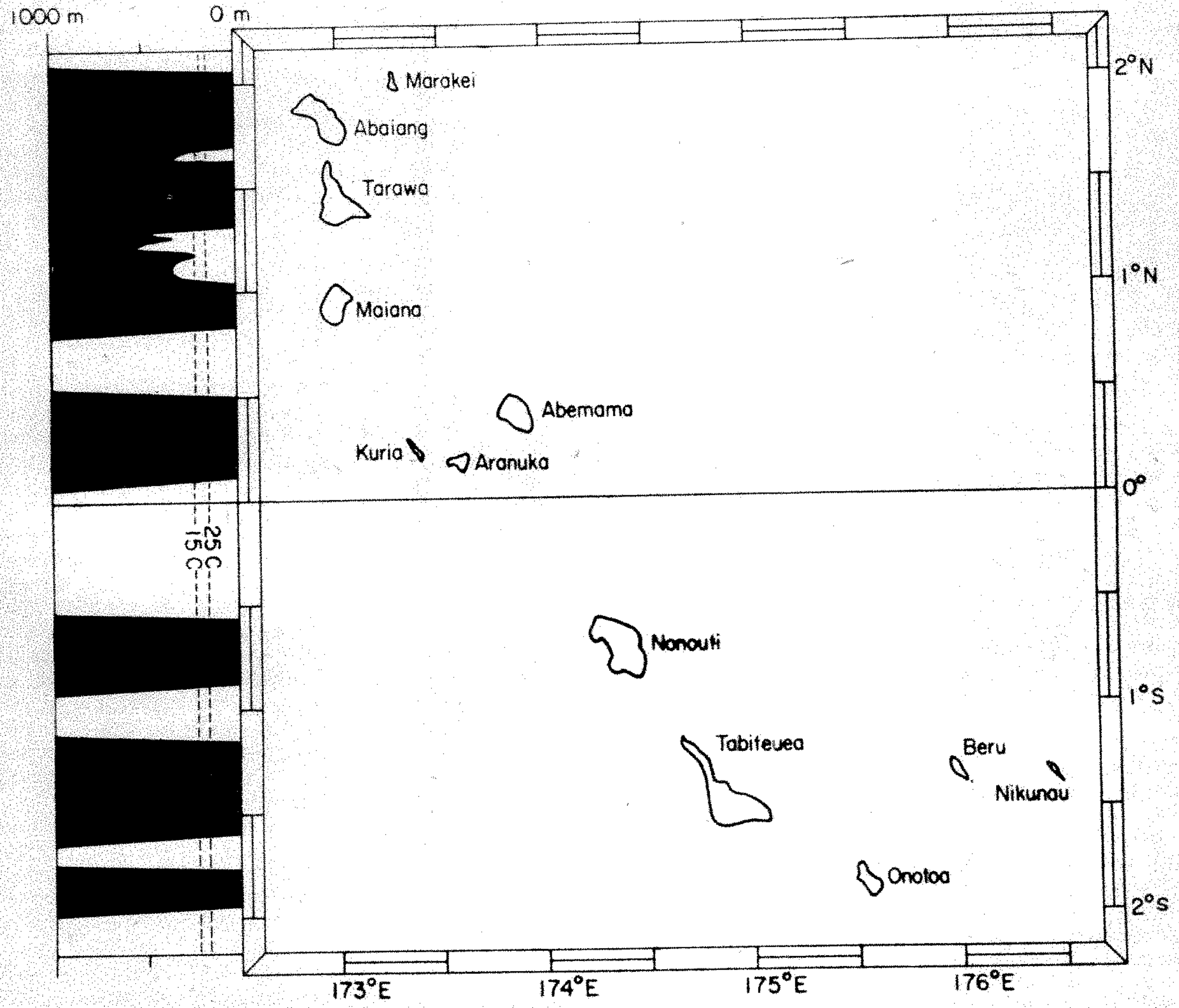

Fig. 1. Right panel, chart of the central Gilbert Islands. The approximate outlines of the shoal areas are shown; the small islets, which mostly lie along the eastern reefs of the atolls, are not depicted. Left panel, profile of the island chain, from the sea surface to a depth of 1,000 m, projected on a meridional plane. Latitude scale same as for right panel; vertical exaggeration 200. Isotherms for 15° C and 25° C are from bathythermograph data taken along 169° 34′ E. Fig. 1 is drawn from U.S.N.H.O. chart No. 0119 and from unpublished recent surveys by H.M.S. *Cook*

The Gilbert group includes both atolls and lagoonless coral islands. Of varied size and shape, the islands rise only a few feet above sea-level and for the most part fall away steeply (and often with spectacular suddenness) to oceanic depths. A chart of the central Gilbert Islands is shown in Fig. 1. This figure also includes the profile of this portion of the island chain, between the sea surface and a depth of 1,000 m, as projected on a meridional plane. The positions of the 15° C and 25° C isotherms included on the profile were observed on a meridional section at the longitude of Ocean Island, some 250 miles west of the Gilbert Islands (see following). These isotherms define the extent of the main part of the thermocline in this region; it is seen that there are no extensive obstructions in the inter-island channels at depths which would interfere with zonal flow at the level of the thermocline.

While the Gilbert Islands lie within the limits of the easterly trade winds, light airs and calms are much more common than in the region along the equator in the central Pacific Ocean. (During the cruise reported here, calms or light airs were reported on 31 of the 46 days spent in the general vicinity of the island group.) The surface currents, while generally setting toward the west, are notorious among island navigators for the occasional occurrence of strong sets in unpredictable directions, causing difficult landfalls on these low islands (personal communications and experience). It should also be noted that the spring tide range in these islands is of the order of 2 m, so that one might expect occasionally to find tidal currents influencing the flow in the vicinity of land.

In the expectation of complex flow in the neighbourhood of the islands, it was decided that H.M.S. *Cook* should first look for the undercurrent by occupying a meridional section well upstream (westward) from the island chain. This section was made along 169° 34′ E., the longitude of Ocean Island (a small, isolated, raised coral island at 0° 53′ S., which H.M.S. *Cook* had, in any event, to visit for bunkers). Stations were occupied every 30 miles from 2° S. to 3° N., with serial measurements of water properties and currents to 800 m. Following completion of the meridional section, three 24-h stations were occupied along the same longitude, approximately at 1° 00′ N., 0° 15′ N., and 0° 30′ S. During each of these three stations the drift of the vessel relative to an anchored beacon was measured by radar. Serial current measurements to 400 m were repeated every 3 h, with serial measurements of water properties to 800 m at the beginning and end of each 24-h station.

The bathythermograph data from the meridional section demonstrate the equatorial spreading of the thermocline associated with a geostrophic undercurrent[1]. The vertical separation between the temperatures of 15° C and 25° C was 60 m at 2° S. and 45 m at 2° N., but at the equator it reached a maximum of 88 m. Preliminary examination of the data from each of the three 24-h stations reveals that easterly flow was present in the thermocline, the mean eastward component not exceeding 1 knot at any depth. While the details of the surface current will have to await a thorough analysis of the effect of the wind on the ship, it can be said that the surface current, regardless of direction, was uniformly slower than the current in the thermocline, and also that during the station at 1° N., which had a preponderance of calms and light airs, the mean surface current had an eastward component.

After completion of the work at 169° 34′ E., H.M.S. *Cook* returned to the Gilbert Islands, where two further 24-h stations were occupied, one in the centre of the Maiana–Kuria channel and the other in the centre of the Aranuka–Nonouti channel. The mean flow in the thermocline was easterly at both stations, with the eastward component not exceeding 1 knot. The surface currents were weaker than the sub-surface flow, and under calm conditions in the Maiana–Kuria channel the surface current had an eastward component.

This work has done little more than make a first identification of the equatorial undercurrent in the Gilbert Islands. It is clear that this area would be a fruitful one for further studies of the equatorial circulation, and of the undercurrent in particular.

The participation of one of us (E. D. S.) in this programme was supported by the U.S. National Science Foundation and Office of Naval Research.

E. D. Stroup

Chesapeake Bay Institute,
Johns Hopkins University,
Baltimore, Maryland.

F. W. Hunt

Commanding Officer,
H.M.S. *Cook*.

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METALLURGY

X-ray Emission Spectroscopic Investigation of Sealing of Anodic Oxide Films on Aluminium

'Sealing' of oxide coatings produced on aluminium and its alloys by anodic treatment in sulphuric acid solutions is the reaction between the amorphous alumina (containing about 15 per cent sulphate) and boiling water or aqueous metal salt solutions. X-ray emission spectroscopy provides a new and useful technique to study the sealing reaction through its quantitative and quick determination of the content of various elements such as aluminium and sulphur in the film and their changes on sealing. Sealing action in metal salt solutions of nickel acetate, cobalt acetate or potassium dichromate can be assessed by the increasing metal ion content of the sealed film. Typical results are discussed here.

The emissivity radiation strength under standard examination conditions is affected by: (*a*) element volume concentration in the oxide film; (*b*) film thickness; (*c*) absorptivity of the film (including action of the water or metal salts taken up in the sealing operation). The volume concentration of the element examined is not changed significantly by sealing since chemical dissolution of the film is very slight, and no volume change can be detected (by normal metallographic techniques). The influence of film thickness remains essentially constant once a minimum film thickness is attained. (Rough calculations indicate that with aluminium and sulphur, 90 per cent of maximum intensity is attained with film thickness of about 4μ.) This leaves absorptivity as the major variable during an examination of sealing. Thus, with anodic coatings produced under standard conditions to an adequate film thickness and sealed in various ways, the aluminium and sulphur counts will decrease on sealing in water, compared with values for the non-sealed film, and will vary with the degree of sealing, that is, with differences in the take-up of water.

The X-ray emission spectroscopy technique has been applied successfully in a study of sealing. A General Electric X-ray spectrograph (*XRD*–5) was employed using the following spectral lines, aluminium *K*–α (8·34 Å), sulphur *K*–α (5·37 Å), nickel *K*–α (1·66 Å), with operating conditions standardized to yield 4,000 c.p.s. for high purity (99·993 per cent) aluminium spectrographic standard.

Aluminium (99·998 per cent) foil samples in groups of 10 panels each were anodized in 15 per cent sulphuric acid solution at 25° C and 15 amp/ft.^{2} for 45 min to yield oxide films with coating weights of 4·5 mg/cm^2 (film thicknesses of about 20μ). Individual weighed panels from a group were sealed in de-mineralized water (*p*H 6·0, 100° C) or nickel acetate solution (nickel acetate 5 g/l, boric acid 5 g/l, *p*H 5 0, 100° C) for varying periods up to 3,000 min The weight gain on sealing was determined, followed by X-ray emission spectroscopy determination of aluminium, sulphur and nickel intensities Panels were stripped of all oxide by treatment in the conventional phosphoric acid–chromic acid solution (A S T M *B* 137-45) at 100° C for 10–15 min, the aluminium panel weight determined and the unsealed coating weight and the per cent weight gain on sealing calculated

The change in aluminium and sulphur radiation intensities as the oxide film was thinned by progressive chemical dissolution in the phosphoric–chromic acid solution was also investigated A group of panels was anodized and sealed together, and then individual panels were immersed in the hot acid solution for varying periods of 15 sec–15 min followed by examination by X-ray emission spectroscopy The loss in weight during dissolution treatment was determined by weighing, after which the samples were treated further to remove the oxide completely The percentage weight of film dissolved was calculated for individual panels

The gain in weight during water sealing (Fig 1) rose with time, but at a steadily decreasing rate, up to 3,000 min, a period more than 50 times longer than the sealing period in commercial use for films of this thickness The aluminium intensity decreased, rapidly at first, and then at a slowly decreasing rate The intensity was linear with the percentage gain in weight on sealing The sulphur emission intensity also decreased, but to a lesser degree and was not linear with the gain on sealing

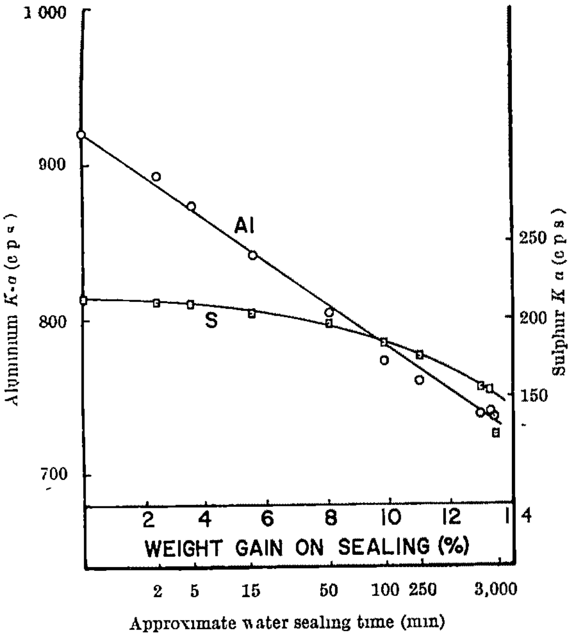

Fig 1 Effect of water sealing time on X-ray emission spectroscopy intensities for aluminium and sulphur (100° C, *p*H 6 0)

The results obtained with nickel acetate solution sealing (Fig 2) were very different from those from water sealing The gain in weight on sealing for the maximum period used (250 min) was much higher than in water sealing (24 per cent versus 11 per cent) due to precipitation of nickel salts during sealing in addition to water take-up The aluminium intensities decreased linearly with percentage gain in weight on sealing during sealing for up to about 30 min and then decreased more slowly Nickel intensities increased linearly during the entire sealing period but with a change in rate after about 30 min of treatment The addition of specific surfactants to the nickel acetate solution has been found to modify greatly the effect of sealing on the X-ray emission spectroscopy values Further work is required to confirm and explain these results

The effect of progressive dissolution of the film on the aluminium and sulphur intensities is illustrated by Fig 3 The values for unsealed films were aluminium, 920, sulphur, 230 c p s These were much higher than the

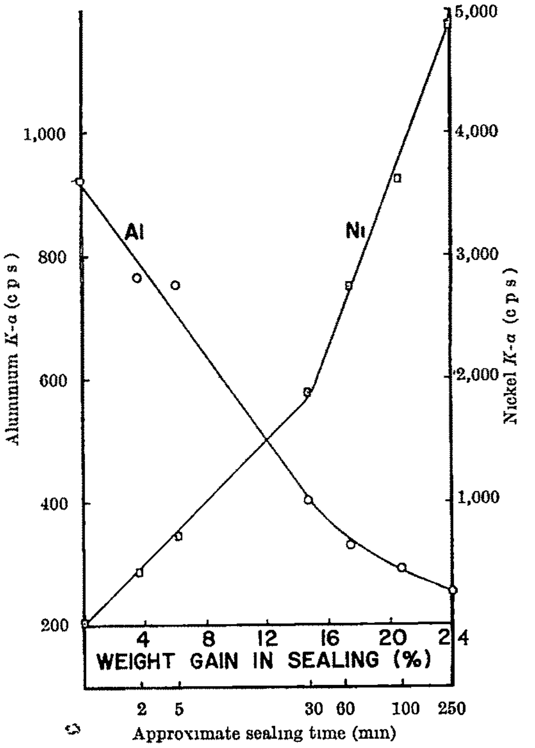

Fig 2 Effect of sealing time in nickel acetate solution on X-ray emission spectroscopy intensities for aluminium and nickel (nickel acetate 5 g/l, boric acid 5 g/l, *p*H 5 0, 100° C)

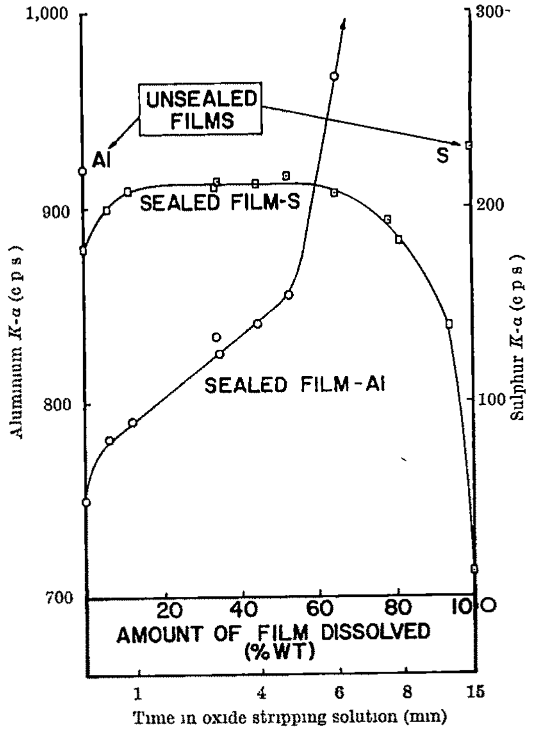

Fig 3 Effect of progressive chemical dissolution of water-sealed anodic oxide film on X-ray emission spectroscopy intensities for aluminium and sulphur (sealed 250 min, 100° C, *p*H 6 0)

counts determined on the samples which had been water-sealed for 250 min, namely, aluminium, 750, sulphur, 180 c p s As individual samples were 'stripped' for increasingly longer periods the aluminium intensity rose to 855 with accompanying loss in weight of about 50 per cent of the oxide film With further stripping the intensity climbed very sharply due to increasing contribution not of the oxide film but of the aluminium substrate which was not screened out by the greatly thinned oxide film

The change in sulphur intensities with increasing dissolution of the film showed an initial rise to a value which was maintained until the increasing thinness of the oxide coating caused a decrease (as compared with the rise with aluminium intensities)

The initial rise of both aluminium and sulphur intensities appears to be due to the presence of a 'skin' of oxide which was sealed to a greater degree than the underlying bulk film The depth and degree of this effect increased with sealing time Similar effects have been noted in work with oxide films sealed in nickel acetate solution Dissolution in the chromic–phosphoric solution probably causes non-uniform thinning due to differential action associated with the pore structure of the anodic oxide coating Non-uniform attack of barrier layer films by the same solution has been discussed by Altenpohl[1], Franklin[2], and Stirland and Bicknell[3] Examination of the location of the dissolution action and of the 'skin' effect is continuing

ROY C SPOONER
W J FORSYTH

Aluminium Laboratories, Ltd ,
Kingston, Ontario

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MINERALOGY

A Mercury Mineral from the British Isles

WE have recently discovered small quantities of cinnabar (HgS) in the galena–calcite–fluorite ore-bodies of Rutland Cavern, Matlock Bath, Derbyshire The mineral occurs in cavities, most often in fluorite, as a brick-red deposit associated with smithsonite ($ZnCO_3$) and iron-staining, and under the microscope appears as minute red specks dusting the surface of smithsonite and fluorite This is the first recorded occurrence of a mercury mineral from the British Isles Since our discovery cinnabar has also been identified on a specimen from Peat Pasture, Greenhow Hill, Pateley Bridge, Yorkshire, in the collection of Sir Arthur Russell, Bart

We thank the staff of the British Museum (Natural History) for carrying out the identifications

R S W BRAITHWAITE
T B GREENLAND

Department of Chemistry,
College of Science and Technology,
Manchester

G RYBACK

36 Hainthorpe Road,
London, S E 27

CHEMISTRY

'Ageing' of Silica Suspensions in Water and its Influence on Flocculation by Polyacrylamide

IT has been found that silica and certain silicates undergo a slow change of surface properties when kept in water, their suspensions becoming resistant to flocculation by polyacrylamide For example, fresh 1 per cent suspensions of the high-purity, amorphous silica 'Aerosil' (Degussa, Germany) are rapidly flocculated by a 0 004 per cent solution of polyacrylamide of high molecular weight, such as 'Cyanamer *P* 250' (American Cyanamid Co , New York), but, after standing for a certain time, 'Aerosil' suspensions can no longer be flocculated, even by larger amounts of polyacrylamide (for example, 0 08 per cent) Fig 1 shows the times required to develop resistance to flocculation in various solutions

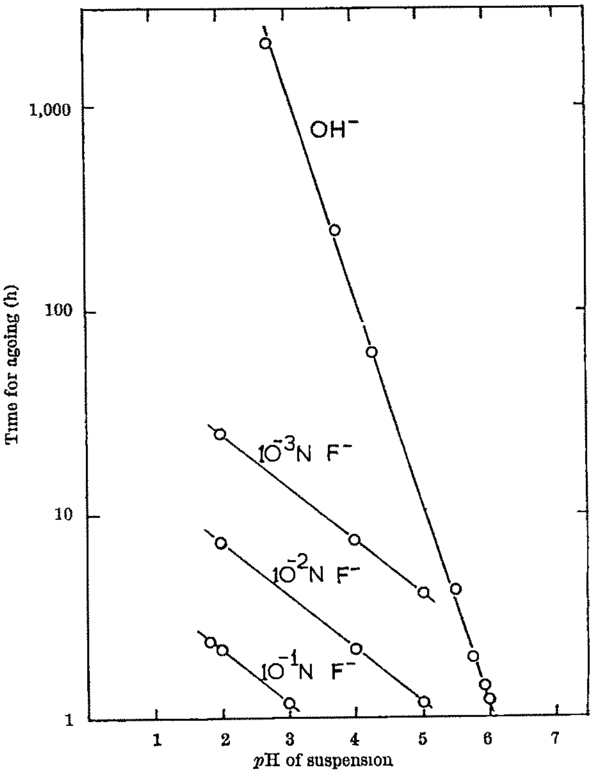

Fig 1 Times of ageing of silica, in aqueous suspensions, required to develop complete resistance to flocculation by polyacrylamide

The 'ageing' effect has been proved not to be due to dissolution of silicic acid or to changes of surface area The silica can be restored to its original (flocculable) condition by heating it to 300° C, this treatment removes chemisorbed water present as silanol groups, whereas removal of physically adsorbed water at lower temperatures does not reverse the ageing Infra-red spectroscopy on samples heated to various temperatures showed that a band at 2 74μ, which has been ascribed to single hydroxyl groups[1], is present in the original and in the 'rejuvenated' silicas but not in the aged forms dried at temperatures below 300° C This band also disappears in fresh silicas that have been flocculated by polyacrylamide

Polyacrylamide is strongly adsorbed by fresh silicas but not by aged ones Adsorption evidently occurs by hydrogen-bonding on to silanol groups, because flocculation was inhibited when competing hydrogen-bonding agents such as alcohols, amides, ketones and ureas were added, furthermore, the relative molar effectiveness of these agents was found to be independent of the concentration of polyacrylamide, and their order of effectiveness agreed closely with their interaction values with silicic acid[2]

Powdered quartz and talc showed a similar ageing, whereas alumina, bentonite and kaolinite showed no ageing, remaining sensitive to flocculation Ageing therefore appears to be associated with surfaces that are predominantly siliceous in composition

In the *p*H range 2–6, the rate of the ageing process is proportional to the concentration of hydroxyl ions (Fig 1) Fluorides also accelerate ageing, though less strongly than OH^-, while chloride is quite ineffective The rate with fluoride is roughly proportional to $[F^-]^{\frac{1}{2}}$

and depends somewhat on *p*H, presumably because hydrogen fluoride is not fully dissociated

From this evidence two conclusions may be drawn

(1) Flocculation of silica by polyacrylamide occurs by hydrogen-bonding adsorption on silanol groups of a particular type—probably isolated OH-groups

(2) Ageing of silica in water is a chemical reaction in which these active groups disappear

A co-ordination number of silicon above four can occur in the presence of hydroxyl or fluoride ions[3], also, an increase of number of OH-groups carried by each silicon atom in the surface layer has been found[4] Although it is not yet clear why adsorption of polyacrylamide is thereby prevented, the hydrogen-bonding ability of surface silanol groups must be involved The presence of a co-ordinately bonded hydroxylic layer seems a plausible hypothesis[5]

The ageing phenomenon may also have important implications for the biological action of silica, fresh silica is flocculated by a very small amount of human saliva, while aged silica is not

One of us (O G) thanks the University of Buenos Aires for a fellowship held during the course of this investigation

O Griot
J A Kitchener
Department of Mining and Mineral Technology,
Imperial College of Science and Technology,
London, S W 7

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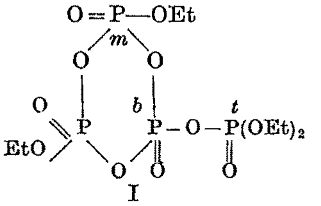
Structure and Mechanism of Hydrolysis of the Product of Reaction of Phosphorus Pentoxide and Ethyl Ether

THE product of reaction of phosphorus pentoxide and ethyl ether which has the composition of a metaphosphate ester $[PO_3(C_2H_5)]_n$ has been the subject of recent discussion Ratz and Thilo[1] have shown that the product of hydrolysis of this ester could result from the splitting of a mixture of the two following compounds



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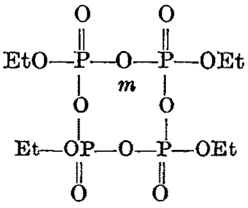

II

However, the nuclear magnetic resonance spectrum obtained by Van Wazer[2] on the crude compound shows the presence of three peaks, the chemical shifts of which are characteristics of terminal (*t*), middle (*m*) and branched (*b*) phosphorus atoms, but in the proportions P_t = 25 per cent, P_m = 72 per cent, P_b = 3 per cent The inequality of P_b and P_t seemed to rule out the proposed structure

The use of this compound as an activating agent in a number of condensation reactions[3] has indicated its extraordinarily high reactivity towards OH groups and raised the question of the mechanism of the condensation reaction The titration of a solution of the polyphosphate ester in water immediately after dissolving shows two primary acid groups for each initial P_4O_{10} molecule (Fig 1, curve 1) Pollmann[4] has examined the changes in infra-red spectra of a solution in chloroform on addition of small quantities of water and suggested an opening of a ring based on the disappearance of a broad peak attributed to cyclic metaphosphate

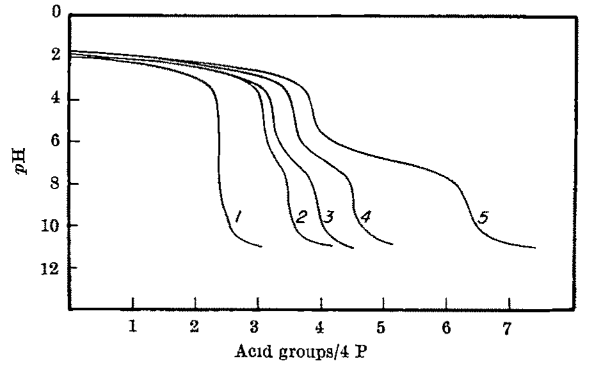

Fig 1 Titration of the polymetaphosphate ester in water as a function of time (1) immediately after dissolving, (2) after 6 h, (3) after 24 h, (4) after 52 h, (5) after boiling for 1 h

The nuclear magnetic resonance technique seemed suitable for a fine analysis of the structure and mechanism of hydrolysis of this compound We have used a Varian spectrometer operating at 24 5 megacycles/sec Phosphoric acid (85 per cent) was used as an external standard and no attempt was made to measure the chemical shift with an accuracy of better than ± 2 p p m

A first attempt to reproduce Van Wazer's experiment on an undiluted sample prepared a few days before the observation has given the result shown in Fig 2 The chemical shifts are identical to those published by Van Wazer $\delta P_t = 16$, $\delta P_m = 29$, $\delta P_b = 42$, and the proportion only slightly different from his with an unequal proportion of P_b (6 per cent) and P_t (32 per cent)

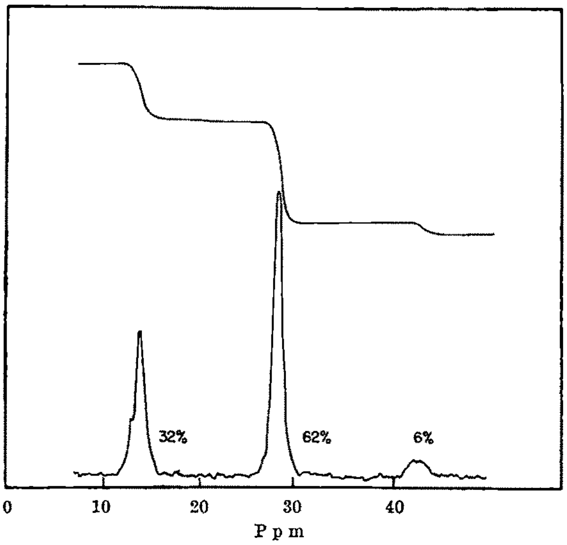

Fig 2 Nuclear magnetic resonance spectrum of a crude sample of polymetaphosphate ester and integration curve (no solvent)

To prevent a partial hydrolysis during the storage or the transfer of the viscous material into the narrow tube, we have introduced directly into the tube a freshly prepared chloroform solution of the compound in the absence of humidity, and evaporated part of the solvent under vacuum The spectrum so obtained (Fig 3*a*) and its integral (Fig 3*f*) show approximately equal proportion of P_b and P_t and therefore permits structure I The relative amounts of phosphorus in structures I and II can then be calculated

$$C_{I} \sim 2 \times (15 + 13 \text{ per cent}) = 56 \text{ per cent}$$
$$C_{II} \sim 44 \text{ per cent}$$

The changes of the spectrum on addition of small quantities of water (Fig 3*b*, *c*, *d*, *e*) reveal the following features

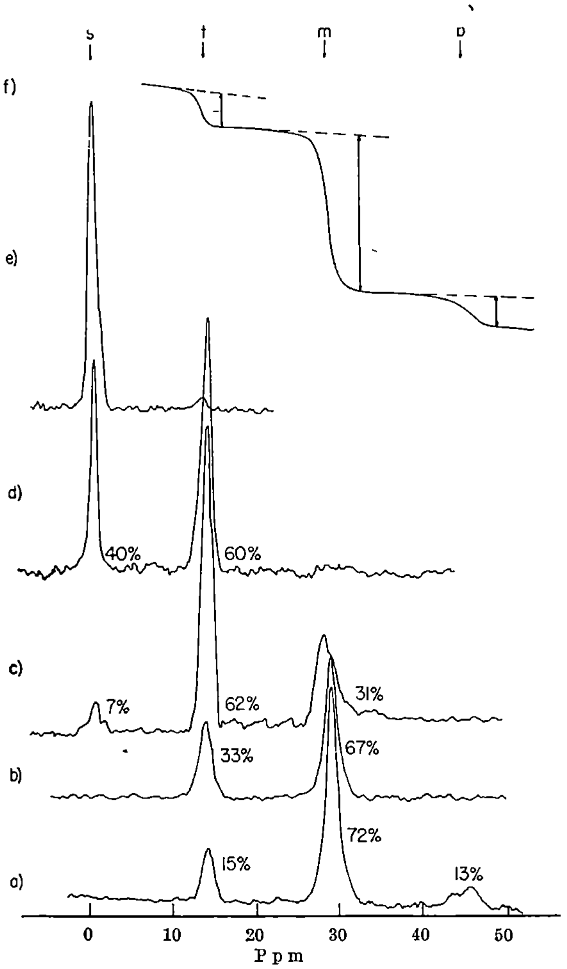

Fig 3 Nuclear magnetic resonance spectrum of a concentrated solution of freshly prepared polymetaphosphate ester in chloroform *a* and *f*, Sample in absence of water and its integral, respectively, (*b*) with less than one equivalent of water per 4 P, *c*, with a large excess of water after 5 h, (*d*) with an excess of water after 20 h, *e*, with an excess of water after boiling (each trace may be at a different amplification)

(*a*) In a first step (*b*) the P_b peak disappears and the P_t peak increases while P_m remains practically constant No resonance corresponding to orthophosphate derivatives appears

(*b*) In a second step (*c*) the concentration of P_m decreases and the concentration of P_t increases until both are roughly equal

(*c*) The concentration of P_t becomes higher than P_m and a small quantity of orthophosphoric derivative (P_s) is found (*c*) followed by a disappearance of P_m with increasing amounts of P_s(*d*)

The same experiment performed with ethyl alcohol gives the same pattern of results From the relative amounts of the different species of phosphorus during these three steps, the following conclusions are drawn

(*a*) The structure I is more sensitive to hydrolysis than structure II The first bond to be split is the bond connecting the 'branched' phosphorus to another phosphorus of the ring

(*b*) The ring opening of structure II follows until transformation of the two compounds into two linear tetrapolyphosphates (P_t/P_n = 1) is complete

(*c*) The hydrolysis of the tetrapolyphosphate proceeds preferentially through formation of two pyrophosphate structures This observation fits with the rate of appearance of secondary acidities evident in Fig 1

It is probable that the relative amounts of structure I and II depend on the preparation and could account for some variation in the behaviour of this material in polycondensation reactions However, a ratio of the order of 1 1 can be expected from the original structure of P_4O_{10} and the observed higher stability of structure II towards hydrolysis

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GILBERT WEILL*
MELVIN KLEIN
MELVIN CALVIN

Department of Chemistry and Lawrence Radiation Laboratory,
University of California, Berkeley.

* Present address Centre de Recherches sur les Macromolecules, 6 rue Boussingault, Strasbourg C N R S Fellow, 1962–63

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Proton Magnetic Relaxation and Protein Hydration

WE have found that ovalbumin dissolved in water decreases the proton spin–lattice relaxation time T_1 and proton spin–spin relaxation time T_2 and we show that this effect is due to an increase of correlation time of part of the water molecules The correlation time τ can be defined as the mean time of persistence of the angular position of a water molecule and is simply related[1] to the Debye dielectric relaxation time τ_d, $\tau = \tau_d/3$ According to the theory of nuclear magnetic relaxation by Solomon[2], T_1 and T_2 are functions of τ In some cases τ can be expressed as a function of the macroscopic viscosity of the liquid[1], but in the case of protein solutions such an explanation is not possible as was shown by Hennel *et al* [3]

The measurements of T_1 and T_2 of ovalbumin reported here were made at 20° C in magnetic field of 3,288 1 gauss using the Carr–Purcell[4] and Meiboom[5] spin echo techniques Recrystallized ovalbumin from hen's egg-white was used and also some measurements were done on a solution of fresh egg-white Care was taken to ensure that the investigated material was sufficiently free from any paramagnetic ions, which could exert a spurious effect on relaxation To remove such ions, versene (ethylenediamine tetraacetate sodium salt) was added to the protein solution in a dialysis bag and dialysis was performed against distilled water The efficacy of this method was controlled

The results obtained show that the reciprocal of relaxation time increases linearly with the concentration

$$1/T_1 = 1/T_{1w} + k_1 c \qquad (1)$$

$$1/T_2 = 1/T_{2w} + k_2 c \qquad (2)$$

where T_{1w} and T_{2w} are respectively the spin–lattice and spin–spin relaxation times of dialysate, practically equal to values for distilled water exposed to the air $T_{1w} = T_{2w} = 2\ 7$ sec The concentration c is expressed in g of dry protein contained in 1 g of solution The constancy of k_1 and k_2 at different concentration is evident from Table 1

Table 1 VALUES OF k_1 AND k_2 OBTAINED FOR OVALBUMIN AT 20° C AND 14 Mc/s

Concentration ($c \times 10^2$)	Fresh k_1	Fresh k_2	Denatured k_1	Denatured k_2
2 32	2 93	7 15	12 1	89 2
4 69	2 77	5 91	12 0	105 5
5 36	3 06	5 92	12 7	109 5
6·03	2 73	7 30	10 3	87 6
7·30	3 08	7 44	13 6	109 0
8·85	3 33	7 25	12 0	97 8
9 46	3 59	6 66	14 3	104 5
mean	3 07	6 80	12 4	100 4

In order to give an explanation of the obtained behaviour of T_1 and T_2 we assume that the protons present in a sample consist of 3 groups (*A*) Protons situated in water molecules which either do not belong to the solvation layer of any protein molecule or, if they do belong, are bound such a way as to enable them to change their position in relation to the protein molecule The correlation time of water molecules of this type is not affected by the presence of protein and is equal to that in pure water (B) Protons situated in water molecules bound to the protein molecules in a rigid, irrotational[6] way and having, therefore, the same correlation time as the protein molecule itself (C) Protons of the protein molecules having very short T_1 and a rather slow exchange rate[7] with the surrounding water escaped our observation

We have, therefore, to consider only groups A and B If there were no proton exchange between these groups one would observe two separate relaxation times T_a in group A and T_b in group B We assume that such an exchange exists and is so fast that the time of life of a proton in any of the two groups is shorter than T_a or T_b In such a case we shall observe single resultant relaxation time T_1 given by the following equation[8]

$$1/T_1 = p_a/T_{1a} + p_b/T_{1b} \tag{3}$$

where p_a and p_b are the fractions of time spent by a proton in groups A and B respectively They are proportional to the amount of water in each group Let w be the mass of water of group B per 1 g of dry protein Then, at low concentration ($c \ll 1$), we have $p_a = (1 - wc - c)/(1 - c)$ and $p_b = wc/(1 - c)$ and remembering that $T_{1a} = T_{1w}$ we obtain from (3)

$$\frac{1}{T_1} = \frac{1}{T_{1w}} + c\left[\left(\frac{1}{T_{1b}} - \frac{1}{T_{1w}}\right)w - \frac{1}{T_{1w}}\right]$$

This is the linear equation (1) where

$$k_1 = \left(\frac{1}{T_{1b}} - \frac{1}{T_{1w}}\right)w - \frac{1}{T_{1w}} \tag{4}$$

In a similar way we obtain

$$k_2 = \left(\frac{1}{T_{2b}} - \frac{1}{T_{2w}}\right)w - \frac{1}{T_{2w}} \tag{5}$$

Expressing T_{1b} and T_{2b} as functions of τ by means of equations (33) of Solomon's paper[2] and substituting this into equations (4) and (5), we obtain a set of two equations which can easily be solved for w and τ by means of a graphical method (The use of equations (33) which are obtained for isotropic random motion is an approximation only, because the shape of the ovalbumin molecule is ellipsoidal with an axial ratio of about 5 and therefore the motion of the irrotationally bound water molecules cannot be regarded as isotropic) Assuming for k_1 and k_2 the mean values given in Table 1 and the value[9] $b = 1\ 515$ Å for interproton distance in water we obtain for ovalbumin

$$w = 1\ 60 \times 10^{-2}, \qquad \tau = 1\ 03 \times 10^{-8} \text{ sec}$$

For egg-white we find $k_1 = 3\ 62$ and $k_2 = 9\ 13$, which yield

$$w = 1\ 97 \times 10^{-2}, \qquad \tau = 1\ 20 \times 10^{-8} \text{ sec}$$

These values of τ should be comparable with 1/3 of the Debye dielectric relaxation time of the protein According to data given by Edsall[10], $\tau_d/3 = 1\ 6 \times 10^{-8}$ sec for the shorter axis and $\tau_d/3 = 6 \times 10^{-8}$ sec for the longer axis of the ovalbumin molecule In view of the obvious shortcomings of the model this agreement is satisfactory

The amount of water irrotationally bound to the protein is in agreement with the results of Buchanan *et al* [6], who found $0 < w < 0\ 07$ from the dielectric constant of ovalbumin solution at very high frequency

We have found that denaturation of protein by keeping a sealed sample at 100° C for 10 min causes an increase of k_1 and k_2, leading to values $k_1 = 12\ 4$ and $k_2 = 100\ 4$ for ovalbumin, and $k_1 = 10\ 36$ and $k_2 = 61\ 43$ for egg-white These values yield $w = 12\ 3 \times 10^{-2}$ and $\tau = 3\ 25 \times 10^{-8}$ sec for ovalbumin and $w = 8\ 7 \times 10^{-2}$ and $\tau = 2\ 63 \times 10^{-8}$ sec for egg-white, showing that the amount of irrotationally bound water increases as a result of denaturation This may be caused by unfolding of the polypeptide chains and exposing a greater number of polar groups to the surrounding water The increase of irrotationally bound water does not preclude that the whole amount of solvation water decreases

We thank Miss K Caputa for her help in preparing the samples and making measurements

O K -DASZKIEWICZ
J W HENNEL
B LUBAS

Institute of Nuclear Physics,
Polish Academy of Sciences

T W SZCZEPKOWSKI

Department of Physiological Chemistry,
Medical Academy,
Cracow, Poland.

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BIOCHEMISTRY

Preparation of a Low Molecular Weight Component with Lactogenic Activity from a Limited Chymotryptic Digest of Ovine Prolactin

THE limited digestion of ovine pituitary lactogenic hormone by chymotrypsin has recently been investigated in this laboratory[1,2] It was found that preparations of prolactin showed lactogenic activity even after 50 per cent digestion with the enzyme Ultracentrifugation investigations revealed that the prolactin core contained a component[3] which had a sedimentation coefficient close to that of the non-treated hormone The digest also contained another protein fraction which sedimented more slowly than the bulk of the material[3] This communication is concerned with the purification and properties of this latter low-molecular-weight component

Prolactin was digested and fractionated by a method essentially the same as that previously described[3] The hormone[4] (3 5 g) was digested to an extent of 50 per cent by incubation with bovine chymotrypsin (Armour Co , Lot No 381092) at 27° C in a *p*H 8 4 buffer containing 0 1 M sodium bicarbonate and 0 01 M *tris* (*tris*(hydroxymethyl)aminomethane) buffer The protein concentration was 1 per cent and the enzyme to substrate ratio was 1 300 The reaction was stopped by carefully adding dilute acetic acid in the cold with continuous stirring until the *p*H of the mixture was 3 The mixture was then dialysed against 0 1 M acetic acid After dialysis the protein was submitted to counter-current distribution in 2-butanol–0 35 per cent dichloroacetic acid[4] The main peak (which had a distribution coefficient of 1 67) was then submitted to chromatography on DEAE (diethylaminoethyl)-cellulose (Schleicher and Schuell Co , Lot No 1196) in *tris* buffer of *p*H 8 2 and ionic strength 0 01 After a prior washing of the column with 0 055 M sodium chloride, two fractions were obtained by elution with 0 1 M sodium chloride (Fraction I) and 0 25 M sodium chloride (Fraction II) Both fractions were again submitted to chromatography on DEAE-cellulose under identical conditions, dialysed and lyophilized

Fig 1 illustrates the results of experiments in which these and other fractions were studied by gel-filtration on 'Sephadex *G*–100' Untreated monomer prolactin (molecular weight 23,000)[3,5] emerged from the column after an elution volume of 50 ml (Fig 1*a*), whereas aggregated

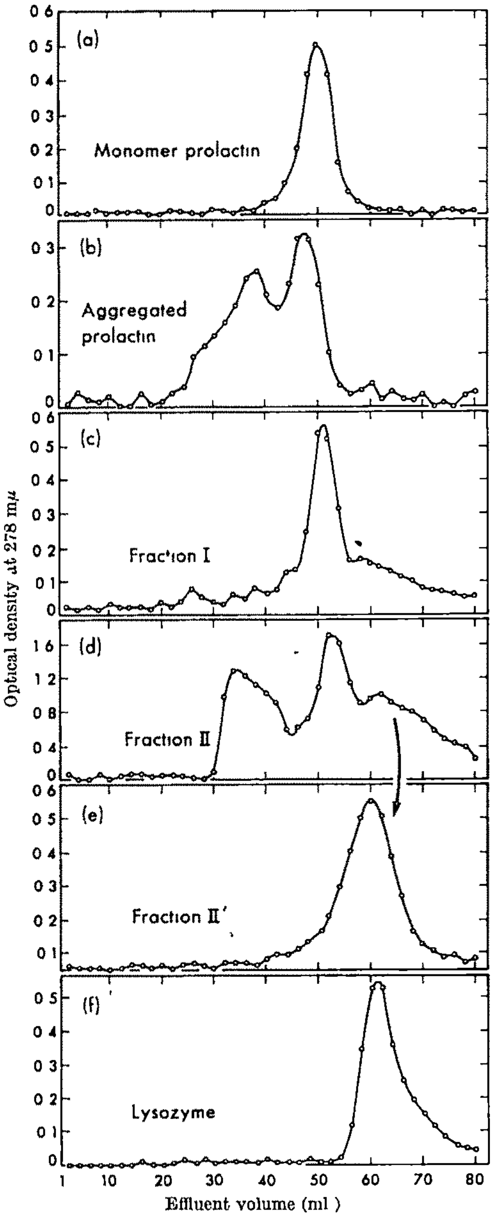

Fig. 1. Gel-filtration on 'Sephadex G-100' in 0.2 M ammonium acetate buffer of pH 8.2 at 20° C. Column size, 107 × 1.6 cm. Fractions of 2 ml. were collected. Flow-rate, 12 ml./h. a, 5.1 mg prolactin monomer (see ref. 3, Fraction A_1), b, 8.6 mg aggregated prolactin (see ref. 3, Fraction C), c, 7.0 mg Fraction I, d, 60.4 mg of Fraction II, e, 10.0 mg of Fraction II′, f, 3.3 mg of egg white lysozyme (Armour Co. Lot 381-187)

prolactin gave rise to multiple components (Fig. 1b). Fraction I, which emerged after the same elution volume as monomer prolactin (Fig. 1c), appeared mainly as a single peak. In contrast, Fraction II appeared to contain three components (Fig. 1d), including one which emerged from the Sephadex column after an elution volume of 60 ml. The latter component (which was designated Fraction II′) was purified by repeatedly submitting it to gel-filtration on 'Sephadex G-100' under the same conditions. Finally, it gave the elution pattern shown in Fig. 1e. In order to obtain a rough estimate of the molecular size of Fraction II′, a sample of egg white lysozyme (molecular weight approximately 14,000) was studied by gel-filtration on 'Sephadex G-100'. Fig. 1f shows that lysozyme was eluted from the column after about the same elution volume as Fraction II′.

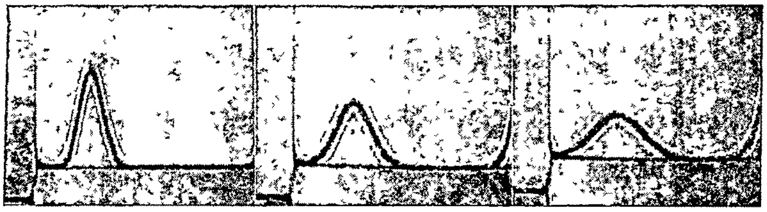

Fig. 2. Sedimentation of Fraction II′ in the ultracentrifuge at 52,640 r.p.m. and 22° C, 13.5 mg protein/ml. solvent, 0.1 M sodium bicarbonate buffer of pH 8.4, sedimentation from left to right, pictures taken at the following intervals after attainment of full speed: 4, 20 and 56 min, artificial boundary-forming cell used

Bioassay of Fraction II′ by means of local crop sac-stimulation in the pigeon[6] showed that it had approximately one half the lactogenic potency of the undegraded prolactin. The result of sedimentation velocity analysis of Fraction II′ in the ultracentrifuge is shown in Fig. 2. In 0.1 M sodium bicarbonate buffer of pH 8.4 the protein sedimented as a single boundary and the sedimentation coefficient ($S_{20,w}$) was found to be 0.88 S, whereas the untreated prolactin monomer has a sedimentation coefficient of 2.18 S under the same conditions[5]. Further investigations on this low molecular weight component possessing lactogenic activity are in progress.

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M. SLUYSER
CHOH HAO LI

Hormone Research Laboratory,
University of California,
Berkeley.

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Identities of the Fatty Acids derived from the Polymyxins and Colistin

Hausmann and Craig[1] first demonstrated that the peptide antibiotic polymyxin B was not homogeneous but could be separated by counter-current distribution into two components, polymyxin B1 and polymyxin B2. On hydrolysis, in addition to the amino-acids αγ-diaminobutyric acid, leucine, phenylalanine and threonine, polymyxin B1 gave (+) 6-methyloctanoic acid, which had previously been isolated and characterized by Wilkinson[2]. Polymyxin B2, although containing the same amino-acids gave an optically inactive acid, $C_8H_{16}O_2$, not identical with n-octanoic acid but which was not otherwise characterized.

We have now demonstrated by gas chromatography that the foregoing C_8 and C_9 acids are present, although in different proportions, in the crude fatty acids derived from polymyxin B, polymyxin E and colistin. In each chromatogram the presence of a trace (less than 1 per cent of the total acid) of a third acid was observed, which has been identified tentatively as n-octanoic acid. By counter-current distribution polymyxins B1 and B2 and polymyxins E1 and E2 were obtained. From the acid hydrolyses of polymyxins B1 and E1, (+) 6-methyloctanoic acid was isolated and characterized. Polymyxins B2 and E2 gave optically inactive acids, $C_8H_{16}O_2$, each of which was converted to an optically inactive amide, shown by the melting point, mixed melting point and infra-red spectrum to be identical with the amide of synthetic isooctanoic acid Further proof of identity was obtained by comparison of the gas chromatograms of the methyl esters and paper chromatograms of the hydroxamates of the natural and synthetic acids

S. WILKINSON
L A LOWE

The Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent

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Uncoupling Action of Chloramphenicol as a Basis for the Inhibition of Ion Accumulation

SUTCLIFFE[1] has reported that the bacteriostat chloramphenicol at concentrations of 2 g/l effectively stops ion accumulation by tissue slices of red beet root and carrot root Respiration and protoplasmic streaming were unaffected The concentration of chloramphenicol required was much higher than that needed for inhibition of protein synthesis in most bacteria (2,000 versus about 10 μg/ml.), but the necessity for high concentrations in plant and animal systems is commonly observed[2]. Sutcliffe assumed that chloramphenicol was acting as a specific inhibitor of protein synthesis, as in bacteria, and interpreted the results as evidence for a close relationship between the process of protein synthesis and salt accumulation as suggested by Steward[3] Jacoby and Sutcliffe[4] later showed chloramphenicol to inhibit uptake of carbon-14-labelled glutamic acid, glycine and proline by carrot tissue The incorporation of the amino-acids into protein was inhibited in short-term experiments, but glycine and proline incorporation was not affected in long-term (24 h) experiments Net protein synthesis was inhibited

This work of Sutcliffe's could have great theoretical importance, and we have accordingly included chloramphenicol in studies of calcium uptake by isolated mitochondria from etiolated maize shoots The experiments were patterned somewhat after those of Brierley *et al* [5] and Vasington and Murphy[6] About 100 g of 3½-day shoots (*Zea mays*, *WF*9 × *M*14) were ground in a cold mortar with about 250 ml of 0 25 M sucrose + 0 05 M potassium dihydrogen phosphate + 0 005 M ethylenediamine tetraacetic acid (EDTA) adjusted to *p*H 7 5 with *tris*(hydroxymethyl) aminomethane (*tris*) Mitochondria were isolated by centrifugation in the cold between 2,000*g* for 6 min and 12,000*g* for 15 min, and were washed once in the grinding medium and once in 0 25 M sucrose Final suspension was in 0 25 M sucrose The mitochondria were incubated for 10 min at 30° C with the additives listed in Table 1 Ice-cold 0 5 M sucrose was layered below the reaction mixture, and the mitochondria centrifuged through the sucrose The pellet was suspended in water and assayed for radioactivity A complete report on the properties of these mitochondria in absorbing calcium is being prepared For now it can be noted that except for some non-specific adsorption the calcium uptake is dependent on respiratory energy or ATP and is accompanied by phosphate, much as with animal mitochondria[5,6]

Chloramphenicol proves to be an inhibitor of calcium binding by mitochondria at concentrations in excess of 0 4 mg/ml At 1 6 mg/ml , which is in the range of concentration used by Sutcliffe, the extra calcium-binding mediated by substrate is virtually eliminated (Table 1)

However, it was difficult to imagine any appreciable protein synthesis occurring in these isolated mitochondria in the absence of added amino-acids In attempting to find an explanation we noted that the inhibitions of calcium binding were very much like those we had obtained with the uncoupling agent, 2,4-dinitrophenol Although chloramphenicol is not considered to be an uncoupling agent, it was conceivable that at these high concentrations some uncoupling of oxidative phosphorylation might occur

Table 1 INHIBITION OF CALCIUM-BINDING IN MAIZE MITOCHONDRIA BY CHLORAMPHENICOL

Substrate	Chloramphenicol	Total ^{45}Ca (c p m /mg N)	Substrate mediated binding (c p m /mg N)
–	–	26,400	—
+	–	65,900	39,500
+	0 8 mg/ml	45,100	18,700
+	1 6 mg/ml	29,000	2,600

Media contained 625 μmoles sucrose, 6 25 μmoles MgCl$_2$ 0 25 μmoles CaCl$_2$ + ^{45}Ca, 17 μmoles phosphoric acid neutralized with *tris*, 0 1 ml ethanol, 0 6 μmoles NAD, 0 4 μmoles thiamine pyrophosphate, 0 1 μmoles coenzyme *A*, 20 μmoles succinate + 40 μmoles pyruvate (as substrate where indicated), 0 335 mg mitochondrial N, and water to a volume of 2 5 ml The *p*H was adjusted to 7 5 with *tris* Chloramphenicol from Parke, Davis and Co (listed as 1,000 μg/mg) was dissolved in ethanol, and equivalent ethanol was added to controls The ethanol is slightly inhibitory to calcium-binding Specific activity of calcium was 172 c p m /mμ mole

Table 2 UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY CHLORAMPHENICOL

Chloramphenicol	Uptake O$_2$ (μ atoms)	Uptake PO$_4$ (μmoles)	P/O
None	16 8	29 2	1 74
0 8 mg/ml	14 8	8 8	0 59
1 6 mg/ml	9 1	1 3	0 14

Warburg vessels contained 425 μmoles sucrose, 5 μmoles MgCl$_2$, 40 μmoles succinate, 40 μmoles pyruvate, 42 μmoles phosphate, 100 μmoles KCl, 6 5 μmoles ATP, 0 6 μmoles NAD, 0 4 μmoles thiamine pyrophosphate, 0 1 μmoles coenzyme *A*, 2 5 μmoles EDTA, 100 μmoles glucose, 25 KM units hexokinase, 0 1 ml ethanol, 0 320 mg mitochondrial N, and water to 2 5 ml The *p*H was adjusted to 7 5 with *tris* Reaction was at 30° C for 15 min Chloramphenicol was added in ethanol as in Table 1, with equivalent ethanol in all vessels The ethanol is slightly inhibitory to phosphorylation

Table 2 shows that uncoupling does occur with concentrations of chloramphenicol which inhibit calcium binding. Oxygen uptake is definitely depressed at 1 6 mg/ml In some experiments 0 8 mg/ml chloramphenicol did not affect oxygen consumption, but phosphorylation was always lowered

Uncoupling agents such as dinitrophenol are known to inhibit salt accumulation by plant tissues[7], although the mechanism of inhibition is subject to various interpretations[8] Uncoupling could also reduce an endergonic process such as protein synthesis. Hence the effects of high concentrations of chloramphenicol ascribed to inhibition of protein synthesis might be due to inhibition of ATP synthesis The fact that respiration rates of tissue are not altered by chloramphenicol (or are even accelerated[1]) would be expected over a certain concentration range if the compound were uncoupling oxidative phosphorylation The depression of mitochondrial respiration by 1 6 mg/ml chloramphenicol (Table 2) is the result to be expected from high concentrations of uncoupler, dinitrophenol in excess of 10^{-4} M also depresses oxygen consumption of these mitochondria Calo *et al* [9] have reported variable effects of chloramphenicol on potato tuber tissue respiration, including an inhibition of 25 per cent

These experiments led us to the opinion that the inhibition of salt accumulation in plant tissue by high concentrations of chloramphenicol could be due to uncoupling of oxidative phosphorylation rather than to the action of the compound as a specific inhibitor of protein synthesis

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J B HANSON
T K HODGES

Department of Agronomy,
University of Illinois,
Urbana, Illinois

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Third Component Participating in the Superprecipitation of 'Natural Actomyosin'

IT has been shown that the superprecipitation of 'natural actomyosin' is subtly controlled by a minute amount of calcium ions[1-3], the superprecipitation is promoted or depressed by the increase or the decrease of calcium ions in the reaction medium respectively This unique action of calcium ions not only affords the basis for the relaxing mechanism of chelating agents[2] as well as physiological relaxing factor[3], but also appears to be closely associated with the link between excitation and contraction[4]

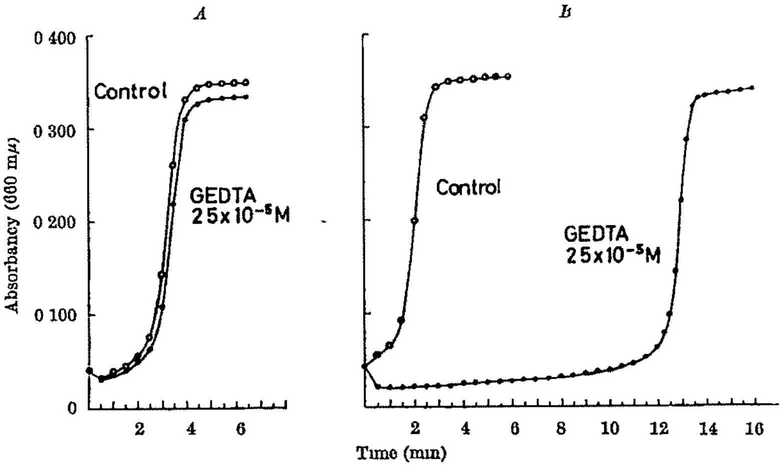

Fig 1 Effect of tropomyosin-like protein on the response of trypsin-treated 'natural actomyosin' to a chelating agent The suspension of 'natural actomyosin', 2 mg/ml , was incubated with trypsin, 10 μg/ml , for 20 min at 20° C After stopping the reaction by trypsin-inhibitor the actomyosin was centrifuged and resuspended for use *A*, The effect of glycoletherdiaminetetraacetic acid (GEDTA) on the trypsin-treated 'natural actomyosin', 0 78 mg/ml , *B*, the effect of GEDTA on the trypsin-treated 'natural actomyosin' plus purified tropomyosin-like protein, 0 08 mg /ml The method for measuring the superprecipitation followed those described in a previous paper (ref 3) For response of untreated 'natural actomyosin' to GEDTA see ref 8

In view of these findings and considerations it is interesting to enquire into a well-known fact[5-8], first noticed by Perry and Gray, that the reconstituted actomyosin is insensitive to the relaxing action of the chelating reagents, in other words, the reconstituted actomyosin lacks an important property of 'natural actomyosin' responsive to calcium ions

Muscle mince was first extracted with Guba-Straub solution according to the routine procedures of actin preparation, then the residue of filtration was exhaustively washed several times at 0° C and finally extracted by water containing 1 mM sodium bicarbonate at about 20° C for 4 h By filtration we obtained a very viscous solution, which was free of myosin and native actin, though it might contain some denatured actin If a fraction of the solution is added to the reaction mixture, the reconstituted actomyosin now behaves like 'natural actomyosin' in its response to calcium ions and chelating agents

Further purification of the active principle was made as follows solid ammonium sulphate was added to the original solution up to 40 per cent saturation Then the supernatant was dialysed against 0 2 mM sodium bicarbonate to eliminate the ammonium sulphate, and the volume of the solution was reduced by evaporating water under vacuum Then the concentrated solution was dialysed against 0 2 mM sodium bicarbonate to reduce its ionic strength below 0 02 Further dialysis against water acidified slightly by carbon dioxide precipitated the essential component After centrifugation the residue was dissolved again by adding a small amount of water by the aid of sodium bicarbonate The protein solution thus obtained has all the properties of tropomyosin found by Bailey[9,10] in every respect· (*a*) the amino-acid composition[9,11], (*b*) the relationship between viscosity and ionic strength[9], (*c*) the sensitivity to trypsin digestion[12]; (*d*) the binding to *F*-actin measured by flow birefringence[13], (*e*) the pattern of ultracentrifuge[10]; the preparation is not yet completely homogeneous but only one peak is appreciable which undoubtedly corresponds to that of tropomyosin In spite of these evidences, usual tropomyosin preparation according to the original method of Bailey[9] is not effective So far, it is not yet decided whether the active principle is the 'native' tropomyosin itself or not

The foregoing facts suggest that the calcium-sensitive nature of 'natural actomyosin' is due to the presence of the active principle in it As noted here, the factor is very sensitive to trypsin digestion Therefore, it is interesting to know whether the nature of trypsin-treated 'natural actomyosin' would become similar to that of reconstituted actomyosin and the addition of the active principle would recover the previous properties of 'natural actomyosin' Fig 1 shows one of the results along this line They are in good accordance with the expectation, indicating that 'natural actomyosin' functionally consists of three protein components—actin, myosin and the factor described here Essentially the same results were obtained with myofibrils

The interaction between actin and myosin in the presence of adenosine triphosphate undoubtedly represents the basic mechanism of muscular contraction However, if we are interested in the physiological contraction of living muscle, where calcium-sensitive property of contractile elements would be of primary importance, the role of the protein factor mentioned here, which resembles tropomyosin in many respects, would be highly evaluated

Details of this communication will be published elsewhere[14].

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SETSURO EBASHI

Department of Pharmacology,
Faculty of Medicine,
University of Tokyo, Bunkyo-ku.

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Cellulase in Tomato Fruits

A CELLULOLYTIC enzyme that reduces the viscosity of sodium carboxymethylcellulose (CMC) solution was found in sodium chloride extracts of ripe tomato fruits using the method of Bell *et al* [1] for determining activity. This is a viscometric method devised for detecting low enzyme-levels by measuring the change in drain time of solutions in Ostwald-Fenske pipettes during a 20-h period

Further extractions were made to compare the activity in the pericarp (wall tissue) and in the locular material (placenta, seeds, jelly and juice) Both salt and non-salt extracts were made The salt extracts were prepared by dissolving 5 g of sodium chloride in 100 g of the blended tissue, holding for an hour at room temperature, and centrifuging out the solids. Non-salt extracts were prepared similarly

In a separate set of salt extractions of the pericarp the activity of an aliquot held in boiling water for 5 min was compared with a non-boiled portion

The salt extracts of the pericarp reduced the drain time during a 20-h period an average of 37 0 per cent, while the non-salt extracts produced essentially no change (Table 1) However, salt had little effect on the extraction from the locular material, which was higher in activity than the pericarp The small difference due to salt with the locular material is probably due to the high juice content of this portion, which would probably contain the enzyme in solution from the breakdown of the cells around the seeds The extracts held in boiling water were found to be inactive

Table 1 PERCENTAGE DECREASE IN DRAIN TIME OF SODIUM CARBOXYMETHYLCELLULOSE DURING A 20-H REACTION PERIOD WITH TOMATO FRUIT EXTRACTS

Extract	Loss in drain time (%)
Pericarp	0 5
Pericarp plus NaCl	37 0
Locular	54 0
Locular plus NaCl	56 7
Pericarp plus NaCl	38 7
Pericarp plus NaCl, boiled	0 0

These results explain why Tracy[2] was unable to detect cellulase in tomato fruits although he did find the enzyme in many portions of the tobacco plant and in several other plants He used expressed sap, which if taken from the pericarp only is shown here to be extremely low in activity In addition, he used a short reaction time of 15 min, which was much too short a period to determine the very low activity

The fact that a cellulolytic enzyme is present in tomato fruits is extremely important in studies on fruit softening since the effect of the cellulolytic enzyme must be assessed as well as the effect of the pectolytic enzymes

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CHESLEY B HALL

Department of Food Technology and Nutrition,
Florida Agricultural Experiment Station,
University of Florida, Gainesville

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Detection of Phosphorothioate Pesticides

THE use of Gibbs's reagent[1] (2 6-dichlor- or 2 6-dibrom-*p*-benzoquinone-4-chlorimine (DBQC)) for locating certain organic thiophosphate pesticides on paper chromatograms was reported by Menn, Erwin and Gordon[2] The reagent forms coloured derivatives with a variety of compounds in aqueous alkaline solution, but the development of a red colour in neutral or slightly acid conditions appears to be quite specific for the P→S group, certainly when linked with solubility of the test substance in petroleum solvents The application of thin-layer chromatography with such a positive identification system offers the possibility of a rapid screening test for these pesticides in toxicological samples

Using a commercially available silica gel/plaster of Paris mixture having a *p*H 6 5 (determined as a 10 per cent suspension in distilled water) only a weak colour reaction was given with quite large quantities of various thiophosphate pesticides on spraying the developed place with 0 5 per cent DBQC in *cyclo*hexane and heating at temperatures up to 120° C However, if hydrochloric acid is added to the mixture used to prepare the plate, to give about *p*H 4, very intense red spots are obtained after spraying and heating to 100° C for 5–10 min, the background remains virtually colourless at this temperature The colour of the spots slowly changes to orange but is then stable for several days, the red colour may be restored by lightly spraying the plate with water

So far as can be ascertained, the reaction is given by all organic phosphorothioates and phosphorodithioates 1 μg produces a very distinct spot with the limit of detection about 0 1–0 2 μg Typical R_F values in benzene-1 2-dichlorethane (1+1) are methyl parathion 0 78, 'Delnav' (2 spots) 0 60 and 0 53, 'Asuntol' 0 37, and 'Diazinon' 0 25

Some variation in the quantity of hydrochloric acid required (which can be determined by titration to *p*H 4) may probably be needed with different silica gels, although a two-fold excess does not seem to cause any adverse effect Normally 10-g silica gel *G* (containing 13 per cent plaster of Paris) is stirred with 10 ml water and 10 ml 0 1 N hydrochloric acid, and the plate (0 75 mm layer) dried at 90°–95° C for 30 min

A concentrated benzene or petroleum ether extract of crops or foodstuffs can usually be applied directly to the plate since interfering substances (which give dark brown or black stains on colour development) remain at or near the origin spot With petroleum ether extracts of stomach contents there is sometimes more interference, but adequate purification can be achieved by partitioning with acetonitrile and spotting the acetonitrile extract.

D P BRAITHWAITE

Veterinary Research Laboratory,
P O Kabete, Kenya

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PHYSIOLOGY

Oxygen Depletion of a Limited Reservoir by Human Conjunctiva

ALTHOUGH the passage of oxygen across the tear-cornea interface is perhaps the best-known example of a direct pathway[1–3] from atmosphere to tissue, other superficial areas can also be demonstrated to receive oxygen in similar manner

An ocular tissue particularly interesting in this respect is the conjunctiva A significant flux across its air-exposed surface might not be unexpected since the maximum oxygen tension of its endogenous[4] vessels can be no more than that of arterial blood, 100 mm mercury, which is considerably less than that of air (155 mm mercury) Also bulbar conjunctiva, especially in close proximity to the cornea, might serve as an oxygen pathway between the atmosphere and the cornea

To measure oxygen flux across the air-conjunctiva interface, a flat surfaced polarographic oxygen electrode (Clark type[3,5], membrane covered) unit, 6 mm in diameter, was placed in direct contact with unanæsthetized human bulbar conjunctiva The polyethylene membrane, fitted over the entire end of the housing served both as an oxygen reservoir and the limiting boundary for a thin layer of *p*H 9 borate buffer solution (0 1 M in potassium chloride) bathing the platinum-reducing electrode This membrane ensures good electrical stability and is the oxygen reservoir of known volume for quantitative analysis By using a 12μ-thick membrane as the oxygen reservoir directly below a 25μ-diameter oxygen cathode, the observed drop in current is almost entirely controlled by the decrease in oxygen tension of the membrane and tissue immediately below the cathode

Current from the electrode was measured with a Beckman model 160 physiological gas analyser and was recorded on a Sanborn model 297 recorder unit The time for 90 per cent response for the entire system was 2 sec

The electrode assembly was allowed to stabilize in air-saturated isotonic saline, maintained at 35° C, for several minutes before each measurement Temperature artefact due to cooling of the electrode while on the conjunctiva was found to be minimal since the polyethylene membrane over the platinum cathode, being small in volume, came quickly to local tissue temperature (35° C) as noted from a thermistor attached to the electrode assembly

Fig 1 shows partial pressure of oxygen in the polyethylene membrane plotted against time while the electrode assembly remained in contact with the conjunctiva, 3 mm distant and 15 mm distant from the edge of the cornea (limbus) This figure provides data from which the rate of oxygen tension decrease in the polyethylene can be calculated These oxygen tension decrease rates can then be converted to ml /sec of oxygen flow from the polyethylene membrane using the Henry's Law constant for oxygen in polyethylene[6], namely, 0 053 c c (S T P)/c c polyethylene atmosphere Because of the relative thinness of the membrane compared with the diameter of the platinum cathode, this flow rate is also the rate at which the conjunctiva withdraws oxygen from its epithelial cells when these cells are in contact with a limited oxygen reservoir, provided by the air-saturated polyethylene membrane

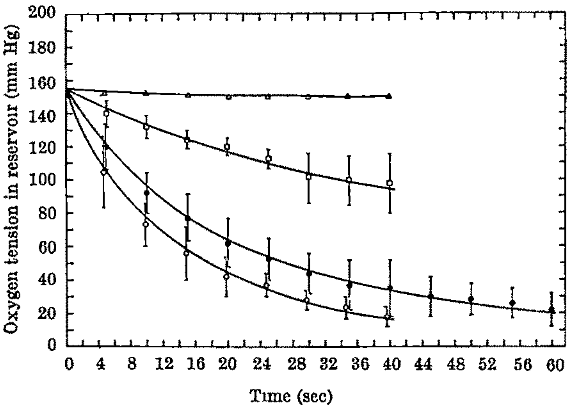

Fig 1 Reservoir mean oxygen tensions plotted against time for bulbar conjunctiva 3 mm (open circles) and 15 mm (closed circles) from cornea For comparison, similar tensions from the forearm (squares) and a synthetic leather surface (triangles) are given

The best estimate of the initial uptake of oxygen by the conjunctiva from this air-saturated membrane at a distance 3 mm from the cornea is 1 9 µl /cm²/h taken from the slope of the appropriate curve at the origin in Fig 1 The range due to scatter of data was 1 6–3 7 µl /cm²/h Rates of uptake at a point 15 mm from the cornea were not found statistically different. For comparison, similar measurements were taken on another air-exposed surface, the forearm—this curve is also given in Fig 1 The initial rate of uptake (at onset of local clamping) for this surface (pO_2 = 155 mm mercury) was 0 55 µl /cm²/h The previously reported value[3] for *in vivo* human cornea was 4 8 µl /cm²/h

Also given in Fig 1 is the control response for the electrode when placed against a moist surface impermeable to oxygen, in this case a soft synthetic leather surface Oxygen consumption of the electrode was in this way found to be negligible

Oxygen diffusion from air into the conjunctiva is too complicated, because of the continuous supply of oxygen by arterial blood and the simultaneous withdrawal by venous blood and tissue consumption, to be described by known solutions of the diffusion equations However, two highly over-simplified models of the air-conjunctiva system permit a very approximate calculation of oxygen flux at the air interface so that some measure of the validity of our experimental data can be obtained

In the first model we assume that for a few seconds after touching the air-saturated polyethylene membrane to the conjunctiva, the surface of the conjunctiva remains at 100 mm mercury, oxygen tension (arterial blood) Diffusion theory then predicts[7] a fall of oxygen tension at the electrode side of the membrane to one-tenth of the initial tension in time equal to 0 94 l^2/D_M, where l is the membrane thickness and D_M is the diffusion coefficient of oxygen in the membrane Fig. 1 gives $D_M = 3{\cdot}8 \times 10^{-8}$ cm²/sec Using this D_M we can calculate the oxygen flux across the membrane-conjunctiva interface for the model we have chosen Unfortunately, this model predicts an infinite flux at zero time, but after 5 sec the predicted flux, from a curve given by Crank[7], is 9 µl /cm²/h, after 20 sec the rate is 3 1 µl /cm²/h and after 30 sec the rate is 1·8 µl /cm²/h These calculated fluxes for the model are in rough agreement with the measured initial flux of 1 9 µl /cm²/h

A second possible model treats the conjunctiva as the surface of a semi-infinite medium with an initial oxygen tension of 100 mm mercury then suddenly exposed at its surface to a constant oxygen tension of 155 mm mercury The oxygen flux per unit area is $\Delta P k_t \sqrt{D_T/\pi t}$, where ΔP is the difference in oxygen tension (55 mm mercury), k is the solubility of oxygen in the tissue, D_T is the diffusion coefficient of oxygen in the tissue, and t is time[7] For this calculation the diffusion coefficient and solubility of oxygen in tissue was assumed to be the same as in 25 per cent protein solution, namely, $D_T = 1 \times 10^{-5}$ cm²/sec and $k_t = 2{\cdot}1 \times 10^{-5}$ ml O_2/ml tissue mm mercury The oxygen flux predicted by this calculation is 7 3 µl /cm²/h at 1 sec, 2 3 µl /cm²/h at 10 sec, and 0 7 µl /cm²/h at 100 sec These values are in reasonable agreement with our experimental value of 1 9 µl /cm²/h for the initial rate of oxygen withdrawal from a limited reservoir

It appears then that we have indeed observed the movement of oxygen into the body across two air-exposed surfaces, the conjunctiva and the skin of the forearm In retrospect our results are not surprising because even in the absence of oxygen utilization the oxygen tension in the body cannot be greater than that of arterial blood 100 mm mercury, and is probably less The gradient between air with an oxygen tension of 155 mm mercury and the body at 100 mm mercury causes a movement of oxygen into all air-exposed surfaces This oxygen is probably not an important source for the body, except in the case of the cornea[3], because it is a minute fraction of the total supply needed At 2 square metres body area and our observed rate of 0 5–2 µl /cm²/h, there is only about 0 01–0 04 l./h moving across the air-skin interface This rate may change at specific locations under pathological conditions of the skin The flux-measuring method we have described may have diagnostic value in these circumstances

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RICHARD M HILL
IRVING FATT

School of Optometry and Department of Mineral Technology,
University of California,
Berkeley

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HÆMATOLOGY

Limiting Factor in the Assay of Aldolase

Recently, investigations were carried out on the activity of aldolase in blood serum and plasma following whole-body irradiation[1]. Before starting further experiments it seemed necessary to determine whether the aldolase assay to be applied in these investigations can be performed with the same accuracy in blood and plasma. For this purpose the following investigations were carried out. Blood was withdrawn from the heart of guinea pigs by puncture and mixed with sodium oxalate to prevent clotting.

One part of this oxalated blood was hæmolysed by adding saponin; in another part plasma was separated by centrifugation. From these two samples and from the oxalated whole blood 1 : 2, 1 : 4, 1 : 8 serial dilutions were made. In each of these dilutions and in the undiluted (1 : 1) sample, aldolase activity was determined using a Unicam *S.P.*500 spectrophotometer, by the method of Tovarnicky and Voluyskaya[2], as modified by Ananyev and Obuchova[3], being based on similar principles to those used by Y. A. Sibley and A. L. Lehninger[4].

Thus, the blood samples were hæmolysed before the determination of activity of aldolase either by addition of saponin or simply by dilution with distilled water.

The results of these determinations show (Table 1) that the activity of aldolase of the plasma decreases with the degree of dilution. On the other hand, the activity of aldolase of the saponinized blood remains constant, being independent of the degree of dilution.

In oxalated whole blood, the aldolase activity never reached the activity found in diluted samples; even the activity of the 1 : 2 dilution was in some cases lower than the activity of the 1 : 4 and 1 : 8 dilutions.

This latter phenomenon found in non-saponinized oxalated whole blood might have its origin in the fact that the undiluted (1 : 1) and sometimes even the 1 : 2 diluted blood samples were not entirely hæmolysed before the aldolase assay. In some cases the aldolase activity of the 1 : 8 dilutions—saponinized and non-saponinized—show a moderate decrease.

To elucidate the cause of this discrepancy between the degree of dilution and aldolase activity, longer dilution series were made from 1 : 1 to 1 : 32 and 1 : 64, and in one part of these dilution samples aldolase activity was determined without centrifugation and in another after centrifugation at 4,000 r.p.m. for 10 min.

From Tables 2 and 3 it can be seen that activity of aldolase has the same value before and after centrifugation of the diluted samples and that, beginning with the 1 : 16 dilution, the activity of aldolase of all blood samples decreases with the degree of dilution. Consequently the

Table 1. Aldolase Activity of (*a*) Plasma, (*b*) Saponinized Oxalated Blood, (*c*) Oxalated Blood in Dilution of 1 : 1, 1 : 2, 1 : 4, 1 : 8

No.	*a*				*b*				*c*			
	1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8
1	—	—	—	—	38·0	35·5	37·6	25·3	10·5	21·2	31·9	31·5
2	—	—	—	—	26·5	24·9	31·5	26·2	9·2	17·1	27·9	26·7
3	42·5	25·6	11·3	6·4	56·8	59·2	58·6	53·0	32·0	28·0	51·2	45·1
4	31·9	22·0	15·2	7·3	46·0	—	—	44·5	18·2	35·9	43·2	39·0
5	25·0	11·7	7·1	3·2	39·4	45·0	48·5	37·9	22.0	39·5	40·6	34·8
6	29·1	14·7	4·8	3·1	53·0	50·8	41·4	35·0	21·0	34·3	38·4	28·3
7	29·5	12·5	8·5	2·7	50·5	61·8	54·2	42·5	21·0	48·5	52·2	36·5
8	32·7	16·6	10·7	4·0	50·0	54·0	54·8	45·4	14·8	20·2	39·7	31·8
9	27·5	16·0	9·2	3·0	40·5	53·5	54·0	55·4	9·4	38·5	46·7	35·8
10	25·8	16·2	8·8	5·3	59·5	69·2	55·8	57·5	17·0	45·5	45·5	37·3
11	29·0	15·4	7·7	4·9	74·5	68·2	67·5	54·6	16·3	54·9	47·7	35·7
12	22·7	9·7	5·0	1·5	60·5	49·0	41·5	—	15·0	51·9	50·0	62·2
13	34·9	18·3	10·8	6·0	68·0	75·7	62·7	49·7	21·1	50·4	47·9	31·8
14	44·8	27·9	19·9	8·7	61·5	64·3	63·1	46·1	29·3	47·7	53·1	39·8
15	37·4	19·0	10·0	6·0	—	—	—	—	30·1	41·2	57·4	55·6
16	34·8	20·0	8·3	4·3	62·4	73·2	78·3	54·5	16·2	48·0	49·0	51·5

Table 2. Aldolase Activity of Saponinized Oxalated Blood in a Dilution Series from 1 : 1 to 1 : 64 : (*a*) without Centrifugation; (*b*) after Centrifugation at 4,000 r.p.m. for 10 min

No.	*a*							*b*					
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:1	1:2	1:4	1:8	1:16	1:32
1	68·8	60·7	61·8	56·0	41·8	—	—	—	63·3	67·2	60·9	41·3	—
2	53·4	58·6	59·2	49·0	30·1	20·9	—	55·3	54·2	58·0	53·8	34·5	21·2
3	65·3	65·8	65·5	52·8	32·0	15·6	—	—	59·2	61·0	54·7	31·9	11·2
4	57·3	62·4	64·0	53·4	36·3	18·7	—	—	59·7	63·3	55·5	32·5	15·1
5	73·2	77·7	74·5	49·4	26·2	13·6	—	—	71·7	71·2	47·5	24·1	—
6	57·5	69·5	66·5	52·9	32·0	18·2	12·9	56·0	67·8	64·5	51·0	—	12·5
7	50·0	47·5	56·0	41·2	25·8	13·4	7·0	56·7	56·1	50·8	41·5	23·5	8·2
8	62·4	53·0	52·0	41·6	25·7	13·9	7·6	55·0	53·5	54·0	42·7	25·1	10·7
9	46·9	48·7	51·0	42·8	25·6	13·9	6·5	45·6	44·7	46·8	40·5	24·3	7·7
10	54·5	61·6	57·3	53·4	32·7	19·5	9·1	—	62·1	61·0	52·1	35·1	17·9

Table 3. Aldolase Activity of Oxalated Blood in a Dilution Series from 1 : 1 to 1 : 64 : (*a*) without Centrifugation; (*b*) after Centrifugation at 4,000 r.p.m. in 10 min

No.	*a*							*b*					
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:2	1:4	1:8	1:16	1:32	1:64
1	24·1	52·7	61·7	57·2	35·0	19·1	—	55·1	54·6	54·9	37·9	19·9	—
2	19·4	39·3	42·4	40·9	28·4	15·4	—	41·0	43·8	42·7	30·6	12·6	—
3	26·0	39·8	41·5	46·7	28·6	14·4	—	46·4	48·2	47·4	30·9	14·7	—
4	30·7	46·2	52·7	49·9	39·7	18·7	—	51·8	54·0	49·2	31·0	16·5	—
5	28·9	55·2	56·0	47·3	24·0	12·2	—	58·6	59·7	44·0	23·4	10·0	—
6	35·2	49·0	52·4	46·1	30·7	13·5	7·2	44·9	52·0	46·0	25·3	10·6	5·6
7	34·2	50·7	56·5	42·7	25·1	13·4	7·3	57·4	54·6	43·7	24·7	9·3	5·4
8	28·8	42·9	46·7	40·3	27·4	13·1	6·4	42·9	47·5	44·3	25·3	10·6	4·6
9	33·4	57·1	51·6	46·6	28·3	14·4	7·1	54·6	56·3	46·7	29·8	13·7	6·1
10	25·4	49·2	58·4	51·0	33·8	18·7	10·3	55·9	56·9	52·5	35·8	16·9	7·6

factor causing the discrepancy between activity of aldolase and degree of dilution is not bound to the stromata but is in the supernatant of the hæmolysed blood, and that from the 1 : 16 dilution this factor causes no discrepancy between activity of aldolase and dilution.

Whether the factor, limiting aldolase activity in whole blood, is the manifestation of enzyme equilibria, or a distinct substance, is to be a subject of further investigation.

These investigations show clearly that: (1) a factor liberated from blood cells by hæmolysis limiting activity of aldolase causes discrepancy between aldolase activity and grade of dilution; (2) the aldolase assay of Tovarnicky and Voluyskaya as modified by Ananyev and Obuchova giving reliable results with blood serum and plasma cannot be applied to whole blood.

BÉLA DALOS

Department of Biochemistry,
'Frédéric Joliot Curie' National Research
Institute for Radiobiology and Radiohygiene,
Budapest.

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Fundamental Function of Ac-globulin and Lipid in Blood Clotting

EVER since the last century when Alexander Schmidt introduced the idea that the clotting of blood can be due to a fibrin ferment which he named thrombin, efforts have been made to understand how this enzyme arises from prothrombin. He already had the view that lipids are concerned, but never knew about Ac-globulin, which is a plasma constituent important for the generation of thrombin activity in association with lipids.

For the purpose of finding out more about the way Ac-globulin and lipids function, work in this laboratory has been directed at the isolation of Ac-globulin from bovine plasma. Even though this endeavour has received constant attention since 1947, we still cannot describe a procedure for obtaining the Ac-globulin as pure as desired for a plasma protein. It is a labile substance. We can, however, achieve 1,000-fold purification in terms of plasma proteins, and by the criteria of immuno-chemistry and the ultracentrifuge (Fig. 1) the product is mainly Ac-globulin and hence very useful for kinetic investigations. Meanwhile thrombin has been purified[1]. A second enzyme was found in prothrombin[2,3] and obtained in pure form[4] (Fig. 1). The substrate, prothrombin, has been available in this laboratory as a purified protein for many years[5] (Fig. 1). With methods of analysis and the strategic proteins (Fig. 1), namely, prothrombin, thrombin, autoprothrombin *C* and Ac-globulin, it was possible to perform some instructive experiments.

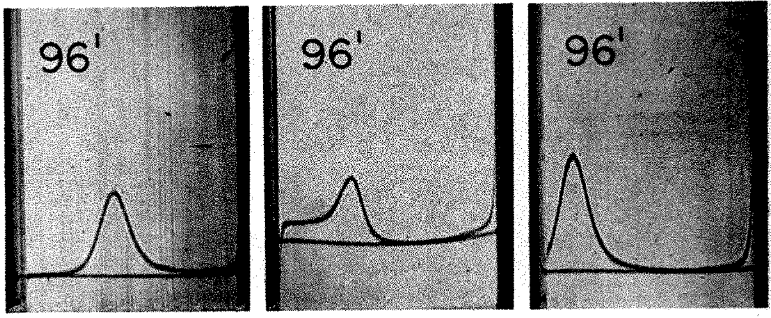

Fig. 1. Ultracentrifuge patterns of purified bovine prothrombin (left), Ac-globulin (centre), and autoprothrombin *C* (right) after 96 min in a 'Spinco' model *E* ultracentrifuge at 50,740 r.p.m. at 20° C. Concentration of protein was 10 mg/ml. We thank Dr. Charles R. Harmison for help in obtaining the ultracentrifuge data

We have come to the conclusion that the conversion of prothrombin to thrombin is fundamentally an autocatalytic process in which either enzyme from prothrombin may be the catalyst. The Ac-globulin alters the substrate specificity of the enzyme so that there are different degradation products of prothrombin when Ac-globulin is present from where it is absent. The lipids are nonspecific in their function and accelerate the complex interactions of the enzyme with substrate.

Considering the experimental facts, the simplest mixture of two proteins was autoprothrombin *C* and prothrombin. The prothrombin became changed to autoprothrombin I (ref. 6). With the same solvent, which contained calcium ions and also Ac-globulin, the prothrombin was converted to thrombin and autoprothrombin III (Fig. 2). Each of these end-products is a substance which we have isolated as a single component. Now when crude cephalin, as commonly prepared[7], was also added to the prothrombin + autoprothrombin *C* + Ac-globulin + calcium ions, the products of the reaction remained the same, but the rate at which they appeared depended on the concentration of lipid used (Fig. 2). Under suitable conditions the yield also increased.

These ideas can be represented by the following equations:

$$\text{prothrombin} \xrightarrow{\substack{\text{calcium ions}\\ \text{autoprothrombin } C}} \text{autoprothrombin I}$$

$$\text{prothrombin} \xrightarrow{\substack{\text{calcium ions}\\ \text{Ac-globulin}\\ \text{autoprothrombin } C}} \begin{array}{c}\text{thrombin}\\ +\\ \text{autoprothrombin III}\end{array}$$

$$\text{prothrombin} \xrightarrow{\substack{\text{calcium ions}\\ \text{Ac-globulin}\\ \text{autoprothrombin } C\\ \text{lipid}}} \begin{array}{c}\text{thrombin}\\ +\\ \text{autoprothrombin III}\\ \text{(rapidly)}\end{array}$$

The quantities of Ac-globulin, autoprothrombin *C*, and lipid in relation to the prothrombin were also varied in all kinds of proportions, including those in which com-

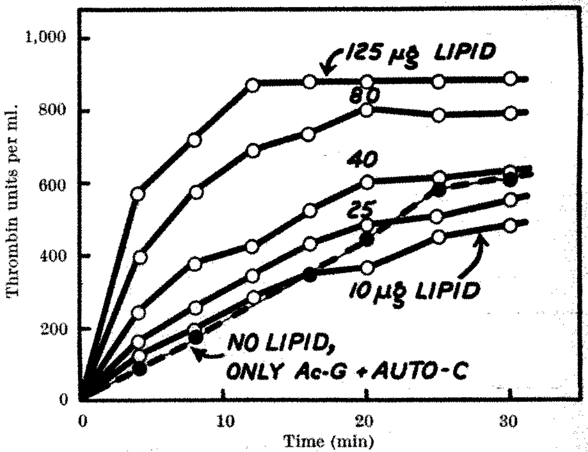

Fig. 2. Activation of purified prothrombin (2,900 U/ml.) at *p*H 7·2 and 28° C with Ac-globulin (80 U/ml.) and autoprothrombin *C* (45 U/ml.) with calcium 0·025 M is represented by lower broken line. No thrombin was formed with autoprothrombin *C* or Ac-globulin alone. The same activation mixture was studied by adding 10, 25, 40, 80 and 125 μg lipid/ml. Assays for prothrombin (ref. 10), thrombin (ref. 10), autoprothrombin *C* (ref. 7) and Ac-globulin (ref. 11) were carried out by previously described methods

plete activation of the substrate occurred in only a few minutes. In a typically slow activation, such as the top curve of Fig. 2, the μg proportions of prothrombin to autoprothrombin *C*, to Ac-globulin and to lipid were respectively 1,500 : 100 : 50 : 125.

Similar to circulating blood, where prothrombin, Ac-globulin, and calcium ions are side-by-side in the plasma medium, they can be together as concentrates in test tubes without changing. The enzyme autoprothrombin *C* does, however, use prothrombin as a substrate and degrades it to autoprothrombin I. The Ac-globulin changes the specificity of the enzyme and prothrombin becomes thrombin + autoprothrombin III instead of autoprothrombin I. The lipid does not change the basic events but alters the rate at which the enzyme does its work. Lipid presumably presents surface interfaces in terms of hydrophilic and hydrophobic structures. Orientation of the protein molecules occurs. The power of the lipid in terms of the rapidity of the reaction is really very impressive. Each component, be it calcium ions, lipid, autoprothrombin *C*, or Ac-globulin, has its purpose and function. What then can be a descriptive term for the function of Ac-globulin? We think the best one introduced thus far was by Ware, Murphy and Seegers[8], who called it a 'co-autocatalyst'. This is what it is in the sense of autoprothrombin *C* coming from prothrombin and catalysing the conversion of prothrombin to thrombin provided Ac-globulin is there. It cannot properly be called a coenzyme since these generally are small molecules and Ac-globulin is a protein.

Landaburu and Seegers[9] stressed their concept of the importance of thrombin in the conversion of prothrombin to thrombin. We have also performed many experiments with that enzyme. The general perspective is the same as with autoprothrombin *C*. First, the simplest combination of thrombin and prothrombin yields autoprothrombin II (with autoprothrombin *C* the product is autoprothrombin I). Then by adding Ac-globulin the enzyme specificity changes and the mixture of prothrombin + calcium ions + thrombin + Ac-globulin yields thrombin and autoprothrombin III. The further addition of lipid does not alter the reaction products, but speeds up their rate of formation and under appropriate conditions may increase the yield. Thus, either enzyme from prothrombin functions in autocatalytic events and Ac-globulin is the co-autocatalyst.

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WALTER H. SEEGERS
EDMOND R. COLE
NOBUO AOKI*
AURELIO OLIVEIRA

Department of Physiology and Pharmacology,
Wayne State University,
Detroit, Michigan.

* Fellow of the Michigan Heart Association.

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PHARMACOLOGY

Site of the Vascular Reponse to Thermal Injury

THE increase of vascular permeability in response to a number of types of mild injury—including thermal injury—is biphasic, consisting of a transient immediate phase, usually lasting 20–30 min, and a delayed phase which comes to a maximum several hours later[1–3]. The alteration of a vessel wall is demonstrable in animals with carbon particles or a vital dye in the circulating blood: in one case the carbon is deposited between the vascular endothelium and the basement membrane under it; in the other, dye-stained plasma exudes into the surrounding tissues. Majno *et al.*[4,5], applying these methods to the rat cremaster muscle, showed therein that venules and fine capillary vessels were readily distinguishable by their characteristic distribution and size, and that, according to the type of injury, venules alone or both venules and capillaries might be affected. We used Majno's technique to locate the site of vascular change during the response to mild thermal injury. A heated copper disk 9 mm in diameter, as used by Wilhelm and Mason[3] in their investigation of thermal injury to the dorsal skin of the rat trunk, was applied to the scrotal skin.

The permeability responses of the cremasteric vessels were similar to those of the dorsal skin. Since, however, the heat had to penetrate the full thickness of the scrotum, they were less intense for a given disk temperature, and they varied more widely in repeated tests because of the varying thickness of the scrotum, not only from animal to animal, but also as a result of unavoidably differing degrees of stretching of the skin during heating. As in the vessels of the trunk, short exposures of 5–10 sec at 54° C in animals under light anæsthesia induced only the immediate response, involving vessels over the whole of the heated area; whereas longer exposures, 40–60 sec, induced an immediate response, often confined to the edges of the heated area, followed by a delayed response involving vessels over the whole area. Moreover, the longer the application the sooner was the onset of the delayed phase.

Fig. 1 records the biphasic time-course of the response to 54° C for 40 sec in the cremaster muscle. Either pontamine blue (65 mg/kg) or 'Pelikan' ink, 1·0 ml./kg, after partial clearance by centrifugation for 30 min at 8,000*g*, was given intravenously when the thermal lesion had reached the desired age. Each point on the graphs represents the mean response in at least three animals. The exudation of dye was estimated by inspection as a percentage of the maximum blueing obtainable with the disk at 54°. The deposition of carbon in the vessels was determined by examination at a magnification of 18 times, and its amount estimated as the percentage of vessels affected, in terms of the total number of vessels

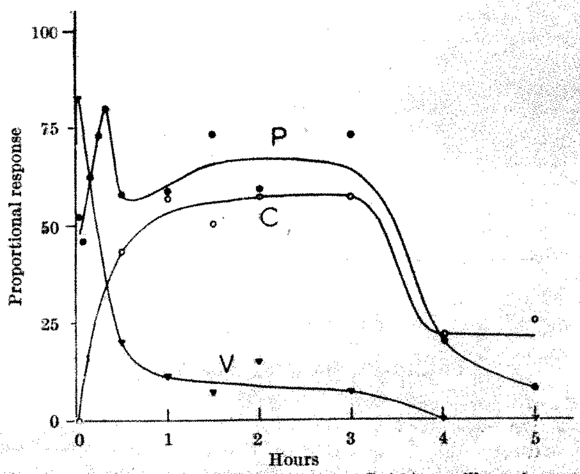

Fig. 1. *P* (●), permeability increase; *C* (○), capillary damage; *V* (▼), venular damage

of each class per unit area revealed by filling all the vessels with an ink–gelatine mixture.

C and *V* in Fig. 1 refer to the deposition of carbon in capillaries and venules respectively. The permeability (*P*) increase is biphasic, and the two phases are strikingly and unequivocally distinguished by the site of vascular damage; in the immediate phase it is venular, in the delayed phase it is capillary. The lag between the venular damage as revealed by carbon and by exudation of dye is noteworthy, and is under investigation by a more detailed analysis of this early phase. A similar but smaller series of tests on the skin of the trunk of mice, in which Prof. L. Kreyberg's strain of hairless (*hrhr*) mice[6] were used to facilitate application of the disk and subsequent observations, gave analogous results—an immediate monophasic response to 54° for 5 sec, associated with venular deposition of carbon and a biphasic response to 54° C for 20 sec. As in the rat, the immediate phase was venular; and in the delayed phase, capillary damage predominated. This clear distinction between venular and capillary damage was first observed by Majno[5], after local application of agents—such as heat, organic solvents and acids—capable of injuring the vascular wall directly. In the lesions induced by each of these agents there was a central area in which capillaries and all other vessels were damaged (direct injury), and around it a ring of venular damage, which he attributed to secondary liberation of endogenous permeability factors, such as histamine (indirect, or mediator-induced injury).

The importance of these observations is two-fold. First, the cremaster technique, and that with hairless mice, offer a means of distinguishing the sites of vascular damage in many forms of injury. We have briefly tested a number of bacterial toxins and living bacteria. All induced a venular followed by a capillary phase, and with some there was in addition a persistent involvement of numerous small veins (not venules) during both phases. In Dr. Majno's laboratory, Dr. Cotran (personal communication) has obtained essentially the same results. Secondly, the demonstration that the major, delayed phase of increased permeability may be associated with purely capillary damage raises doubts about the participation in that phase of some of the endogenous permeability factors proposed as mediators of inflammation[7,8]. In Dr. Majno's hands and ours, histamine, serotonin, bradykinin, globulin *PF* (ref. 8) and lactic acid induce venular changes of short duration. None of these substances, in some instances even when injected repeatedly, induced the capillary change that characterizes the delayed permeability phase of thermal injury. There is clearly at least a *prima facie* case against their participation in this phase of the vascular response to injury.

F. R. WELLS
A. A. MILES

The Lister Institute of Preventive Medicine,
London, S.W.1.

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PATHOLOGY

Thalidomide and Neoplasia

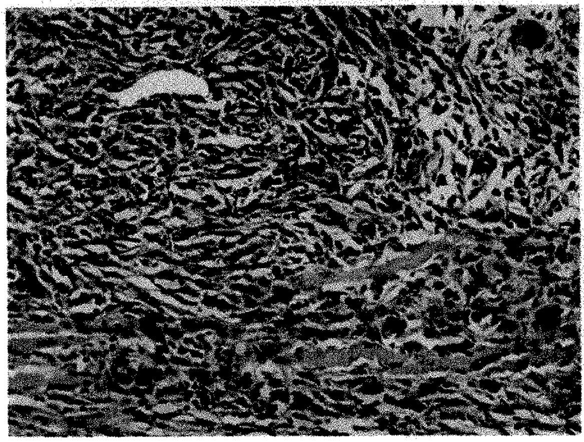

Fig. 1. Sarcoma penetrating voluntary muscle in a mouse injected repeatedly with thalidomide suspended in arachis oil

EARLY in 1962 Prof. Alexander Haddow suggested that we should test thalidomide for carcinogenicity. Accordingly, 20 male mice of the Chester Beatty stock strain were injected subcutaneously in the flank once weekly with 15-mg thalidomide suspended in 0·2 ml. arachis oil. A similar number of mice was injected weekly with arachis oil only and a further group left untreated. Injections of thalidomide were stopped at 57 weeks (total dose of thalidomide = 855 mg), but injections of arachis oil were continued for 90 weeks.

At the time the injections were stopped in the thalidomide-treated mice rapidly growing masses were observed at the injection site in two of the five surviving mice. One of the tumours proved to be a moderately well-differentiated spindle cell sarcoma and the other a more anaplastic sarcoma (Fig. 1). The latter was transplanted subcutaneously into five mice of the same stock strain: the transplants grew progressively in three. The mouse which bore the more anaplastic tumour also had an adenomatous tumour of the lung. No tumours were seen in the 15 mice which died prior to the fifty-seventh week, nor in the remaining three mice which survived beyond that time. One mouse in this group is still alive. No injection-site tumours have arisen in the arachis oil-treated controls, eight of which survived beyond the fifty-seventh week, and three of which are still alive. Two mice in this group were found to have both malignant lymphoma and parenchymal cell hepatomas at post-mortem; such neoplasms occur not infrequently in untreated mice of the same strain. Fifteen of the untreated controls died prior to the development of the first injection-site tumour in the thalidomide-treated group; two with malignant lymphoma. No other neoplasms were seen in these mice, four of which are still alive.

As part of another experiment designed for a different purpose, one male and two female mice were injected subcutaneously daily for seven days each week with 7·5 mg thalidomide in 0·1 ml. arachis oil until they had received a total of 220 injections (total dose of thalidomide = 1·65 g). During the eleventh month one of the females developed a spindle cell sarcoma at the injection site. The remaining two animals developed no tumours and were killed at 12 months.

While we are confident that the three injection-site sarcomas seen in these experiments may reasonably be attributed to thalidomide treatment, we stress that the carcinogenic effect is very weak. Despite the injection of very large doses the incidence of tumours was low and the induction-time long. Furthermore, we point out that the method of administration was not that used when thalidomide was used pharmaceutically. This communication is presented solely because it is felt that our observations may have some basic importance and implications. There is no case for extrapolating the findings to the human situation, and no reason to suspect that thalidomide administered orally in much lower doses, or acquired via the placenta, has any influence on the likelihood of developing neoplastic disease.

There is another cogent reason for doubting the biological significance of the experimental results presented here Certain types of biological activity, in addition to teratogenicity[1], are commonly found in association with carcinogenicity, particularly antimitotic activity, tumour-inhibitory activity, and mutagenicity If thalidomide possesses such activities it does so only in small degree The suggestion that thalidomide may possess tumour-inhibitory activity[2,3] met with negative results in practice Bach *et al* [4] saw no inhibition of two transplantable mouse tumours, and, at the Chester Beatty Institute, we saw no inhibition of growth of the transplantable Walker rat carcinosarcoma 256 in response to thalidomide administered orally or intraperitoneally in repeated large doses Roath *et al* [5,6] observed "an inhibition of some aspects of cellular activity" in leucocyte cultures exposed to thalidomide, but the significance of their findings has been disputed[7] Luers[8] found no evidence that thalidomide causes mutations in *Drosophila*

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F J C Roe
B C V Mitchley

Chester Beatty Research Institute,
Institute of Cancer Research
Royal Cancer Hospital,
Fulham Road,
London, S W 3

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IMMUNOLOGY

Template Requirement in the Primary Antibody Response

In most considerations of the role of the antigen in antibody formation the assumption is made that a template is either formed or activated A template, by definition, is a system whereby many replicas are produced The need for a template is based largely on the secondary response in which large quantities of antibodies are produced in response to small quantities of antigen over a long period of time This, indeed, does appear to require a template I wish to question whether the information available indicates that primary antibody formation requires a template

In earlier papers[1-3], we have discussed quantitative aspects of the antibody response In one of these papers[2] a graph was constructed relating antigen dosage to antibody response for diphtheria toxoid and poliovirus in man The secondary responses showed much more antibody formed than antigen administered 10^{-12} moles of diphtheria toxoid/kg produced about 10^{-6} moles of antitoxin/l while 10^{-14} moles of poliovirus/kg produced about 10^{-12} moles of antibody/l The primary responses were in marked contrast to this 10^{-9} moles/kg of toxoid produced about 10^{-9} moles of antitoxin/l and 10^{-14} moles/kg of poliovirus produced about 10^{-14} moles of antibody/l

It is true that serum protein antigens do not appear to follow this pattern For example, rabbits given a primary intravenous injection of 8×10^{-8} moles/kg of bovine-γ-globulin produced 6×10^{-6} moles/l. of antibody[4] Similarly, chickens given 2×10^{-7} moles/kg of bovine or human-γ-globulin produced respectively 2×10^{-5} and 1×10^{-5} moles/l of antibody while 4×10^{-7} moles/kg of bovine or human albumin produced 2×10^{-5} and 1×10^{-5} moles/l of antibody[5] In all these cases the amount of antibody formed is approximately two orders of magnitude greater than the amount of antigen given It should be stressed, however, that serum proteins given as single injections are always used in considerably larger doses than are antigens of microbial origin This raises the question as to whether a primary response is being measured or a combination of primary and secondary responses The latter concept gains some support from the fact that a secondary response to bovine-γ-globulin is only about twice the primary response[4]

While such calculations cannot be precise, it is difficult to believe that two antigens used in very different amounts, of different origins and of different antigenicity should both produce in the order of 1 molecule of antibody for 1 molecule of antigen as a purely accidental phenomenon If on primary immunization this is true, then a template need not be postulated While direct use of the antigen for a single moulding of antibody is attractive, this appears inconsistent with present-day knowledge of protein synthesis mediated by RNA The clonal selection theory sees antigen as playing a purely stimulatory part in the replication of lymphoid cells already producing the specific antibody It is difficult to see how this would be consistent with a 1 1 ratio of antigen and antibody

K M. Stevens

Department of Medicine,
College of Medicine,
University of Kentucky,
Lexington

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Active Immunization of a Human Being against Cobra (*Naja naja*) Venom

Because of the complexity of venom proteins and their great local and systemic effects, active immunization has been confined to animals utilized in the production of commercial antivenins In work recorded here, the feasibility of a safe and practical method of immunization of man against snakebite has been explored

Parrish and Pollard[1] found that immunity against the effect of snakebite did not exist among 14 human subjects, each of whom had been bitten twice or more by crotalid snakes However, the development of a persistent immunity might not be anticipated in this group because envenomation occurred from 2-year to 14-year intervals, and the members of the group were also treated with antivenins following snakebite The first observation of active immunization against snakebite was reported by Haast and Wiener[2] A subject (human) was given repeated injections of crude cobra venom over a period of 2 5 years A maximal injection of 40 mg of venom was tolerated, this quantity of venom is lethal in the absence of immunity Immunological tests were not performed with the serum or globulin to determine the degree of protection afforded by these injections, but the 40 mg immunizing dose of venom he tolerated is significant

Wiener[3] described in one human subject the titre rise and persistence effected by repeated injections of Australian tiger snake venom This subject survived the bite of a snake of this species with little difficulty subsequent to his immunization

In this laboratory, in an attempt to measure the development of neutralizing antibodies, a human subject

Table 1 IMMUNIZATION SCHEDULE OF HUMAN SUBJECT

Days	0	6	10	14	20	27	33	40	47
Dose	0 050 mg	0 100 mg	0 150 mg	0 500 mg	0 250 mg	0 350 mg	0 500 mg	0 750 mg	1 mg
Days	54	61	72	86	100	30-day rest	138	130	145
Dose	2 mg	4 mg	6 mg	8 mg	10 mg	30 day rest	6 mg	8 mg	10 mg

was given 17 injections containing increasing amounts of Indian cobra (*Naja naja*) venom. Venom solutions were passed through a 'Millipore' filter and then added to an equal volume of a 4 per cent sodium alginate adjuvant (Colab Laboratories, Box 66, Chicago Heights, Illinois) The 17 injections were given over a period of five months (Table 1) 10 ml of blood was withdrawn from the subject after the fourth, seventh and tenth injections 500 ml of blood was withdrawn after the eleventh injection and 1 litre after the seventeenth Globulins were separated from serum by ammonium sulphate precipitation, and the antivenin titre was determined by neutralization of *Naja naja* venom in 18–20 g Swiss mice Identical neutralization tests were conducted by a commercial laboratory (Table 2)

Table 2 DRIED GLOBULIN RESTORED TO FLUIDITY 1 G PLUS 8 0 C C DILUENT FOR ANTIVENIN NO 349207

Venom, LD_{50}	168 mg (1 LD_{50} = 0 00595 mg)
Neutralization	Globulin versus venon
	1 c c of human globulin solution as prepared here neutralizes 36 LD_{50} cobra venom (0 244 mg neut /c c)

The results of this work show a development of active immunization against cobra venom 1 ml of the globulin solution neutralized 30 mouse LD_{50}'s (0 244 mg venom) Since it required 100 ml of whole blood to produce 1 g of the crude globulin, it is estimated on a blood volume basis that 40 g are present in the subject's circulation This circulating globulin alone, therefore, should neutralize a dose of 100 mg cobra venom This calculation does not take into account the extravascular globulins present in the area of envenomation, which themselves are capable of venom neutralization

The subject receiving cobra venom was under observation by a physician Periodic blood and blood chemistry examinations were conducted to determine possible pathological conditions arising from the injections Examination of the subject during and after the injections indicated the absence of pathological response to the venom except for local swelling and pain, which persisted from one to three days in the area of injection For a 2-week period after the fifteenth injection of venom the subject's blood pressure was elevated slightly

The potential advantages of active immunization of man against envenomation have been recognized for many years The presence of locally necrotizing enzymes in some venoms still presents a formidable obstacle However, in venoms with little or no local toxicity it appears that man may be safely immunized

I thank Wyeth Laboratories, Marietta, Pennsylvania, for their assistance in neutralization tests described here

HERSCHEL H FLOWERS

Pathology Division,
U S Army Medical Research Laboratory,
Fort Knox, Kentucky

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RADIOBIOLOGY

Cæsium-137 Content of Fresh-water Fish in Finland

INVESTIGATIONS of the diets of the Finnish Lapps[1,2] have revealed that fish is the second important source of radiocæsium for several Lapp groups Unexpectedly, high radiocæsium values, especially in pike, were obtained in the first analyses of Lapland fish[2] Therefore it seems important to carry out substantial research on the hydrospheric chains of fall-out nuclides both in Lapland and other parts of Finland In the following the results of our fish analyses up to the present time are briefly reported These include analyses of cæsium-137 and potassium in 23 samples of fish from the Finnish Lapland and a number of samples from 2 lakes in southern Finland

The fish were caught during the period 1961–63 The main part of the samples, 21 in number, were taken from Lake Inari (28° E , 69° N), one sample from the Kemi river (24° E , 66° N) and one from the Nakkala river (24° E , 69° N) The samples taken for comparison are from southern Finland, from the Pyhajarvi (23° E , 61° N) and Nasijarvi (23° E , 62° N) lakes, and were caught in the spring of 1963 In addition, we have analysed 3 fish samples from the Varanger fjord on the Arctic Ocean (30° E , 70° N), caught in May 1962 At the fishing place the fish were weighed and their lengths were measured After this the entrails and heads were removed and the fish were soaked in formalin so as to preserve them Later a scale sample was taken for age determination Also, the 'teeth' of the gill arches of the whitefish were counted in order to determine the subspecies The fresh weight of the samples was usually 1 5–2 kg The fish were dried at 100°–110° C They were ground and their activity was measured in a cylindrical plastic container, the diameter of which was the same as that of the crystal on which it was placed The volume of the container was 0 5 l

The 512-channel pulse-height analyser was made by the Finnish Cable Works, and Harshaw's 3 in × 5 in sodium iodide crystal was used The calibration of the system for the determinations of cæsium-137 was made with a Radiochemical Centre standard solution *CDR* 4 2/11/60 For the potassium calibration Merck's potassium chloride was used In the cæsium-137 range (0 60–0 72 MeV) the sensitivity was 13 9 c p m /nc. and in the potassium range (1 38–1·56 MeV) 7 38 c p m /g potassium The corresponding backgrounds were in the cæsium-137 range 2 90 c p m and in the potassium range 1 49 c p m The potassium correction in the cæsium-137 range was 0 26 c p m /g potassium The lead shield used for the reduction of the background count was 12 cm thick The system was calibrated for the density 1 0 and the correction for the variation of the density was 3–4 5 per cent Each sample was measured for 1 h and in the cæsium-137 determinations the value of 2σ varied as follows When the cæsium-137 content was 0 1 nc /kg (fresh weight) $2\sigma = \pm$ 14 per cent and when the cæsium-137 content was 5 0 nc /kg (fresh weight) $2\sigma = \pm 1$ per cent In the potassium determination 2σ was about ± 6 per cent The results of the measurements are presented in Table 1 The samples taken in January 1962 were analysed at Lund[2]

An examination of the Lapland samples reveals that the cæsium-137 content of various fish species vary rather much (0 6–6 nc /kg) If the samples are divided into two groups on the basis of their activity using 2 nc /kg as the dividing line it will be noted that the fish containing more than 2 nc /kg are fish of prey and that the low activity group includes only grayling and whitefish, which feed on insects, insect larvæ and plankton

Several sub-species of whitefish are distinguished in Lake Inari, three species of which were analysed separately (Table 2) Considerable inconsistency prevails regarding the Latin names of these sub-species Those used in Table 2 were given by Jarvi[3] For the *C fera* Jur f *inarensis* Jarvi the name *C pidschian* Gmelin is also used The samples were taken in July 1962

Table 1 CÆSIUM-137 BURDEN OF LAKE FISH IN LAPLAND

When caught (month–year)	Where caught	Fish species	Age (year)	Cæsium-137 (nc /kg fresh weight)	Potassium (g/kg fresh weight)	Cæsium-137 nc /g potassium
				2σ	2σ	
Sept '61	Kemijoki	Pike (*Esox lucius*)		3 90 ± 0 05	3 7 ± 0 4	0 95
Jan '62	Inari	Pike		2 78 ± 0 05	2 9 ± 0 4	0 96
,, ,,	,,	Whitefish (*Coregonus lavaretus*)		0 64 ± 0 03	1 2 ± 0 3	0 53
May '62	,,	Perch (*Perca fluviatilis*)		2 41 ± 0 05	2 9 ± 0 4	0 83
,, ,,	,,	Whitefish		0 83 ± 0 03	3 0 ± 0 4	0 28
July '62	,,	Brook trout (*Salmo salvelinus salvelinus*)	6	3 22 ± 0 05	3 4 ± 0 4	0 94
,, ,,	,,	Perch	10	2 81 ± 0 05	3 0 ± 0 4	0 93
,,	,,	Lake trout (*Salmo trutta lacustris*)	6	2 27 ± 0 04	3 6 ± 0 4	0 63
,, ,,	,,	Pike	8	1 74 ± 0 07	3 5 ± 0 4	0 50
,, ,,	,,	Grayling (*Thymallus vulgaris*)	3	1 01 ± 0 04	4 0 ± 0 4	0 25
,, ,,	,,	Whitefish	6	0 95 ± 0 06	3 5 ± 0 4	0 27
,, ,,	Nakkalanjoki	Brown trout (*Salmo trutta fario*)	5	2 70 ± 0 05	4 6 ± 0 4	0 59
Sept '62	Inari	Pike	4	5 90 ± 0 06	3 3 ± 0 4	1 79
,, ,	,,	Lake trout	6	3 46 ± 0 05	3 2 ± 0 4	1 08
,, ,,	,,	Grayling	4	1 82 ± 0 04	4 3 ± 0 4	0 42
,, ,,	,,	Whitefish	4	1 11 ± 0 04	3 6 ± 0 4	0 31
Mar '63	,,	Lake trout		4 65 ± 0 06	3 2 ± 0 4	1 45
,, ,,	,,	Arctic char (*Salmo salvelinus alpinus*)		4 65 ± 0 06	3 3 ± 0 4	1 41
,, ,,	,,	Brook trout		3 84 ± 0 05	2 6 ± 0 3	1 48
,, ,,	,,	Whitefish	8	1 56 ± 0 04	2 8 ± 0 4	0 56

Table 2 CÆSIUM-137 (NC /KG FRESH WEIGHT) IN THREE SUB-SPECIES OF WHITEFISH FROM LAKE INARI, JULY 1962

	Average no of gill arch teeth (a) acc to Järvi	Average no of gill arch teeth (b) in sample	Average age (yr)	Cæsium-137 (nc /kg)
Coregonus fera Jur f *inarensis* Järvi ('Jokisiika')	21 6	22 0	5	0 57
Coregonus wartmanni Bl f *borealis* Järvi ('Siika')	33 1	32 0	6	0 95
Coregonus holsatus Thien f *inarensis* Järvi ('Lehtisiika')	24 0	24 3	7	1 34

Table 3 CÆSIUM-137 IN FISH CAUGHT IN THE ARCTIC OCEAN AND LAKES IN SOUTHERN FINLAND

When caught (month–year)	Where caught	Fish species	Age (year)	Cæsium-137 (nc /kg fresh weight)	Potassium (g/kg fresh weight)	Cæsium-137 nc /g potassium
				2σ	2σ	
May '62	Arctic Ocean Varanger Fjord	Salmon (*Salmo salar*)		0 15 ± 0 02	4 4 ± 0 4	0 034
,, ,,	,,	Halibut (*Hippoglossus vulgaris*)		0 07 ± 0 02	3 8 ± 0 4	0 0053
,, ,,	,,	Cod (*Gadus callarias*)		0 02 ± 0 02	3 8 ± 0 4	0 0018
Nov 62	Nasijärvi	Bream (*Abramis brama*)	15	0 54 ± 0 03	3 8 ± 0 4	0 14
Feb '63	,,	Perch-pike (*Lucioperca sandra*)	4	1 86 ± 0 04	3 1 ± 0 4	0 60
,, ,,	,,	Burbot (*Lota vulgaris*)		1 82 ± 0 04	3 2 ± 0 4	0 57
,, ,,	,,	Pike		1 80 ± 0 04	2 8 ± 0 4	0 64
,, ,,	,,	Whitefish	5	1 40 ± 0 04	3 2 ± 0 4	0 44
,, ,,	Pyhajarvi	Roach (*Leuciscus rutilus*)	8	0 25 ± 0 02	2 7 ± 0 4	0 093
,, ,,	,,	Pike	4	0 24 ± 0 02	3 3 ± 0 4	0 070
,, ,,	,,	Ide (*Leuciceus idus*)	6	0 18 ± 0 02	2 7 ± 0 4	0 067
,, ,,	,,	Silver bream (*Abramis farenus*)	7	0 17 ± 0 02	3 0 ± 0 4	0 057
,, ,,	,,	Burbot		0 17 ± 0 02	2 0 ± 0 3	0 085
,, ,,	,,	Bream	8	0 10 ± 0 02	2 4 ± 0 3	0 073

The results show a tendency in the direction of diminishing activity when the feed changes from plankton to bottom animals In general the activity of the whitefish unambiguously seems to have risen during the year 1962 An analysis of the activity of pike and trout also supports this fact

If the Lapland samples are compared with Arctic Ocean samples (Table 3) there is a clear difference between the cæsium-137 values The activity of the Arctic Ocean samples is only about 1/20th of that of the Lapland samples It also seems that the cæsium-137 values of the fish in southern Finland are somewhat lower than in Lapland So far as the samples from southern Finland are concerned, however, comparison is difficult for the reason that the Nasijarvi and Pyhajarvi samples represent quite different levels of activity For example, if the cæsium-137 activities of fish of the same species, burbot and pike, are compared in the two lakes, 8–10 times higher activities are observed in the Nasijarvi samples These two lakes are reported to represent two different lake types, Nasijarvi being a humus type of lake, Pyhajarvi a low-humus 'rich' lake Humic acids are weak cation exchangers and some limnologists consider them to represent an important dietary item for the zooplankton It thus seems that the fish get the main part of their radiocæsium through their food intake even though a smaller part may come directly from the water through the mechanism in the gills which regulates the salt balance Also, it seems plausible that the high cæsium-137 contents found in the fish of prey in the two lakes of humus-type is due to a food chain humus—zooplankton—small fish—big fish

Age determinations were made of 22 fish samples dealt with here No clear correlation between the age and the cæsium-137 content of fish of the same species could be observed Fresh-water fish have been analysed since 1961 for radionuclides in Sweden too, with results of a similar type[4]

The results presented here will form a basis for the planning of more comprehensive studies of the cæsium-137 content of fish in which attempts will be made to clarify the enrichment chains of cæsium-137 from the water through plankton and bottom animals to the fish

Limnological advice by Dr Reino Ryhanen and aid in acquiring some of the fish samples by Mr Jorma Toivonen are gratefully acknowledged The age determinations were made by Mr Lauri Peippo

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ERKKI HASANEN
JORMA K MIETTINEN

Department of Radiochemistry,
University of Helsinki,
Finland

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BIOLOGY

Liberation of Extracellular Nitrogen by Two Nitrogen-fixing Blue-green Algae

In a previous communication[1] it has been shown that certain nitrogen-fixing blue-green algae isolated from marine environments liberate appreciable quantities of fixed nitrogen into the medium during healthy growth. This feature has been observed for many blue-green algae by other workers[2-4] and investigations on the production of extracellular nitrogen have also been carried out[5-7] This communication provides information on the relationship between growth and liberation of extracellular nitrogen by the two nitrogen-fixing species *Nostoc entophytum* and *Calothrix scopulorum*, and on the nature of the extracellular products

The strains utilized were those used in previous investigations[1,8] and were available in pure culture The correlation between growth and liberation of extracellular material was investigated by growing the algae in nitrogen-free medium under conditions which, apart from a more vigorous air supply (3 5 c c /flask/sec) and higher temperature (mean 27° C), were similar to those described elsewhere[1]. The rate of production of extracellular nitrogen was studied over a 24-day period, the nitrogen liberated being determined at 4-day intervals by collecting the contents of triplicate flasks and obtaining nitrogen[9] and dry weight[1] data Alga and medium were separated through a bacteria-proof filter and the algal filaments gently washed twice with distilled water, the washings being added to the filtrate The results obtained are presented in Fig 1 It is evident that vigorous fixation of nitrogen by both species occurred during the experimental periods and microscopic examination revealed no unhealthy filaments Thus, the extracellular nitrogen did not appear to be due to cell autolysis Both algae show a high initial exudation rate, which with *Calothrix* decreases with age up to the 12–16-day stage and thereafter markedly increases The pattern of exudation by *Calothrix* is very similar to that recorded for *Anabaena*[5], and while *Nostoc* showed no latter increase in extracellular nitrogen it is possible that had the experiment been continued further an increase would have occurred

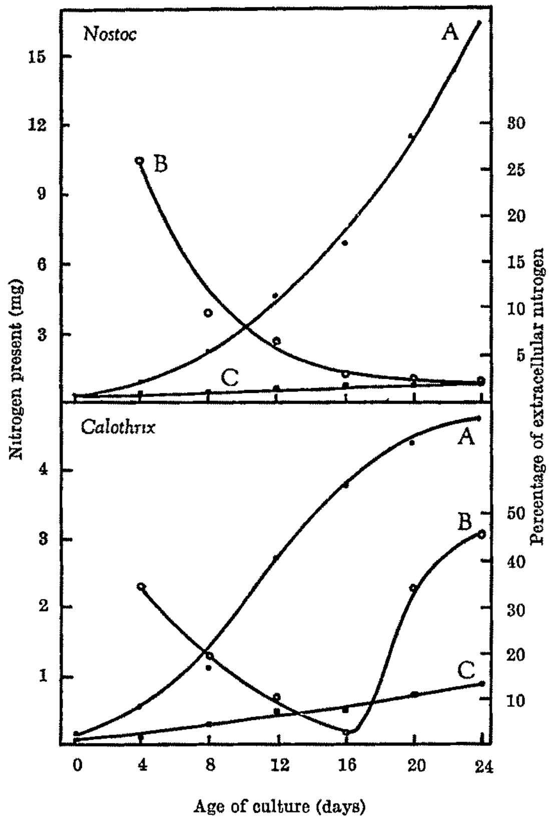

Fig 1 Liberation of extracellular nitrogen by *Nostoc* and *Calothrix* *A* represents total nitrogen fixed, *B* represents nitrogen liberated during preceding 4-day periods as percentage of total nitrogen fixed during same period, *C* represents mean extracellular nitrogen

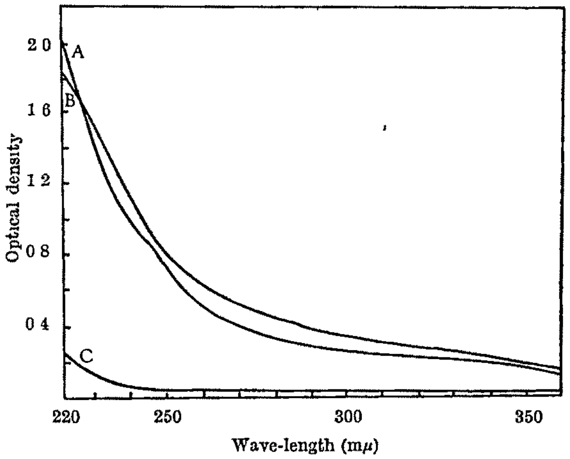

Fig 2 Light absorption by *Nostoc* filtrate (*A*), *Calothrix* filtrate (*B*), artificial sea-water (*C*)

It has been recorded by other workers[10] that filtrates from several species of algae, none of which was blue-green alga, show high absorption of ultra-violet light and thus for comparison data were obtained on light-absorption in the range 220–680 mμ by filtrates of the algae under investigation Readings were carried out on a Unicam *S P* 500 spectrophotometer (220–360 mμ) and Unicam *S P* 600 spectrophotometer (360–680 mμ). It was not possible with the particular instrument used to record absorption below 220 mμ The results obtained on absorption in the range 220–360 mμ by 24-day-old filtrates are presented in Fig 2 These show that maximum absorption both by the artificial sea-water medium used and by the algal filtrates occurs in the ultra-violet region and that absorption by the filtrates is much greater than by the sea-water medium alone The absorption spectra obtained are very similar to those of natural sea water and filtrates of various other algae[10], suggesting that the shape of the absorption curves of natural sea water may be due partly to the extracellular products of algae and other micro-organisms In the region 360–680 mμ absorption by the filtrates decreased with increase in wavelength while an overall increase in absorption occurred as the cultures aged

Analyses of medium from 24-day-old cultures for the presence of amino-acids were carried out, as it has been shown that these substances form an appreciable part of the total nitrogen liberated by blue-green algae, where they are present in the free state[5,6] and in the form of peptides[5] After desalting the filtrates[11] they were concentrated *in vacuo* and applied before and after hydrolysis to two-dimensional paper chromatograms and the amino-acids estimated semiquantitatively[12] The results showed that, for *Calothrix*, alanine, threonine and leucine were present in largest quantity together with traces of aspartic acid, glutamic acid, phenyl-alanine, serine, glycine and proline After hydrolysis the same amino-acids and also valine were present but in very much higher quantities, alanine, glutamic acid and leucine being most abundant In *Nostoc* filtrates the basic amino-acids and threonine were most abundant while there were traces of aspartic acid, glutamic acid, serine, alanine and glycine In the combined form these amino-acids increased substantially in quantity, glutamic acid, threonine and the basic amino-acids being most abundant, while leucine and valine, which were not present in the free state, were detected There appeared to be little qualitative or quantitative correspondence between the amino-acids liberated by the two species, and there is evidence[7] that the production of extracellular nitrogen may vary according to the conditions of growth

These results suggest that the nitrogenous substances liberated by the foregoing algae may be of biological importance in Nature for it has been shown that many micro-organisms utilize amino-acids as nitrogen sources while others require amino-nitrogen for growth Droop[13] has shown, for example, that the cryptomonad *Hemiselmis virescens*, from the same locality of seashore as the algae under consideration, is dependent on amino-nitrogen for growth There is also evidence[14] that the extracellular nitrogenous compounds of blue-green algae may be important as chelating agents in fresh-water, and it is probable, therefore, that in marine environments the extracellular products of marine blue-green algae exert a similar function

I thank Prof G E Fogg for his advice

W D P. Stewart*

Department of Botany,
University of Nottingham

* Present address Department of Botany, Westfield College (University of London), Hampstead, London, N W 3

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Seedling Inoculation with *Ustilago nuda* (Jens.) Rostr.

The vacuum method of seedling inoculation with chlamydospores of *Ustilago nuda* already described[1] was the first non-floral method to give high infection percentages in barley This method has the disadvantage, where large numbers of seedlings are concerned, that the coleoptiles must be cut individually by hand before being placed under vacuum in the chlamydospore suspension It has now been found possible to infect barley seedlings without the tedious and time-consuming necessity of cutting the coleoptiles.

When barley seeds were germinated at about 20° C until the coleoptiles of the seedlings, which were then usually 2–3 cm in length, were burst by the emerging first leaf and were then inoculated by the vacuum method but without cutting of the coleoptiles, a low percentage of smutted plants resulted (6 per cent) as compared with that of plants from seedlings the coleoptiles of which were cut when they reached a length of about 1 cm and were then vacuum inoculated (57 per cent) An experiment to induce natural breaking of the coleoptiles at a shorter length by exposing the seeds to light during germination resulted in the coleoptiles being burst through at various lengths all shorter than the controls in total darkness which were still unburst at 5 cm

A mercury vapour lamp suspended 15 in above the water agar plates on which barley seed was germinating at 17° C, the seed having first been allowed to absorb water at 3° C for five days, resulted in the first leaf bursting the coleoptiles when the latter were only about 1 cm in length When such seedlings were placed under a vacuum of 25 in of mercury in a chlamydospore suspension for three successive periods of 1 min, 79 per cent smutted plants resulted

This limited trial indicates that equally good results may be expected from the inoculation of uncut seedlings germinated in the foregoing manner as were previously obtained by the original method

T. Kavanagh*

Plant Pathology Department,
University College,
Dublin

* Present address Agricultural Institute, Kinsealy, Malahide, Co Dublin

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Immobilization of Elephant Seals using Succinylcholine Chloride

Biologists of the Australian National Antarctic Research Expeditions have been investigating the ecology and physiology of the southern elephant seal, *Mirounga leonina* Linn, at Macquarie Island for many years[1] Present work requires the animals to be handled for a variety of procedures, but their large size (mature cows 2½ m long and 500 kg in weight, mature bulls 5 m long and 3,000 kg in weight) makes physical restraint of any but small pups impossible

Muscular relaxants have long been used for restraining wild animals in captivity[2,3] and extension of these techniques to the field has facilitated studies of a variety of land mammals[4] One pinniped, the California sea lion, *Zalophus californianus* Lesson, has been immobilized in captivity[3] This communication reports the extensive use of succinylcholine chloride for immobilizing elephant seals, *Mirounga leonina* Linn, the establishment of reliable dose rates, and the elephant seal's tolerance to high dosages

Intramuscular injections of a concentrated (200 mg/ml) solution of succinylcholine chloride were administered by means of an extension syringe This comprised a 20-ml veterinary syringe screwed to a light metal tube, 3 ft long, down which a rod passed to actuate the plunger The injection was made on undisturbed animals just anterior and lateral to the base of the tail The straight-line length from nose to tail of each animal was estimated by eye and converted to a total body-weight by reference to a regression curve of Laws[5] Exact dose rates were then calculated Subsequent weighing and measuring of immobilized seals generally confirmed Laws's figures

In 42 attempts, we have successfully immobilized 31 seals up to 4 5 m long and weighing an estimated 2,500 kg. Nine of the eleven failures to immobilize occurred with large adult male seals, the blubber of which may not have been penetrated by the needle. Dose rates of 0 5 mg/kg (0 23 mg/lb) and 1 mg/kg (0 45 mg/lb), while sometimes effective, were not as reliable as 2 mg/kg (0 91 mg/lb) This rate greatly exceeds doses used on domestic animals apart from pigs, which require a similar quantity for complete relaxation[6], and is 4–8 times the effective dose rate for the California sea lion[3]

Paralysis often occurred in less than 0 5 min and usually within 1 min of injection Its duration varied from a few sec to 45 min and no ill-effects were observed after the animals recovered Muscle paralysis followed a definite sequence which was similar to that observed in other animals[4,6] Facial muscles were affected first, followed by those of the neck and hind flippers; then the fore flippers and finally the body musculature relaxed Paralysis of the diaphragm may also have been induced, since apnœa appeared to last for periods up to 10 min, sometimes to the extent of cyanosis of the tongue Hyperventilation of similar duration preceded muscle recovery, the sequence of which occurred in the reverse order to that of paralysis

Immobilized elephant seals have been subjected to a number of procedures to which, judging by the absence of distress or after-effects due to handling and other treatment, they seem ideally suited We have taken skin biopsies and blood samples, injected vital stains, and measured, weighed and transported seals to enclosures for observation The immobilization technique should greatly facilitate certain surgical procedures on suitably anæsthetized animals and the administration of hormones and drugs can also be tried

The ease with which elephant seals of almost any size can be restrained by injections of succinylcholine chloride has considerably extended their use as field and experimental animals for biological research There is good reason for supposing that other Antarctic phocids would react to this drug similarly, thereby allowing them to be more readily examined Improvement of the technique against the very largest elephant seals is proceeding

JOHN K LING
DAVID G NICHOLLS

Antarctic Division.
Department of External Affairs,
Melbourne

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Evolution of the Giraffe

THE mode of evolution in the giraffe has been a favourite subject for discussion Darwin[1] wrote " it has been supposed that all the parts must have been simultaneously modified, and it has been argued that, on the principle of natural selection, this is scarcely possible but it cannot be denied that an animal might have its neck, or head, or tongue, or forelimbs elongated a very little without any corresponding modification in other parts of the body". Graham Cannon[2], very properly I consider, rejects this, concluding "It is this idea of co-ordinated variation that is, to my mind, the central core of the whole problem of evolution".

Doubts on the extent of the survival value conferred by ability to reach high twigs arise when it is considered, as Graham Cannon (p 139) pointed out, that the male giraffe is some 2 ft taller than the female and thus by natural selection the females would tend, disastrously, to be eliminated in times of drought, Himmelfarb[3] similarly objects that the young would tend to be eliminated likewise

Schreider[4] discusses the value, to certain human races living in hot climates, of dolichomorphism as an aid in achieving heat loss So also to the giraffes, living as they do in hot climates, their dolichomorphic structure will serve a similar purpose to young or old, male or female continuously and not merely in times of drought, while at the same time enabling them to achieve that size and tallness which confers greater ability to evade, or defend against. predators and to reach a source of food otherwise unavailable to them Which factor has been of greatest evolutionary value is uncertain Darwin[5] wrote in this connexion "The preservation of each species can rarely be determined by any one advantage, but by the union of all, great and small"

To the best of my knowledge and belief, no one has ever suggested that dolichomorphic races of man evolved by elongation of first one part then another, and evolution by pattern mutation is very much the more probable.

A BROWNLEE

Agricultural Research Council Field Station,
Compton, Nr Newbury, Berks

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Monolayer Tissue Culture on 'Cellophane' Squares

'CELLOPHANE' has for some years been recognized as a material on which monolayers of many cell types can be grown[1] When a sheet of 'Cellophane' is so covered with cells it can be cut up into small pieces which are suitable for a number of uses

For example, they can be distributed into tissue culture tubes and used as stationary or roller cultures for virus isolation and titration They can be frozen and stored, probably indefinitely, in a liquid nitrogen refrigerator and remain ready for use on reconstitution

The types of cell grown and stored satisfactorily so far include the following (*a*) primary cultures of human embryo kidney and bovine testis, (*b*) secondary cultures of monkey kidney and human amnion , (*c*) continuous cell lines, that is, HeLa (2 strains), *MK*2, *HEp*2 and *L* cells

No unsatisfactory cells have so far been encountered, but it has been found that too dense a cell sheet may detach from the 'Cellophane' when it is cut

Ordinary thin 'Cellophane' sheet sold in rolls for wrapping purposes is used It is cut into rectangles about 7 cm × 3 cm which will fit inside a 2-oz. 'medical flat' screw-cap bottle. Each piece is cleaned and sterilized by immersion in ether followed by ethanol and sterile distilled water after which it is introduced as a roll into a bottle with sterile precautions and kept moist with a little sterile buffered saline

Each bottle is inoculated with about 8 ml of cell suspension of a density suitable for culture on glass, and care is taken to ensure that the 'Cellophane' is completely wetted and submerged before incubation To inspect the growth the bottle is gently tilted so that the 'Cellophane' sticks to the glass whereupon the bottle can be turned and examined microscopically

When the monolayer is nearly confluent the medium is poured off and the 'Cellophane' extracted with fine pointed forceps and placed, cells uppermost, on a little medium in a Petri dish

It is then cut into strips about 7 mm wide and these are cut into squares and washed off the scissors into a bottle of medium One sheet yields about 40 squares If not required immediately they may be kept satisfactorily in 10 ml of medium in a screw-cap bottle and remain usable about as long as ordinary tube cultures

Squares can be stored, probably indefinitely, in a liquid nitrogen refrigerator such as the Linde *LNR–25–B* 20 or more can be put into 1 ml of dimethyl sulphoxide freezing solution and frozen slowly in the manner described previously[2,3] Reconstitution is by rapid thawing at 37° C, and the squares are then distributed as required without washing The medium should have a low bicarbonate content to prevent temporary excessive alkalinity[4] Freezing and thawing do not seem to cause detachment of the cells. When detachment has occurred it has been the result of the manipulations If the damage is not too great the cells usually grow over the 'Cellophane' again within a day or two

Squares put into 1–2 ml medium in tubes which are incubated vertically behave like comparable monolayers on glass Inspection of a monolayer is easily performed by sloping the tube then giving it a twist so that the square sticks to the glass. A variety of virus strains have been grown in this way, for example, vaccinia, herpes simplex, poliovirus, influenza, and respiratory syncytial virus, and the cytopathic change (CPE) was in each case indistinguishable from that seen in monolayers on glass. Sub-culture can be made by transfer of the square or the medium or both. Infectivity titrations and neutralization tests are performed with economy in glassware by the addition of squares to the tubes containing the virus or virus–serum mixtures.

In roller tubes the squares stick to the glass within the area traversed by the medium and seem to suffer no deterioration. Human embryo kidney cells gave CPE at the same times as parallel tube cultures when inoculated with several strains of common cold viruses, including wild type strains as well as *FEB* and *HGP*.

The number of cells per square is approximately ⅛ that of a normal monolayer. This has the disadvantage of presenting fewer cells for examination than in a monolayer on glass and it gives a lower virus yield. However, an advantage was that the medium needed to be changed only once a week instead of daily.

Preservation of monolayer fragments offers a number of possibilities which have so far been only partially explored. It is a means of preserving viable fragments of cell sheets in prolonged experiments.

Reconstitution is so quickly carried out that monolayers are available on demand and this might enable them to be used for the isolation of labile viruses from clinical material. It could be especially useful in rendering available monolayers of cells of unusual sorts or kinds required infrequently.

J. Nagington

Public Health Laboratory Service,
Cambridge.

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CYTOLOGY

Asynapsis of the Salivary Chromosomes of Hybrids between *Anopheles punctipennis* and *Anopheles freeborni*

Burgess[1] showed by crossing *Anopheles punctipennis* with *A. freeborni* that these two species were more closely related to each other than was generally thought. In crossing experiments between these two species when *A. punctipennis* was the female, 8·3–15·2 per cent of the eggs hatched, from which only one adult emerged, although it died because it could not free its tarsi from the pupal case. In the reciprocal cross with *A. freeborni* females, 2·2–25·1 per cent of the eggs hatched, but showed high mortality in the first and second instars and only one larva reached fourth instar.

While making salivary gland chromosome maps of *A. freeborni* and *A. punctipennis*, we found that there were considerable similarities in the banding patterns of certain regions of the chromosomes. The ends of the chromosomal arms of the two species were very similar. Of particular significance was the great similarity of the banding pattern in the left arm of chromosome III of *A. freeborni*[2] to that of *A. punctipennis*; the similarity was so close that the map of III–*L* for one species fit that of the other species. Therefore, the chromosomes of the hybrids would be of special interest.

Reciprocal crosses using induced copulation[3] were made between the two species. Nine chromosomal smears[4] were made of the resulting fourth instar larvæ for each of the two possible crosses. We were surprised to find little or no chromosomal synapsis in either cross. Fig. 1 shows the typical configuration of the chromosomal complement of the hybrid between the *A. freeborni* females × *A. punctipennis* males. Even in the left arm of chromosome III little or no synapsis occurred although the similarity of the banding pattern can be followed in both the maternal and paternal chromosomes. Of interest was the close proximity of all five arms to each other in all squash preparations although they are not synapsed.

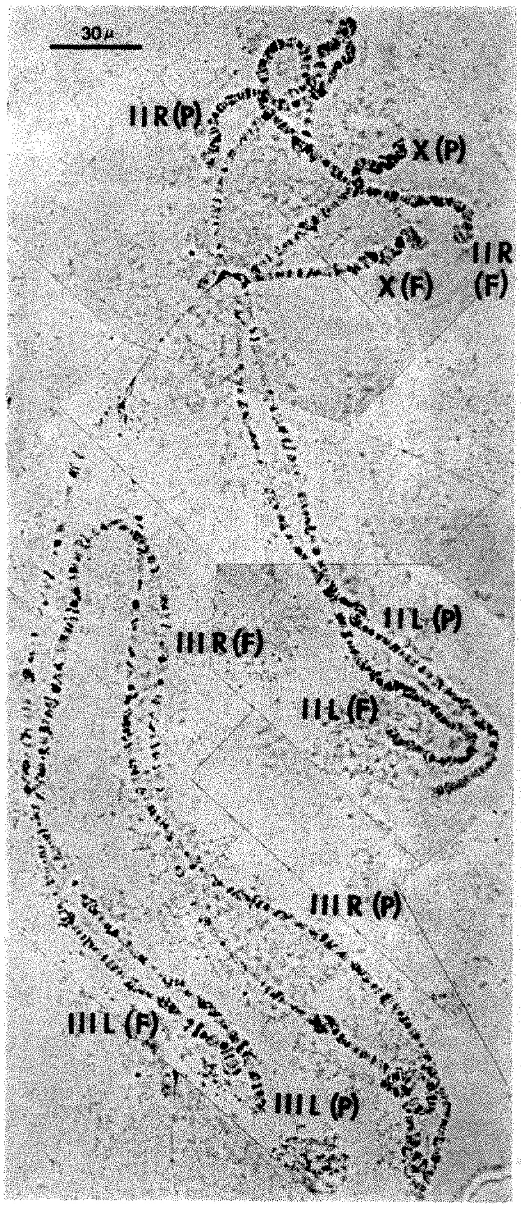

Fig. 1. Salivary chromosomes of hybrid of *A. freeborni* ♀ × *A. punctipennis* ♂. Chromosome arms are designated *X*, II-*R*, II-*L*, III-*R*, III-*L*. *P* = *A. punctipennis*; *F* = *A. freeborni*. (Composite photo)

Each of the hybrid chromosomes seemed almost as wide as a normal synapsed pair of chromosomes in *A. punctipennis* or *A. freeborni*. We thought it possible that the hybrids might be allotetraploids. This could explain the width and the proximity of the chromosomes of the two species. Allotetraploidy would seem to be ruled out by the observation of the normal diploid number of six chromosomes in the brain of a hybrid fourth instar larva. Probably the close approximation of the unsynapsed chromosomes is a manifestation of the general phenomenon of somatic pairing.

The asynapsis and easy identification of the chromosomes from both species offer a sure method of identification of naturally occurring hybrids between these two species.

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RICHARD H. BAKER
JAMES B. KITZMILLER

Department of Zoology,
University of Illinois,
Urbana, Illinois.

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ENTOMOLOGY

Oviposition in Relation to the Accumulation of Bloodthirsty Black Flies (*Simulium* (*Gnus*) *arcticum* Mall. (Diptera)) prior to a Damaging Outbreak

THE black fly *S. arcticum* is notorious for its destructive outbreaks along the downstream sections of the North and South branches of the Saskatchewan River in Canada. Outbreaks during 1944–46, inclusive, killed an estimated 800 animals[1]. Since 1948, annual treatments of the rivers with DDT larvicide have generally prevented spring outbreaks.

The adults of *S. arcticum* emerge daily from May until October in numbers affected by various conditions in the preceding adult and larval environments. Usually only the earliest adults in the season appear suddenly in overwhelming numbers; throughout the remainder of the summer the adults continue to emerge from the river and to attack animals, but in relatively uniform small numbers from day to day. On only two occasions were late summer attacks heavy enough to kill cattle. The sudden appearance of excessive numbers of flies during spring outbreaks was attributed to the simultaneous emergence of large numbers of black fly adults[1]. However, observations during periods of emergence did not support the 'mass emergence' theory, and an additional possibility, the accumulation of newly emerged adults awaiting suitable attack weather, was considered. This theory, too, no longer seems adequate because attacks have been observed over a wide range of weather conditions including high winds. Evidence here presented indicates that, in an autogenous generation, the accumulation of nulliparous females in the gravid state, awaiting precise environmental conditions for oviposition, is an important prerequisite to a mass attack.

During 1960–62 attacking populations of black flies at Saskatoon near the Saskatchewan River were sampled and their oviposition experiences determined. Adults were collected at half-hourly intervals, as they came to bite horses, throughout several diel cycles. Examination of these flies for follicular relics showed that more than 99 per cent of the adults in the initial mass outbreaks in the springs of 1961 and 1962 had already oviposited prior to seeking their first blood meal. In August, 1960, only 65 per cent of the flies coming to take blood had previously oviposited. Thus *S. arcticum* is apparently a 'facultative blood sucker', a category, according to Rubtzov[2], which includes most blood-sucking species. In May and June when the larval environment of *S. arcticum* apparently favours the accumulation of large nutrient reserves, the emerging females can be autogenous and thus not require a blood meal until they have deposited their first batch of eggs. When the larval environment is less favourable (as in August) a large proportion of the emerging females are anautogenous and require a blood meal before they can develop their first batch of eggs.

Despite larviciding, the 1962 spring outbreak was severe enough to kill cattle. Judging by the sizes of the follicular relics of flies attacking animals at this time, most had laid eggs only a few hours previously. These occurrences of autogeny and mass attack in a single generation support this theory that the emerging black flies accumulate in a gravid condition in the vicinity of the emergence site until weather favourable for oviposition occurs. These black flies oviposit while in flight over the surface of a river during calm mild evenings[3]. Only after depositing their first batch of eggs do they venture forth in search of a blood meal. The range of meteorological conditions suitable for oviposition appears to be much narrower than that for blood seeking and thus they are more likely to accumulate in the gravid state than in the post-gravid state. In the laboratory, fully gravid females will retain their eggs for many days in the absence of an environment suitable for oviposition.

The observations recorded here also show that the flies often mate and feed on nectar before they approach an animal for a blood meal. However, these two apparent essentials are often dispensed with, leaving oviposition as the only main adult event that must occur before autogenous flies are free to search for a blood meal.

This theory is potentially important in behaviour investigations of all 'mass-outbreak species' the females of which are autogenous in the first ovarian cycle, because it suggests that there is a greater urgency to investigate the meteorological requirements for oviposition than for blood feeding if this basis for predicting outbreaks is sought. Until now, behaviour investigations of these black flies have been concerned only with meteorological requirements for blood feeding.

I thank Michael Ivanochko for sorting and dissecting the large number of black flies used in this investigation.

F. J. H. FREDEEN

Entomology Section,
Canada Agriculture Research Station,
Saskatoon, Saskatchewan, Canada.

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VIROLOGY

Use of a Combined Complement-fixing Antigen to detect Arthropod-borne Viral Infection

IN an area where several arthropod-borne viruses are active, the examination by complement fixation of acute and convalescent human sera to detect possible infection with local agents may be tedious and time consuming. Sometimes the convalescent sera alone are screened and those that react with one or more antigens are then paired with the acute specimen and retested.

To avoid screening large numbers of convalescent sera against several antigens, preparatory to testing the paired sera, I have used in the initial test a combined group *B* arthropod-borne viral antigen, as follows.

The five group *B* viruses identified in South India, dengue types 1, 2 and 4, Japanese encephalitis and West Nile, are combined after treatment of infected baby mouse brains with sucrose and acetone according to the method of Clarke and Casals[1]. Antigens are diluted so as to give 2–4 units of each, based on box titrations with homologous hyperimmune mouse sera, in the final combination. Antigen serum mixtures are held overnight at 4° C prior to the addition of the hæmolytic system. One unit represents that dilution of antigen giving nearly complete fixation of two units of complement with the highest dilution of serum[2].

In complement-fixation tests with known hyperimmune mouse sera, the reaction of the combined antigen with each serum has resembled that using the homologous antigen alone One such test, a box titration using individual antigens as well as the combination, is presented in Table 1 In other tests marked serological crossing has been noted, particularly within the dengue and Japanese encephalitis–West Nile groups The heterologous reaction has never, however, exceeded the homologous

Table 1. COMPLEMENT FIXATION USING INDIVIDUAL AND COMBINED ANTIGENS AGAINST KNOWN IMMUNE SERA

Antigen		Hyperimmune mouse serum				
Type	Initial dilution in combination	Dengue 1	Dengue 2	Dengue 4	Japanese encephalitis	West Nile
Group *B* combination		128/256*	128/256	256/256	512/512	64/512
Dengue 1	1 8	128/128	—	—	—	—
Dengue 2	1 16	—	128/256	—	—	—
Dengue 4	1 8	—	--	256/128	—	--
Japanese encephalitis	1 16	—	—	—	512/512	—
West Nile	1 16	—	—	—	—	128/512

* Reciprocal of serum titre/reciprocal of antigen titre

Using the combined preparation, with antigens diluted as indicated in Table 1, 36 convalescent sera were tested from patients with dengue-like illnesses or pyrexias of unknown origin that did not yield viruses Twenty of these combined with the grouped antigen to fix complement at a dilution of 1 8 or greater When these 20 sera were paired with the acute samples and retested against the individual group *B* antigens, a four-fold or greater rise to one or more viruses was demonstrated for 11 patients With the other nine patients complement-fixing antibody-levels in both specimens were equivalent

The 16 convalescent sera not reacting with the combined antigen were next tested against the individual antigens In no case was complement fixed at a dilution greater than 1 4

The combined antigen thus indicated in every instance those sera which would react with the individual antigens Conversely, no false positive reactions were noted In work now under way, the principle of a combined antigen is being applied to several additional, unrelated Vellore agents

DONALD E CAREY

Virus Research Centre,
Poona 1, India

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PSYCHOLOGY

Saline Preference Curve for Mice: Lack of Relationship to Pigmentation

Two lines of investigation have led to the experiment described here A large body of data has been gathered on the preference behaviour of animals presented with a choice between saline solutions of various concentrations and distilled water Most species studied seemed to manifest a preference for saline solutions of low (hypotonic) concentration and an aversion to solutions of higher (hypertonic) concentration[1,2] A much less thoroughly investigated problem arises from the recurrent but apparently little-known observation that darkly pigmented animals have greater taste sensitivity than their lightly pigmented conspecifics

Hoshishima *et al* [3] have recently examined this phenomenon in mice presented with various taste substances These workers report no saline preference and report unusually high discrimination (aversion) thresholds for all strains used In addition, their black strains appeared to have higher thresholds than their white strains, while the hypothesis that pigmented animals are more sensitive to taste would predict a difference in the opposite direction Since the statistical techniques used required extremely large differences in order to reach significance, initial preferences may have been overlooked and only the more pronounced aversions taken into account If a saline preference of dark mice did exist, and was maintained at higher concentrations than that of white mice, and only aversion thresholds were observed, it might be erroneously concluded that the discrimination threshold of black mice was the higher

Because melanocyte-stimulating hormone has adrenocorticotrophic properties[4], and because of possible structural relationships between melatonin and Farrell's 'adrenoglomerulotropic' factor[5], one purpose of this experiment was to ascertain more conclusively whether pigmentation may be specifically related to sodium chloride appetite It was also deemed important to determine whether or not mice manifest a saline preference and to re-evaluate the saline discrimination threshold since no adequate investigation of these phenomena in mice has been reported to our knowledge

Thirty-six male mice, 5–6 weeks of age and of approximately equal body-weight, were used The mice were obtained from the Roscoe B Jackson Laboratory and comprised six separate strains with six mice per strain There were three albino strains (*SJL/J*, 129/*J*, *CAF/J*) and three black strains ($B_6D_2F_1$, B_6AF_1, *C57BL/6J*) Pairs of animals of each strain were placed in wire mesh cages on to which were symmetrically attached two inverted 50-ml graduated test-tubes with standard curved metal drinking spouts Mice were maintained in a temperature controlled room and fed Purina laboratory chow *ad lib*

Following a 10-day period during which distilled water was presented in both tubes, a saline solution was substituted for the water in one of the tubes Five concentrations (0 003, 0 010, 0 033, 0 100, and 0 333 molar) were presented in an ascending order of concentration with the concentration increased on each Friday of five successive weeks Twice weekly (Tuesdays and Fridays) readings were taken, tubes were emptied and refilled with fresh

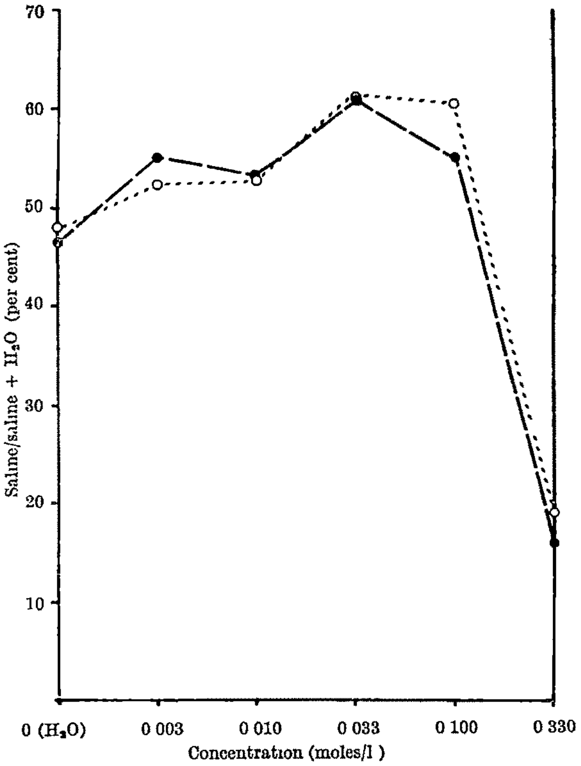

Fig 1 Saline preference-aversion functions for black and white mice Relative preferences computed in terms of saline ingestion as percentage of total fluid intake ——, Black, - - -, white

solutions, drinking nozzles were exchanged randomly, and relative positions of saline and water were reversed It was hoped that this relatively long period between position alternations would allow more stable preference behaviour to develop

Fig 1 shows the preference-aversion functions of the black and white strains Differences between black and white animals did not approach significance at any saline concentration There were no observable differences between individual strains However, because a few animals temporarily escaped and on recapture could be identified with certainty for pigment but not strain, statistical analyses of strain differences were not performed

At each concentration, t tests were applied to the differences between amounts of water and saline ingested using the pooled data of all animals The discrimination (preference) threshold falls between 0.01 and 0.033 molar, which is within the range of that found for the rat[1] Significant saline preferences were obtained at concentrations of 0.033 molar ($P < 0.01$) and 0.10 molar ($P < 0.05$) A significant saline aversion occurred at 0.333 molar ($P < 0.001$) These 'P' values represent two-tailed tests

These results indicate (*a*) that mice produce a typical saline preference-aversion function when offered saline solutions of different concentration in addition to distilled water, (*b*) the suggested relationship between pigmentation and taste sensitivity or appetite for sodium chloride was not confirmed

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GEORGE WOLF

Department of Psychology,

GEORGE H LAWRENCE

Department of Psychiatry,
Yale University,
New Haven, Conn

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Heart Rate during Auditory Vigilance Performance

THE work recorded here is the first in a series of experiments designed to investigate the relationship between cardiac activity and the performance of monotonous tasks and tasks involving high rates of information processing

Since drowsiness is commonly reported by subjects of vigilance experiments it is to be expected that this would be reflected in a decrease in heart rate during a vigil, and it is possible that this decline will be related systematically to the decrease in the number of signals detected correctly during different periods of the vigil Furthermore, Lacey and Lacey[1] have suggested that variability in resting heart rate may be indicative of the quality of performance shown by subjects engaged on perceptual-motor tasks They differentiate between those subjects who show high variability (labiles) and those who show low variability (stabiles) and suggest that each group is liable to show consistent modes of responding and to commit specific types of errors Although they have not used vigilance tasks in their investigations the implication seems to be that the more variable the heart rate at rest the more are commissive errors likely to occur in vigilance situations

In the present work, electro-cardiographic recordings were taken from eight subjects (mean age 21.5 years) in an auditory vigilance situation The task consisted of a tape-recording of a series of digits, 1–9 inclusive, recorded in random order at the rate of 1/sec The signal to be detected consisted of a series of three successive odd digits which were all different There were 24 such signals during the 60-min task, six occurring at random intervals during each 15-min period Subjects responded by pressing a key which was attached to an event recorder whenever they considered a signal to have occurred Heart rate measures (the number of R waves/min) were taken during the following periods (1) a 20-min pre-task resting period, (2) the first 7 min of the task, (3) the ninth, twelfth, fifteenth . . fifty-first min of the task, (4) the last 8 min of the task, (5) during a 10-min post-task resting period

The mean heart rate for each subject for each 15-min period was divided by his mean heart rate during the pre-task resting period These 'working-resting' ratios were then transformed into Z-scores for the group Performance scores (the number of correct detections) for each 15-min period were also transformed into Z-scores for the group These data are given in Table 1, from which it will be seen that heart rate and performance both decline during the course of the vigil

Table 1 RELATIONSHIP BETWEEN HEART RATE AND VIGILANCE PERFORMANCE DURING THE COURSE OF THE EXPERIMENT

15-min periods	Z scores	
	Performance	Heart rate
1	+ 1.60	+ 1.40
2	0.00	+ 0.20
3	− 0.50	− 0.30
4	− 1.00	− 1.30

The results seem to indicate a systematic relationship between heart rate and vigilance performance It is not possible, however, to interpret this easily in terms of decreasing activation during the course of the vigil, since for this to be the case the beginning of the task should be accompanied by a substantial increase in heart rate In the case of only one subject was this observed, and all mean working-resting ratios were less than unity

Lacey[2] considers that a deceleration in heart rate is associated with the uptake of information It seems possible that the deceleration observed in this work may be due to increased attempts by subjects to take up information from the task and the environment as the vigil proceeded

Subjects were ranked in terms of variability of resting heart rate, in terms of total error score (that is, errors of omission and of commission combined), and in terms of errors of commission only The rank-order correlation between heart rate variability and total error score is given by $\tau = 0.69$ ($h = 2.02$, $P < 0.01$), and between heart rate variability and commissive error score by $\tau = 0.42$ (non-significant)

It is difficult on the basis of the results of this work, in which only eight subjects participated, to draw any reliable conclusions about individual differences in detection ability and their prediction, but the significant correlation between cardiac lability and total error score suggests that heart rate variability may be some prediction of vigilance performance, although it seems unlikely that it will efficiently predict commissive errors in performance

This work is supported by a grant from the Medical Research Council

S GRIEW
D R DAVIES
A C C TREACHER

Department of Psychology,
University of Bristol

[1] Lacey, J I , and Lacey, B C , *Res Pub Assoc Nerv Ment Dis* , **36**, 144 (1958)

[2] Lacey, J I , *J Comp Physiol Psychol* , **43**, 338 (1950)

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 9

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W C 1), at 5 30 p m —Dr I Hacking "On the Foundations of Statistics"

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Measurements at Very Low Frequencies with particular reference to Noise" opened by Dr J L Douce, Mr H Sutcliffe and Prof J C West

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Dr F Fallside and Mr M R Patel "Effect of a Back E M F Non-linearity on Responses of a Second-order Position Control System", Dr F Fallside and Dr J O C Ezeilo "Application of the Second Method of Lyapunov to the Stability of Certain Position Control Systems containing a Back-E M F Non-linearity"

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 6 p m —"The Pitcairn People" (colour films)

Tuesday, December 10

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N W 1), at 5 p m —Scientific Papers

Royal Society of Arts Commonwealth Section (at John Adam Street, Adelphi, London, W C 2), at 5 15 p m —Dr T W Tinsley "The Ecological Approach to Pest and Disease Problems of Cacao in West Africa" (Inaugural John Curtis Lecture)

Institution of Civil Engineers (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr H R Boyce "Swansea Docks—Entrance Works"

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Materials for Hall Effect Devices" opened by Mr R P Chasmar

Institution of the Rubber Industry, London Section (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W 1), at 5 30 p m —Mr M S Evans "Mould Design" 7 p m —Panel Discussion on "Accelerators"

Royal Society of Medicine, Library (Scientific Research) Section (at 1 Wimpole Street, London, W 1), at 5 30 p m —Dr Gordon Wolstenholme "Ethiopia in a Modern Africa"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Prof J N Davidson "Recent Developments Among the Nucleic Acids" (Sixteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Institution of Chemical Engineers, South-Eastern Branch (joint meeting with the Chemical Engineering Group of the Society of Chemical Industry, at 14 Belgrave Square, London, S W 1), at 6 p m —Mr T W G Rowe "High Vacuum"

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Mr G V. Cleare "Factors Affecting the Performance of High-Speed Tracklayers"

Wednesday, December 11

Geological Society of London (at Burlington House, Piccadilly, London, W 1), at 5 p m —Dr E P Saggerson and Mr B H Baker "Post-Jurassic Erosion Surfaces in Eastern Kenya and their Deformation in Relation to Rift Structure", Dr R Nicholson "The Structure and Metamorphism of the Mantling Karagwe–Ankolean Sediments of the Ntungamo Gneiss Dome and their Time-Relation to the Development of the Dome"

Royal Microscopical Society (at Tavistock House South, Tavistock Square, London, W C 1), at 5 30 p m —Prof M Francon (Professeur à la Faculté des Sciences de Paris et à l'Institut d'Optique) "Polarizing Interference Microscopes"

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Mr J E Austin and Mr J R Palfrey "Product Mixing During Serial Flow in Pipelines"

Royal Institute of Chemistry, London Section (at Shell Mex House, Strand, London, W C 2), at 6 p m —Annual General Meeting, followed by a film show

Royal Society of Arts (at John Adam Street, Adelphi London, W C 2), at 6 p m —Sir Ifor Evans "Education in London" (Edmund Rich Memorial Lecture)

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W C 1), at 6 15 p m —Mr A G Readett "The Organization and Administration of a Technical Translation Department"

Society of Chemical Industry, Food Group—Nutrition Panel (at 14 Belgrave Square, London, S W 1), at 6 15 p m —Prof Sir Alister Hardy "Can We Get More Food from the Sea?"

Institute of Metals (joint meeting with the London Branch of the Institute of British Foundrymen, at the National Liberal Club, Whitehall Place, London, S W 1), at 7 p m —Mr G Swinyard "Quality in Copper Based Castings"

Oil and Colour Chemists' Association (at the Institution of Electrical Engineers, Savoy Place, London, W C 2), at 7 p m —Prof R N Haszeldine "Inorganic Polymers—Fact or Fiction"

Society of Instrument Technology, Automation Section (at Manson House, 26 Portland Place, London, W 1), at 7 p m —Prof M W Thring and Mr D F Nettell "Domestic Automation"

Society of Chemical Industry, Plastics and Polymer Group (joint meeting with the Liverpool Section, at the Donnan Laboratories, University of Liverpool), at 7 30 p m —Dr R N Haward "Additives in Thermoplastics"

Wednesday, December 11—Thursday, December 12

Institution of Chemical Engineers (at Church House, Great Smith Street, London, S W 1), at 9 30 a m daily—Symposium on "Productivity in Research"

Thursday, December 12

Institution of Electrical Engineers, Electronics Division (joint meeting with the Medical and Biological Electronics Group of the British Institution of Radio Engineers, in the Department of Physiology, University College, Gower Street, London, W C 1) at 10 30 a m and 2 30 p m —Discussion on "The Brain"

Institution of Civil Engineers, Traffic Engineering Group (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Informal Discussion on "Practical Applications of Traffic Theories" introduced by Mr J G Wardrop

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Performance of Large A C Motors for Industrial Drives" opened by Mr B Barker, Mr A B D Reed, Mr D D Stephen and Mr A C G Witts, also Mr D D Stephen "Connecting Large Machines to Power Systems"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Dr P Brookes "Chemical Aspects of Mutation" (Seventeenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Chemical Society (at the Royal Institution, Albemarle Street, London W 1) at 6 p m —Prof J S Anderson, F R S "Some Contemporary Problems of Solid State Chemistry" (Liversidge Lecture)

Institution of Mechanical Engineers, Internal Combustion Engines Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on "Residual Fuels—Is Their Use Justified ?"

Society for Analytical Chemistry, Biological Methods Group (at "The Feathers", Tudor Street, London, E C 4), at 6 30 p m —Nineteenth Annual General Meeting, followed by a Discussion Meeting on "The Detection of Substances with Teratogenic Properties" opened by Mr K E V Spencer

Society of Chemical Industry, Microbiology Group (joint meeting with the Birmingham Section, at Haworth Building, The University, Birmingham), at 6 30 p m —Dr K Burton "Structure and Action of Deoxyribonucleic Acid"

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, Adelphi, London, W C 2), at 7 p m —Mr Paul Thompson "Teaching by Electronics"

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W 1), at 7 30 p m —Mr D P Choyce "Personal Experience of Ocular Onchocerciasis in Central America, Africa and the British Isles" followed by a discussion

Thursday, December 12—Friday, December 13

Biochemical Society (in the Science Lecture Theatre, The University, Leicester), at 2 p m on Thursday—Colloquium on "Regulation of Microbial Metabolism", 10 10 a m on Friday—Original Communications

Saturday, December 14

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E 23), at 3 30 p m —Dr P H Gulliver "Village Life in East Africa" *

Monday, December 16

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Mr W F Gibbons "Ceramic Sealing Techniques Applied to Electron Tubes and Semiconductor Devices"

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Mr A Stevenson "Analysis of Dithiocarbamates and Thiuram Disulphides Used in Agriculture A Collaborative Study of Macro-Methods Available for the Evaluation of Technical and Formulated Products"

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W C 2), at 6 30 p m —Sir Albert Mumford, K B E "Communications and Radio"

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 8 15 p m —Dr L S B Leakey "Exploring Man's Prehistory in East Africa"

Monday, December 16—Tuesday, December 17

British Biophysical Society (at the Institution of Electrical Engineers, Savoy Place, London, W C 2)—Symposium on "Control Mechanisms"

Monday, December 16—Wednesday, December 18

Institute of Physics and Physical Society, Electronics, Electron Microscopy and Analysis, and Low Temperature Groups (at Imperial College, London, S W 7)—Conference on "The Electrical and Magnetic Properties of Thin Films in Relation to Their Structure"

APPOINTMENTS VACANT

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Lecturer in the History of Science—The Registrar and Secretary University of Durham, Old Shire Hall, Durham (December 14)

Technician in the Department of Agronomy, University of New England for duties which will include the preparation of experimental material in glasshouses, the sampling of plant materials, and assistance with chemical laboratory work—The Registrar, University of New England Armidale, New South Wales, Australia, and The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, December 15)

Senior Lecturer, Lecturer, or Assistant Lecturer in Mathematics in the School of Mathematical and Physical Sciences—The Registrar, University of East Anglia, Earlham Hall, Norwich, Nor 05G (December 16)

Professor and a Senior Lecturer or Lecturer in Botany, and a Senior Lecturer or Lecturer in Zoology at the University of Khartoum —The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 20)

Senior Lecturer or Lecturer in Engineering Mathematics at the University of Khartoum—The Registrar University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 20)

Senior Lecturer or Lecturer in Electrical Engineering at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (December 20)

Assistant in Pathology in Queen's College, Dundee—The Secretary, University of St Andrews, Queen's College, Dundee (December 21)

Lecturer in Anatomy and Histology in the Department of Agriculture, Sutton Bonington, near Loughborough—The Registrar, The University, Nottingham (December 21)

Lecturer in the Department of Medical Physics—The Secretary, The University, Edinburgh (December 21)

Lecturer in Pure Mathematics—The Registrar, The University, Manchester 13 (December 28)

Research Officer (Biochemist) (with a Ph D degree or postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability, and preferably training and experience in mammalian biochemical research) at the McMaster Animal Health Laboratory, Commonwealth Scientific and Industrial Research Organization, Sydney, to undertake research in some aspects of chemical pathology, particularly in relation to the effects of parasitism—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C 2, quoting Appointment No 202/214 (December 28)

Chair of Chemistry—The Secretary, The University, Aberdeen (December 31)

Graduate in Mechanical Engineering or Applied Mathematics, to conduct research on an aspect of vibration concerned with transport—The Secretary, University College, Gower Street, London, W C 1 (December 31)

Lecturer (with an honours degree in pharmacy or the equivalent, and preferably subsequent specialization in physical chemistry) in Pharmaceutics at the University of Adelaide, Adelaide, South Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia, December 31)

Lecturers or Assistant Lecturers in Philosophy (including Philosophy of Science), Sociology, and Social Psychology—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (December 31)

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Lecturer/Senior Lecturer in Archæology (Greek and Roman Archæology) at the University of Sydney, Australia—The Secretary, The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 15)

Senior Lecturers or Lecturers in Anatomy (including Histology) and Physiology (including Biochemistry) at the University College of Rhodesia and Nyasaland Medical School—The Assistant Registrar, The Medical School, Birmingham 15 (January 15)

University Assistant Lecturer (preferably with a knowledge of South Asia, that is, Pakistan, India, Ceylon) in Social Anthropology—The Secretary of the Appointments Committee, Faculty of Archæology and Anthropology, The University, Downing Street, Cambridge (January 15)

Biochemist (Basic grade) at the City General Hospital, to work with, and assist, the Senior Biochemist—The Group Secretary, Stoke-on-Trent Hospital Management Committee, Princes Road, Stoke on-Trent

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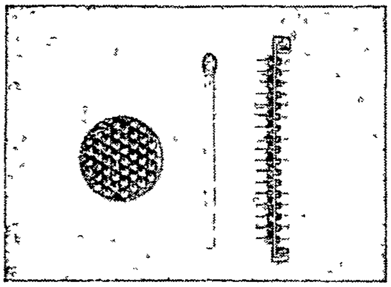

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DEPARTMENT OF MEDICAL PHYSICS

LECTURESHIP

Applications are invited for the post of Lecturer in the above Department Salary scale Lower, £1,250 by £110 to £1,690 , Higher, £1,805 by £115 to £2,150 by £110 to £2 700 per annum, with placement according to qualifications and experience, and with superannuation benefit and family allowance where applicable The successful applicant will include among his duties those of University Radiation Protection Officer and will be expected to undertake and supervise research in this and related fields

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than December 21, 1963

CHARLES H STEWART,
Secretary to the University

EPSOM GROUP MANAGEMENT COMMITTEE

Senior Biochemist required for the Department of Chemical Pathology in the Group Laboratory at Epsom District Hospital duties principally in laboratory supervision with opportunities for conducting original work Whitley Council salary and conditions £1 520 to £1,880 per annum

Applications stating age qualifications and experience, and giving names of two referees, to Group Secretary Epsom District Hospital, Epsom Surrey as soon as possible

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UNIVERSITY OF EAST ANGLIA

SCHOOL OF MATHEMATICAL AND PHYSICAL SCIENCES

Applications are invited for posts of Senior Lecturer, Lecturer, or Assistant Lecturer, in Mathematics in the School of Mathematical and Physical Sciences from October 1, 1964 Preference may be given to applicants whose interests lie in continuum mechanics (in particular fluid dynamics), analysis, or statistics The salary scales are Senior Lecturer, £2 175 by £80 to £2 575 by £75 to £2,650 , Lecturer, £1 250 by £60 to £1 670 by £80 to £2 150 , Assistant Lecturer, £1 000 by £50 to £1 150, plus family allowance and F S S U benefits

Further particulars may be obtained from the Registrar University of East Anglia Earlham Hall, Norwich, Nor 05G to whom applications (four copies) (one copy only from overseas), should be sent not later than December 16, 1963

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* **Application forms can be obtained from T. J. Lunt, Staff Manager, Ferranti Limited, Hollinwood, Lancs.**
Please quote reference JRP/15.

The Commission of the European Atomic Energy Community (EURATOM)

invites applications for the post of

SCIENTIFIC OFFICER

in the Biology Department of the Directorate-General for Research and Training, Brussels Candidates must be nationals of one of the Member States of the Community (Belgium, France, Germany, Italy, Luxembourg, the Netherlands), selection will be based on qualifications

Principal Qualifications Required

University degree, preferably in Physics Thorough knowledge of dosimetry of ionizing radiations with reference to biological systems

Maximum Age · 50 years

Languages:

Thorough knowledge of one of the following languages Dutch, French, German, Italian

Satisfactory knowledge of one other of these languages and, if possible, English

Closing Date for Receipt of Applications December 31, 1963

Further information, candidature requirements and **compulsory application form** are published in the Journal officiel des Communautés europeennes, No 165, November 16, 1963, which can be obtained on application to

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UNIVERSITY OF OXFORD

DEPARTMENT OF ENGINEERING SCIENCE

There is a vacancy for a University Lecturer to take part in the research and teaching programmes of the Department The appointment will be at a salary in the range £1,250 to £2,285 (according to age), plus child allowances and F S.S U The post may be held in conjunction with a College Fellowship, and the attention of applicants is drawn to the advertisement for a Fellowship in Engineering Science at Lincoln College which also appears in this issue

Applications (five copies) should reach the Administrator, Engineering Laboratory, Parks Road, Oxford, from whom further particulars may be obtained, by December 31, 1963

APPLICATIONS ARE INVITED FROM recent graduates (male) in zoology or horticulture specializing in entomology for the post of Assistant Entomologist to work on the screening of new organic chemicals as potential insecticides Salary according to age, qualifications and experience —Applications, with full details, to Personnel Officer, The Murphy Chemical Co Ltd, Wheathampstead, St Albans, Herts

---

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF APPLIED MATHEMATICS

Applications are invited for a post of Lecturer or Assistant Lecturer in Applied Mathematics to begin duties on October 1, 1964, or such earlier date as may be arranged Initial salary according to qualifications and experience on the scales Lecturer, £1,250 by £60 to £1,670 by £80 to £2,150, Assistant Lecturer, £1,000 by £50 to £1,150, with F S S U provision and family allowance A grant towards removal expenses will be made

Further particulars may be obtained from the Registrar, to whom applications (four copies) should be sent by December 31, 1963

UNIVERSITY OF HULL

DEPARTMENT OF CHEMISTRY

Technician Glass Blower (over 21) required for the Department of Chemistry Salary on scale £540 to £740 per annum, plus qualification allowance where appropriate Position on scale dependent on age and experience

Applications to Superintendent of Laboratories before December 13, 1963

THE AUSTRALIAN NATIONAL UNIVERSITY

HEAD TECHNICIAN FOR DEPARTMENT OF GENETICS

Applications are invited for the position of Head Technician in the Department of Genetics, The John Curtin School of Medical Research, Australian National University, Canberra, A C T The successful applicant will be responsible to the head of the Department and his duties will include supervision of technical staff, ordering of chemicals and apparatus, general administrative duties and laboratory service The Department is concerned only with research A wide experience of bacteriological and biochemical laboratory practice is required, and experience in the handling of laboratory animals would be an advantage The salary offered will be within the range £A1,884 to £A2,324 per annum The University will pay reasonable removal expenses and will provide assistance in obtaining housing for an appointment from outside Canberra The position carries superannuation, long service leave and normal fringe benefits

Applications should be forwarded to the undersigned at Box 4, G P O, Canberra, A C T, Australia by December 20, 1963

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FELLOWSHIP IN ENGINEERING SCIENCE

The College invites applications for the above Fellowship, the Fellow to take up his duties as soon as possible and in any case not later than October 1, 1964 The appointment will be for three years in the first instance Duties to engage in full-time research, on a topic related to the scientific interests of the C E G B, in the University Engineering Laboratory, to assist in supervising research students in that Laboratory and to undertake a limited amount of teaching of undergraduates in the College Stipend in the range of £1,250 to £2,285, fixed according to age and the rules governing a comparable university post The Fellow will be entitled to free rooms and dining rights in the College He will be a member of the Governing Body of the College If married, the Fellow will be entitled, in lieu of free rooms to a marriage allowance of £150, and an allowance of £50 for each child

Applications and inquiries should reach the Principal, Hertford College, Oxford, not later than January 17, 1964

APPLICATIONS ARE INVITED FROM Physicists with Ph D for a position in the Department of Physics, at the University of Victoria, Victoria, British Columbia, Canada Duties, which will include teaching and research, will commence on September 1, 1964 Salary will depend upon qualifications and experience Salary floors at present are $12,000 for Professor, $9,000 for Associate Professor, and $7,000 for Assistant Professor —Applications and inquiries should be addressed to the Head of the Department of Physics

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(B) *to Staff Officer* (*Reference FRR/243A*),
UNILEVER RESEARCH LABORATORY,
COLWORTH HOUSE, SHARNBROOK, BEDFORD

UNILEVER RESEARCH

UNIVERSITY OF EAST ANGLIA

SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited from plant physiologists or physical scientists interested in plant physiological problems for a post as a Senior Research Associate in the School of Biological Sciences The person appointed would be expected to work under the general direction of Professor J Dainty on a programme of ionic and water relations of plant cells and tissues financed by a grant from the Department of Scientific and Industrial Research The appointment would start as soon after January 1, 1964, as possible and would normally terminate on July 31, 1967 The salary scale is that for Assistant Lecturers in the University, namely, £1 000 by £50 to £1,150, plus family allowance and F S S U benefits

Further particulars may be obtained from the Registrar, University of East Anglia, Earlham Hall, Norwich, Nor 05G, to whom applications (two copies), should be sent not later than December 16, 1963

UNIVERSITY OF EDINBURGH

DEPARTMENT OF VETERINARY PHYSIOLOGY

Applications are invited for the post of Electronic Specialist to establish and take charge of an electronic workshop from January 1, 1964 Preference will be given to candidates holding Higher National Certificates The duties will include the construction, modification, and maintenance of high quality electronic apparatus used in biological research Knowledge and experience of both valve and transistor methods will be required Salary in the range £765 to £900 per annum, with placement according to qualifications and experience

Applications, stating age, qualifications and experience, should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge Edinburgh as soon as possible

---

UNIVERSITY OF ALBERTA

Research Assistantships and Graduate Teaching Assistantships from $3,700 to $4,300 per year are available to students studying for degrees of Ph D and M Sc in Chemistry Travel allowance up to $250 is provided

For further information write Dr H E Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

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DIVISION OF CLINICAL CHEMISTRY

SENIOR SCIENTIFIC OFFICER (BIOCHEMIST)

Applications for appointment to this position are invited from graduates in science, or persons with equivalent qualifications, whose major subjects include biochemistry and/or chemistry and/or physiology A minimum of five years experience in clinical chemistry is required, and special training or experience in enzymology would be an advantage, though not essential Duties include assistirg with the maintenance and expansion of the clinical chemical services of the hospital (which is the main teaching hospital of the University of New South Wales Medical School), and with special investigations Current research interests include the clinical and laboratory aspects of protein separation, aromatic metabolites, metal-chelating agents, vaso-active peptides and enzymes of tissues and serum Additional information is available from the Head of the Division, Dr R J Bartholomew The range of salary, subject to variation with the male basic wage, is £A2,307 to £A2,382 to £A2,501, the initial salary being determined by qualifications and experience Conditions of service are in accordance with the Scientific Officers (Public Hospital Scientists) (Metropolitan) Award

Applications, in writing, showing age, marital state, and full details of qualifications and experience, together with names and addresses of two referees, should be addressed to the Chief Executive Officer, Prince Henry Hospital, Sydney, Australia Closing date January 31, 1964

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for two Lectureships in Physical Chemistry in the Department of Chemistry Preference may be given to applicants with a special interest in the study of synthetic high polymers or in the application of radioactive tracer techniques Initial salary in the scale £1 250 by £60 to £1,670 by £80 to £1,830 , £1,830 by £80 to £2,150, according to age, qualifications and experience, together with F S S U and family allowance benefits

Applications (three copies), naming three referees, should be sent by December 31, 1963, to the Assistant Registrar (Science), the University, Birmingham 15, from whom further particulars can be obtained

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Broodbank Fellowships: Notice

Ordinances, pp 495 and 511 , *Supplementary Ordinances,* p 790

The Managers of the Broodbank Fund for the furtherance of research in Biochemistry or Biophysics with special reference to the Principles and Practice of Food Preservation give notice of their intention to proceed to the election of a Broodbank Fellow in the Easter Term 1964 The tenure of the Fellowship will be from a date to be determined by the Managers in consultation with the Fellow A Fellow will be required to devote himself to full-time research in a subject approved by the Managers

Candidature is open , but a Fellow must become a member of the University if he is not already a member of it, and of the Superannuation Scheme The tenure of a Fellowship, which will not normally exceed three years, will be determined by the Managers The research work will be carried out in Cambridge (or in part elsewhere with the Managers' consent) and will be subject to such conditions as the Managers may impose

The annual pensionable stipend of a Fellow is determined by the Managers, provided that it shall normally be not less than £750 nor more than £1,250, according to the Fellow's experience and qualifications In addition grants may be made by the Managers to cover expenses incurred by the Fellow in his work A Fellow must inform the Managers of the award to him of any other emolument or of his intention to undertake work other than work approved by the Managers, and he may in consequence suffer a reduction in his stipend He may, with the permission of the Maragers, undertake not more than six hours' teaching a week for remuneration without suffering any deduction from his stipend

Applications (ten copies) must be sent to the Registrary, at the University Registry, The Old Schools, Cambridge They must contain an outline of the candidate's proposed research, indicating how in his view this bears on the principles and practice of food preservation, and must be accompanied by a statement of his career and his date of birth, by copies of any papers he may have published or references thereto, and by the names of at least two referees Not more than two testimonials may also be sent No applications received after April 8, 1964, will be considered

---

MANCHESTER COLLEGE OF SCIENCE AND TECHNOLOGY

(FACULTY OF TECHNOLOGY IN THE UNIVERSITY OF MANCHESTER)

Applications are invited for new appointments as Lecturer or Assistant Lecturer in the Department of Mathematics, with corresponding title and status in the University of Manchester The sections of the Department especially interested in these appointments are those concerned with (a) pure mathematics, (b) statistics, and (c) engineering mathematics, and applicants should have already completed some research in one of these fields Commencing salary according to qualifications and experience in the scale Lecturer, £1,250 by £60 to £1,670 by £80 to £2,150 per annum , Assistant Lecturer, £1,000 by £50 to £1,150 Superannuation under F S S U Family allowances

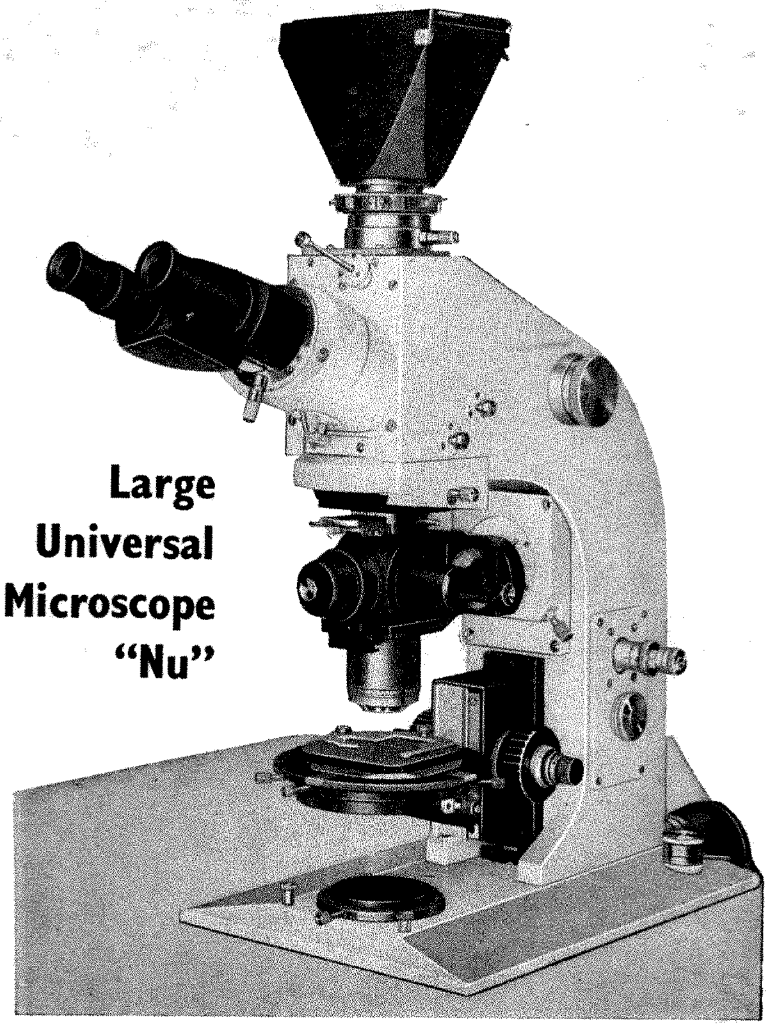
Conditions of appointment and forms of application may be obtained from the Registrar of the College, Sackville Street, Manchester, 1, to whom completed applications should be forwarded by Saturday, December 21, 1963

UNIVERSITY OF KHARTOUM

FACULTY OF PHARMACY

Applications are invited for a Reader or Senior Lecturer to act as Head of the Department of Pharmacognosy in the Faculty of Pharmacy at the University of Khartoum Salary scales Reader, £S 2 400 per annum , Senior Lecturer, £S 2,052 by £S 75 to £S 2,277 per annum Cost of living allowance approximately £S 180 per annum, at present Outfit allowance £S 50 Family allowances Wife, £S 60 per annum first child, £S 90 per annum , second and third child, £S 30 per annum each (£S 1=£1 0s 6d sterling) Passages for appointee and family on appointment, termination and annual leave There is a superannuation scheme and arrangements can be made to maintain F S S U policies Direct appointment on contract normally for five years with possibility of renewal (secondment welcomed if possible) Unfurnished accommodation provided at rent (excluding rates) up to 7½ per cent of salary

Applications (ten copies), detailing qualifications and experience and naming three referees, by December 20, 1963, to Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1, from whom further particulars may be obtained



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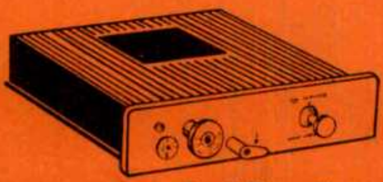

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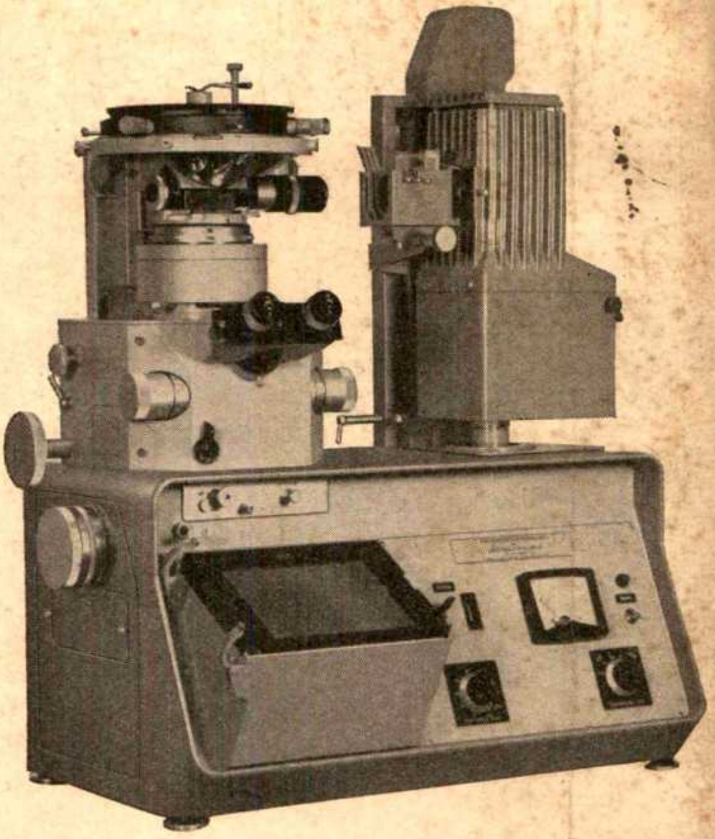

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SUPPLEMENT: INDEX TO VOLUME 199

NATURE

A Weekly Journal of Science

Vol. 200, No. 4911 SATURDAY, DECEMBER 14, 1963 6s. 0d.

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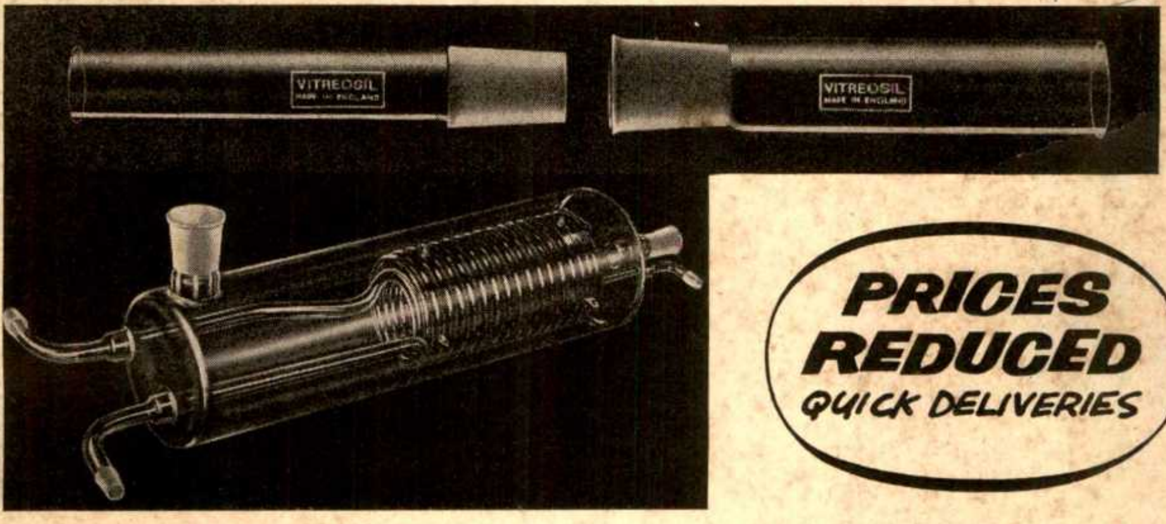

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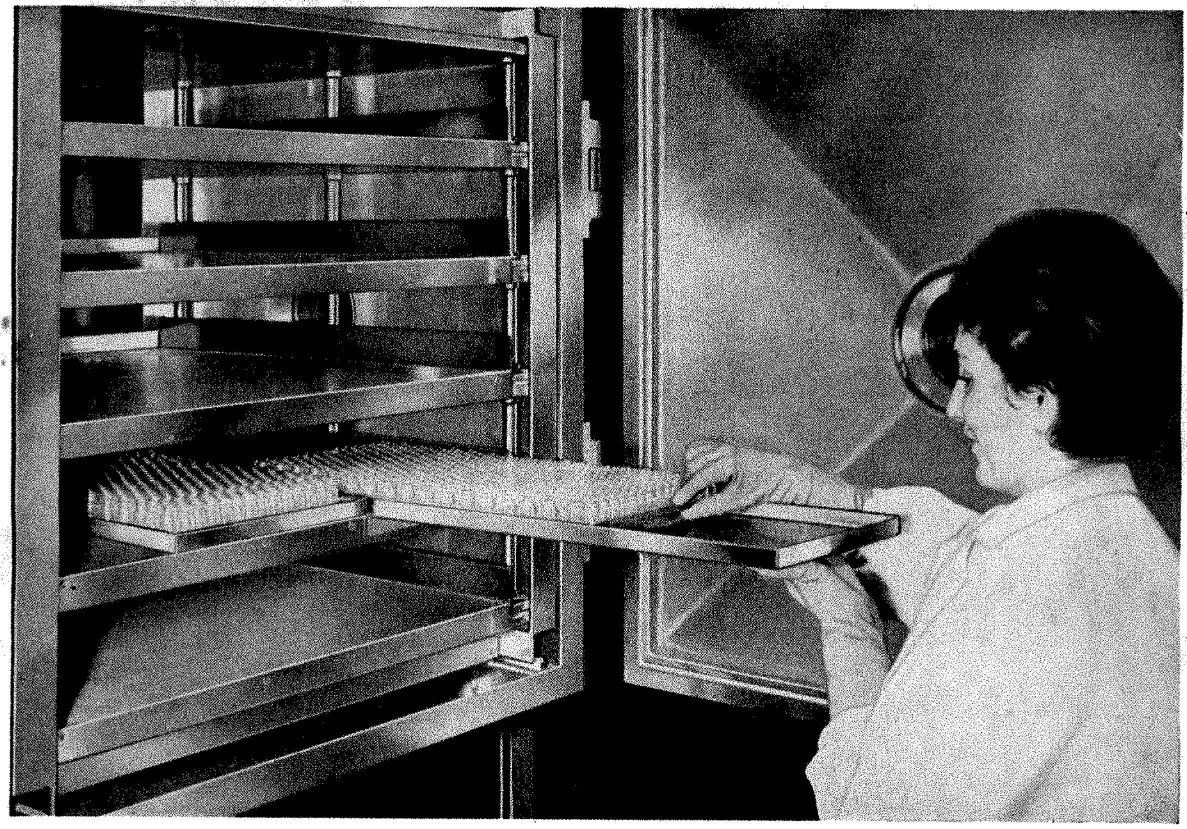

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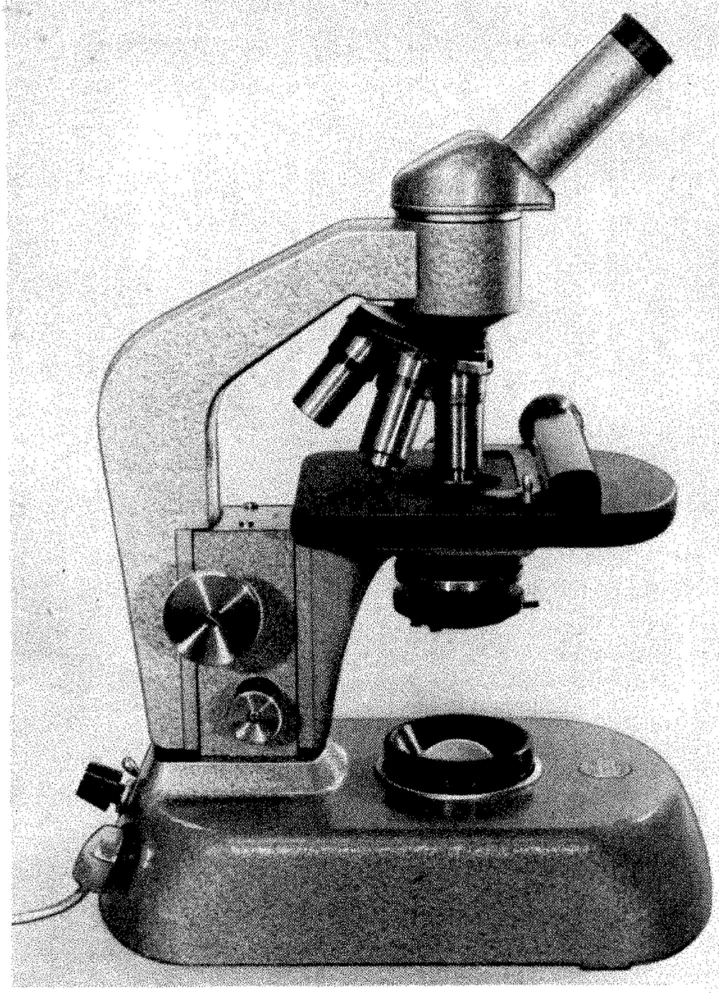

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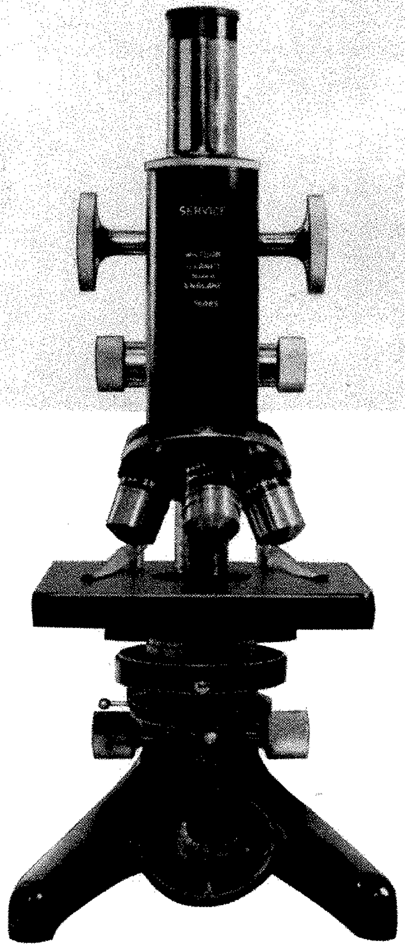

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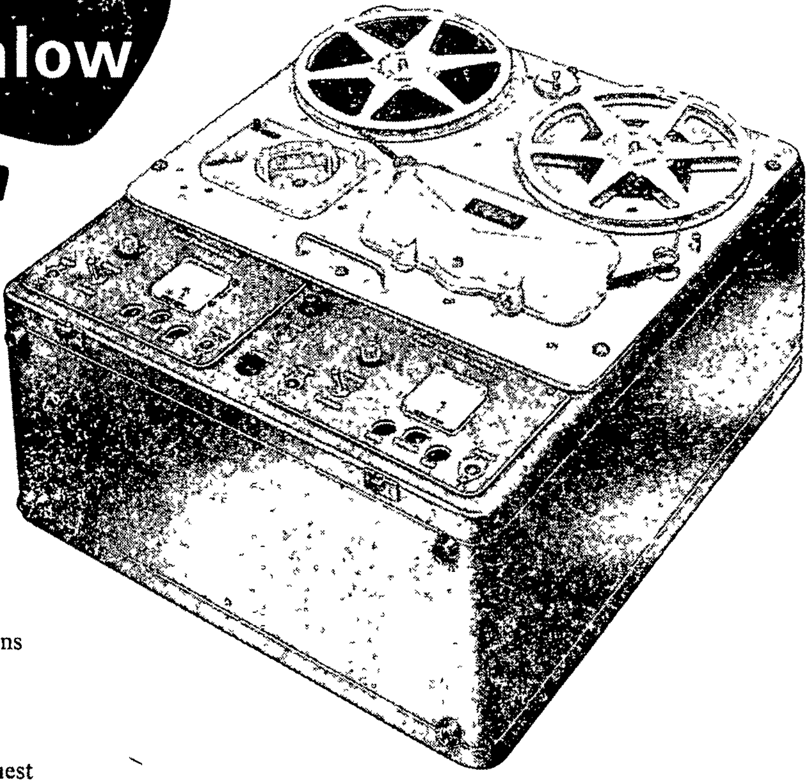

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OVERHAULING BRITAIN'S SCIENCE MACHINERY

IT is a remarkable tribute to the insight and thoroughness of the *Report of the Machinery of Government Committee*, prepared by a Committee under the chairmanship of Lord Haldane in 1918, that after nearly fifty years its views on the Government organization for scientific research should not only be quoted at length by Sir Burke Trend and his colleagues* but should also be regarded by them as providing a firm basis for meeting the needs of to-day, at least so far as the Research Councils and their relations to a Minister for Science are concerned This does not in any way detract from the value of the work which has now been carried out by the Trend Committee of Enquiry into the Organization of Civil Science, though it could not be suggested that its Report is a State paper comparable in significance with that of the Haldane Committee (see *Nature*, 103, 85, 1919) This is not because the Trend Committee has interpreted its terms of reference too narrowly or because it attempted to provide neat solutions without delving deeply into difficulties and underlying problems There is, indeed, one astonishing blind spot, the Report can be fairly criticized for not examining the problem of co-operative research in any depth Moreover, it does not consider more than rather superficially the problem of departmental organization of science, though the Committee in its remarks on the location of the Building Research Station and the Road Research Laboratory shows itself well aware of the essential conditions. Apart from this, the Report provides a fair and balanced review of the existing organization and the factors of which account must be taken However, it does not examine, as the Haldane Report did, the fundamental nature of the problem of tendering scientific advice in the process of Government and the principles to be observed

The Committee was appointed in March 1962 to consider (1) whether any changes were desirable in the existing functions of the various agencies, for which the Minister for Science is responsible, concerned with the formulation of civil scientific policy and the conduct of civil scientific research, and whether any new agencies should be created for these purposes, (2) what arrangements should be made for determining, with appropriate scientific advice, the relative importance in the national interests of the claims on the Exchequer for the promotion of civil research in the various fields, (3) whether any changes were needed in the existing procedure for financing these agencies or accounting for their expenditure It did not examine either issues of policy or consider the scale or content of research programmes, and the Report does not enter into the theory of organization The survey is based on the assumption that the Government's organization for promoting research and development in the civil field must be such as to ensure that the resources provided are used to the best advantage, that unnecessary duplication of effort is eliminated, and that new 'growing points' are fostered and encouraged

In its survey of the existing organization in Britain, the Report estimates Government expenditure on civil research and development in 1962–63 at £151 6 million, compared with £6 58 million in 1945–46 The major items are £50 million for the Atomic Energy Authority, £24 7 million for the Ministry of Aviation, £24 million in the universities (and learned societies), £21 4 million through the Department of Scientific and Industrial Research Besides expenditure of £6 million and £6 5 million, respectively, on the Medical Research Council and the Agricultural Research Council and £4 6 million on the Agriculture Departments, some £2 8 million is expended in other civil departments, £1 2 million on space research and £1 million each by the Admiralty on observatories and oceanography and by the Air Ministry on meteorology and aviation medicine Some £800,000 a year is expended by the Development Commission on scientific research

By and large, the factual survey led the Committee to conclude that in the aggregate the agencies concerned with promoting civil science do not constitute a coherent and articulated pattern of organization and that the arrangements for co-ordinating the Government's scientific effort and apportioning available resources on a rational basis are insufficiently clear and precise It does not suggest any criteria of coherence but considers that any satisfactory structure should permit and encourage common interests and shared purposes to transcend artificial limits of organization At present it sees some danger that the proper distinction between the roles of the universities and of the Research Councils may become obscured as a result of post-war developments which diminish the self-sufficiency of the universities as centres for promoting scientific research The Committee instances the inconsistency between the Admiralty's continuing responsibility for the Royal Observatory and the responsibility of the Department of Scientific and Industrial Research for other research in astronomy The division of function and responsibility for promoting development contracts between that Department and the National Research Development Corporation is also criticized, as well as the arrangements for co-ordinating the activities of that Department with the Ministry of Aviation and other departments

The present arrangements are not conducive to the concentration and flexibility which are becoming increasingly important in scientific research and development, nor does the Advisory Council for Scientific Policy, with its present inadequate staff and lack of responsibility for finance, possess effective means of influencing priorities. The composition of the Council is also criticized, the presence on it of official members who necessarily represent the several policies of the organizations which they serve is regarded as inconsistent with the conception of an advisory council The Trend Committee recognizes that the issues involved in deciding alike the nature, number and functions of the agencies required and of the organization to provide a comprehensive and coherent research strategy and advise on the distribution of resources, are complex, and it stresses the need for a revised form of organization to hold a fair balance between the freedom of thought and action desiderated by responsible scientific opinion and the national need for efficient use of resources Nevertheless, the attitude hitherto maintained by the

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* *Committee of Enquiry into the Organization of Civil Science* Pp 59 (Cmnd 2171) (London H M S O, 1963) 4*s* net

Minister for Science can no longer prevail without modification, and some new arrangements must be concerted to correct the manifest deficiencies

So far as the universities are concerned, like the Robbins Committee†, the Trend Committee upholds the principle of the University Grants Committee and assumes that the universities' general income, from which their teaching and research functions are mainly financed, will continue to reach them in block grants, covering a period of years Nevertheless, it also considers that the system of additional earmarked grants, at present operated mainly through the Research Councils, is vital for the proper development of scientific research in the universities and should be continued It offers the indispensable safeguard to centralized provision as well as meeting the unforeseeable contingencies arising out of the rapid advance of science The Trend Committee does not believe that it would be a satisfactory arrangement for the University Grants Committee to assume the full responsibility for university research, including the functions at present exercised by the Research Councils

On the contrary, the Committee considers that, subject to certain safeguards, the present system provides the most satisfactory arrangements for the support of science in the universities, and it suggests that the partnership between the universities and the Research Councils should be extended to deal with the problems that arise from the increasing costliness of certain types of essential equipment Safeguards are needed, however, to ensure that the earmarked grants do not become so large in scope and scale as to distort the pattern of university research The Trend Committee was not satisfied that Research Council grants for specific projects always covered the real costs of these projects, including the overheads and provision of technical assistance and accommodation. It thought that this question required further consideration by Research Councils and welcomed the recent decision to allow Research Councils to supplement grants for postgraduate training by contributions towards the incidental costs of equipment, etc Further, it insists that, in apportioning their general income, the universities must bear in mind the needs of the country in research no less than in teaching, and that an effective means must be provided for ensuring that financial responsibility for research projects is transferred from the Councils to the general income of the universities when the research which the Councils have been supporting becomes an integral part of the work of the universities and when new subjects, developed from these researches, ought to be incorporated into the general university structure

Such a procedure has already been devised and used for the transfer to university general funds for the 1962–67 quinquennium of 16 per cent of expenditure previously financed by the Research Councils, and the Trend Committee is satisfied that this procedure provides the necessary safeguard It is also satisfied that the universities generally accept their responsibility for national needs in research, and although the Committee is well aware that the research needs are liable to be overlooked when universities are being asked to concentrate their resources on increasing the number of places available for students, the Robbins Report lays sufficient emphasis on the expansion of postgraduate activities and on the development of research to suggest that the danger is not very serious Further, the Trend Committee urges the Research Councils to consider the establishment of units within, or in association with, universities as an alternative to the creation or expansion of separate research institutes It also suggests a modest expansion of the system of Research Professorships of the Royal Society and that the universities might direct attention to the provision of a larger number of professorships and other senior posts in their science departments—the Committee insists that, in the long-term, university finance should be on a scale sufficient to permit the creation of such posts out of general income

The Report recommends no change in the present functions of the Medical Research Council or the Agricultural Research Council, though the Committee suggests that when the latter Council has completed a current review of facilities for research in relation to processing imported food, existing arrangements for research on food-processing should be examined in more detail On the other hand, the Committee finds the present combination of responsibility for scientific research with that for industrial research and development in the Department of Scientific and Industrial Research unsatisfactory It unhesitatingly recommends the dissolution of that Department and the distribution of its functions and responsibilities between three new agencies, all responsible, like the other two Research Councils, to the Minister for Science Two of these will be Research Councils—a Science Research Council and a National Resources Council—and the other, in view of the executive character of some of its functions, which will include those now exercised by the National Research Development Corporation, will be an autonomous agency, the Industrial Research and Development Authority

If the Committee's recommendations were followed up, the Science Research Council would assume the responsibility for the support of relevant research in universities and similar institutions as well as for postgraduate awards in science and technology, and its activities would not be so narrowly interpreted as to hinder it from fostering the expansion of technological research, whether in universities or other institutions, on which the Robbins Committee lays emphasis It is not to be confused with the Advisory Council on Scientific Policy, which is to be reconstituted with the authority and resources that its status as the chief advisory body to the Minister for Science demands Initially, the Science Research Council is conceived as being organized in six divisions, based on scientific disciplines, and including responsibility for financing the National Institute for Research in Nuclear Science, for the scientific aspects of the United Kingdom's relations with the Council for the Encouragement of Nuclear Research, for the supervision of the scientific space research programme and for the United Kingdom's interest in the European Space Research Organization It would assume direct control over the Royal Observatories, but responsibility for the Meteorological Office should remain with the Air Ministry The Report makes no attempt to define the precise nature of the organization required, but it points out that the new Council must also be prepared from the start to encourage activities devoted to the synthesis of scientific experience, including new applications of scientific method, and that additional divisions for the purpose should be established as required Any means of providing the scientific basis for working out policy such as Dr S Toulmin has suggested could presumably be fitted in at this point (see *Nature*, 200, 503, 1963).

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† Committee on Higher Education *Higher Education Report of the Committee appointed by the Prime Minister under the Chairmanship of Lord Robbins* Pp xxi + 335 (Cmnd 2154) (London H M S O, 1963) 15*s* net See also *Nature*, **200**, 925 (1963)

The recommendation for the establishment of a National Resources Research Council follows the recommendations of the Report of the Committee set up in 1961 under the chairmanship of Sir William Slater by the Advisory Council on Scientific Policy This Council should be responsible for all the functions now exercised by the Nature Conservancy, including the management of nature reserves, the Trend Committee agreeing on this last point with the Advisory Council rather than with the majority of the Slater Committee It would also be responsible for research into hydrology, fisheries and related aspects of aquatic biology, though not for the management of the Departmental fisheries laboratories and the Torry Research Station, for the Geological Survey and the Soil Surveys, for long-term forestry research, and for research into oceanography, although administrative responsibility for the National Institute of Oceanography would remain with the Admiralty The Development Commission's research activities in fisheries, oceanography and marine biology should also be transferred to agencies under the new Council, but further consideration of the future of the Commission is called for, particularly with reference to its responsibilities in rural development and rural sociology

In some details, as indeed is already indicated, these proposals do not follow the recommendations of the Slater Committee, as the Trend Committee had reservations about some of the detailed recommendations, but it agreed that existing arrangements for promoting scientific research in ecology are deficient and that it would be right to bring together, in one organization, work which is now the responsibility of various agencies The Trend Committee does not propose to transfer to the new Council at present either the Water Pollution Research Station or the Hydraulics Research Station, but the Hydrological Research Unit should be transferred, and the new Council should also be responsible for the existing Committee on Hydrological Research The majority of the existing research stations of the Department of Scientific and Industrial Research would be transferred to the new Industrial Research and Development Authority, and one of the six divisions of this Authority envisaged would be devoted to their administration

Another division of the Authority would be responsible for grants for applied research or specific research projects in the industrial field, and it is suggested that the Human Sciences Committee of the existing Department might form part of this division The support of industrial research associations would fall to a third division, and it is suggested that a first task of the new Authority would be to consider whether any changes in the organization of the associations, the scale of their operations or the method of financing are required The Trend Committee seems to accept the view that some of the smaller associations could be amalgamated with advantage, and it proposes the same division should be made responsible for the unit created for promoting operational research in industrial operations Another division would continue the work at present sponsored by the Department's Economics Committee of technico-economic surveys of certain industries in relation to their needs for research and development, and this division in particular would have close relations with the National Economic Development Council

A main reason for advocating the establishment of the new Authority is the Trend Committee's belief that considerable development of the Department of Scientific and Industrial Research's activities in industry is required, particularly in relation to the activities of the National Economic Development Council and, in promoting development contracts, the National Research Development Corporation Such development the Committee regards as of major and growing importance, and its proposal that the work of the Corporation and the Department's activities in this field should both be transferred to a Development Division of the Authority, operating on similar lines to the Corporation and with a like measure of independence but relieved of the statutory obligation that its operations should break even, is obviously sound Further, the Trend Committee suggests that this Development Division should be authorized to stimulate Departments to place development contracts for new inventions and equipment, playing a positive part and encouraging initiative

The Committee's recommendations for the dissolution of the Department of Scientific and Industrial Research and for the re-grouping of its functions are logical and seem fundamentally sound They should eliminate some of the present weaknesses and should also increase the effectiveness of the National Research Development Corporation Moreover, the Committee is well aware of the importance of effective co-operation between the various Research Councils themselves and between the Councils and Government departments and other organizations, including the defence departments Although no specific arrangements are suggested, the desirability of a close link between the Ministry of Defence and the Industrial Research and Development Authority is particularly desiderated Further, the new Advisory Council to the Minister for Science, which, it is recommended, should replace the present Advisory Council on Scientific Policy, with similar terms of reference but consisting entirely of independent members, half of whom should be scientists, some industrialists and some with wide experience of public affairs, provided with adequate staff and resources, should supply the necessary oversight and co-ordination

That Council, however, is part of the new arrangements proposed in regard to the Minister for Science, but there are two further matters which should be noted first One of the six divisions initially suggested for the Industrial Research and Development Authority is concerned with the dissemination of scientific and technical information, including the co-ordination of information activities throughout the Authority It is recommended, however, that the Office of the Minister for Science should take over the management of the National Lending Library for Science and Technology and the support of the Association of Special Libraries and Information Bureaux This again is an improvement on the present arrangements, but it is still only a first step For all its recognition of the value of information work, the Trend Committee shows no appreciation of the importance of a national library policy Arrangements which leave the management of the National Lending Library for Science and Technology and of the new National Reference Library for Science and Invention in different hands are not ideal, and the proposals here are only acceptable as an expedient while the wider issues of ministerial responsibility are being decided

The second matter is one on which the Trend Committee displays an extraordinary lack of insight It blandly proposes that the Overseas Research Council, which in 1959 replaced the Colonial Research Council, should be dissolved, suggesting that it has now discharged its functions The Overseas Research Council has, in fact, never issued a report and it would be more accurate to suggest that it has never functioned Since the Colonial Research Council was dissolved, the Government has drawn a veil over overseas research in spite of its increasing importance and urgency, and the importunities of those who recognize what is at stake have had no effect on either Government or Opposition The two great political parties have both been content to watch the leadership, which Britain once gave and should still be giving in this field, pass to the United States, the Department of Technical Co-operation has so far been unable to break the silence or end the neglect. While the Trend Committee does, in fact, admit that research in less-developed countries should not be neglected entirely and that the Department of Technical Co-operation is a less suitable means for furthering such efforts than a research council, its proposal that this should be included among the responsibilities of the Minister for Science for the Government's international relations in scientific matters is entirely inadequate The Committee's lack of vision and appreciation of Britain's place in the world of to-morrow is a blot on an otherwise sound and constructive Report

Of the further changes recommended in regard to the Minister for Science, it will suffice to note here that it is proposed he should assume additional substantive responsibilities for promoting civil scientific research and development, particularly as regards the allocation of available funds, and that the power to appoint the governing bodies of the several research agencies and the authority to issue formal directions should be vested in him and not, as at present, in Privy Council Committees The Report shatters the attempt which the Minister has so long maintained to operate with a minimal Office and recommends strengthening his staff and facilitating exchange of personnel between his Office, the headquarters of the research agencies and the stations which they operate Here, generally, it is in line with the recommendations for a Science and Policy Office considered at the Ministerial Meeting on Science in Paris in October (*Nature*, 200, 719, 1963)

Leaving for later discussion the question of arrangements at ministerial level in the light of any decision regarding responsibility for higher education, etc , the Trend Committee's proposals offer opportunities for immediate action to improve the present arrangements However, while that action is being taken, the matters which the Committee has left on one side, such as co-operative industrial research and the future of the research associations and the location of some of the research stations, could be further studied There is no *a priori* reason why the new Advisory Council, the Councils for Science Research and National Resources Research and the Authority for Industrial Research and Development should not be established as quickly as possible, though this will require legislation, and the process may well provide a touchstone of the sincerity of the support which the political parties promise to scientific and technological work There are points on which considerable difference of opinion is possible, but the differences do not lie on party political lines, and the proposals of the Trend Report offer a means of rectifying some of the present weaknesses in the organization of civil research in Britain which calls for firm support from all political parties

RESEARCH MANAGEMENT

Scientists in Industry

Conflict and Accommodation By Dr William Kornhauser, with the assistance of Warren O Hagstrom Pp xii+230 (Berkeley and Los Angeles University of California Press, London· Cambridge University Press, 1962) 6 dollars, 48*s*

Managing Creative Scientists and Engineers

By Eugene Raudsepp Pp xvi+254 (London and New York Collier–Macmillan, Ltd , 1963.) 55*s*

BOTH books are concerned essentially with the theory and practice of research management, but in approach, as well as in style and treatment, they are in sharp contrast Dr. Kornhauser examines the conduct and management of research as an example of the relations between professional workers and the industrial and other organizations by which they are employed, and if he is inclined at times to exaggerate the conflict and strain which may arise between professional interests and those of Government or industry, his book is an important contribution to the discussion of the place and functions of the professions in the technological society of to-day He takes up the examination where Carr-Saunders and Wilson left it thirty years ago, but with due acknowledgment to the subsequent investigations of Lewis and Maude Dr. Kornhauser does not accept without reservation Carr-Saunders and Wilson's view of the professions nor does he give any precise definition to put in its place Nevertheless, he recognizes the importance and value of that earlier work, if he seems rather to overlook the stress which they laid on a profession as providing the permanent anchorage and shelter which are the prerequisite for independence and integrity in professional service to society

Dr Kornhauser's book is based on the findings of an investigation he conducted as a member of the Institute of Industrial Relations of the University of California among a group of scientists and engineers engaged in industrial research, mostly employed by industry, but some by Government and independent research organizations At the outset he outlines the ways in which tension is liable to arise between professional ideals and interests and the objectives of the organization in which the scientist or engineer is employed This is followed by discussions of professional goals in industry, the ways in which professional controls can be exercised in industry—and here he discusses the management of research more specifically—professional organizations and their relations with industry, professional incentives in industry, professional influence in industry, and, finally, the adaptations of the professions and industrial organizations towards one another Dr Kornhauser is writing in an American context and it is important to remember this in reading his forthright rejection of the Trade Union, but he is not unmindful of conditions in Britain, though he makes no reference to the work of such bodies as the British Association of Chemists, the Association of Scientific Workers or the Institution of Professional Civil Servants

Such omissions apart, Dr Kornhauser deals fairly enough with the main problems that arise in the relations of professional men with industry or indeed with society in general, and with the functions of the professions to-day now that such a high proportion of their members are now no longer self-employed but employees The implications for the future are indicated clearly enough, and while, as already noted, Dr Kornhauser's first preoccupation is with the American scene, his vision is much wider, and an excellent bibliography is not limited to American sources but includes the major contributions from Britain Well written and substantially free from jargon, it will be read with interest by scientists and professional men generally and not simply by those concerned with the practice or management of research

While the research manager will find many points for reflexion if not suggestions for practice in Dr Kornhauser's book, that of Dr Raudsepp, directed specifically at the research manager, appears to be addressed also to the managed, at least to judge by an appendix, "Increasing your creative problem solving ability", which seems somewhat out of place, though in themselves, if adopted, some of the suggestions might assist the scientist in adjusting himself to industry Dr Raudsepp, in fact, does not seem quite sure of himself or of the type of reader he is addressing He begins with two chapters dealing with the creative process and the characteristics of the creative professional, but it is never clear whether he is concerned essentially with discovery or with invention. Failure to make the distinction confuses much of his writing throughout the book, and this part also illustrates another main weakness While an understanding of the nature of discovery and invention and the conditions most favourable to them is a logical starting point in considering the management of research workers, the examination of the creative process is far too superficial and is limited to American sources Apart from Ghiselin, scarcely a major contribution is mentioned, and for an understanding of the creative process itself one must still turn, for instance, to the writings of J L Lowes and J J Thomson and Sir Henry Dale, to Mrs Jacquetta Hawkes's talk on her father, Sir F Gowland Hopkins or to C Nicolle's little book, *Biologie de l'Invention*

For the research manager, however, the essential thing is to understand the conditions for creative work in which the mind has free play, and the second part of the book discusses the factors in the general climate of culture and industry in the United States which inhibit or stifle creative work or that favour its expression In the second of these chapters, Dr Raudsepp makes several suggestions for changing the industrial conditions so as to promote creative work and initiative, but it is in the third part of the book that he addresses himself specifically to the problem of managing creative research, and by that he means essentially fundamental research Here again there is some confused thinking, and particularly when he comes to discuss the work of teams or groups, where a number of assumptions are open to question Nor does he attempt to distinguish between supervision and management of research, though what reads like a deliberate attempt to obliterate a distinction recently emphasized by a Working Party of the Federation of British Industries seems, on the contrary, to demonstrate the validity of the distinction

His concluding chapter is entitled "Supervising Creative Professionals" and is concerned with what in Britain would be generally understood as the management of fundamental research Much in this chapter, and also in the suggestions made regarding incentives in the preceding chapter on motives of creative workers, is essentially sound, though sometimes it verges on the trivial and at other points is controversial, if not provocative

By and large, Dr Raudsepp is inclined to be too sweeeping in his generalizations, particularly about people, and his heavy, sometimes staccato, style does not make for easy reading Here and in his excessive use of jargon, not always self-explanatory, the absence of any bibliography, and the fragmentary character of his references, his book contrasts sharply with Dr Kornhauser's Industrial research managers who read this book will doubtless find some useful suggestions, though some may have considerable reservations, and its practical value is likely to be in direct rather than in inverse proportion to their own experience and vision

R BRIGHTMAN

MONOPHYLETY VERSUS POLYPHYLETY

The Origin of Races

By Carleton S. Coon Pp xli + 724 + xxi + 32 plates. (London Jonathan Cape, 1963) 63*s*. net.

THE classical view of the origin of the present-day races of man is one of comparatively recent diversification from a single stock However, as ever more fossil remains are discovered, it becomes increasingly likely that the species *Homo sapiens* did not arise in a restricted area, and that there never was a single stock in the sense of a particular geographical group of populations There is still considerable controversy as to what fossil hominids should be included within the taxon *Homo sapiens* Many of the arguments result from the difficulty of recognizing palæospecies It is evident that if the fossil record were complete the making of taxonomic groups within the evolutionary continuum must involve putting father and son in different categories at some level or other When there are data on the ecology and size of fossil populations it is occasionally possible to bring some objectivity to a taxonomy of evolutionary clades, but in most cases, and certainly so in palæoanthropology, classifications must perforce be essentially arbitrary In the past, much use was made of the 'gaps' in the fossil record, but many of these 'gaps' have now been filled and the ancestry of present-day man can be traced back to at least the Middle Pleistocene with a reasonable degree of certainty There is an increasing tendency to refer all these ancestral forms to the species *sapiens* not only because of the cladal continuity but also because of the many otherwise unique features which are shared with present-day man

If then the species, in this sense, is quite old the possibility exists that the races of man are also old, but there is variable opinion as to how old Coon, in this book, maintains that the major races are extremely old and that they were differentiated at least as early as the base of the Middle Pleistocene Unfortunately, he does not systematically present and evaluate the evidence for this view, and in the main it is left to the reader to extract the relevant information from the wealth of data which is given in a descriptive way about not only all fossil hominids but also the rest of the Primates It would seem, however, that particular importance is attached to two facts In the first place, Coon points to the immense differences which exist between the languages of the world to-day, and considers that such diversity must indicate long separation This theme, however, is not developed, and his remark that "it takes 20,000 years for two sister languages to lose all semblance of relationship" would seem to indicate that the evidence is not of any great relevance anyway It is doubtful if anyone thinks that the differentiation of the major present-day races did not begin considerably earlier than this The second type of evidence is more compelling and rests on the occurrence of distinctive racial characteristics in both the present-day populations of large geographical areas and in fossil populations from the same areas Coon considers that there is a more or less complete morphological sequence of forms connecting the present-day Australoids with Java man, and he recognizes distinctive mongoloid features in the fossil hominid remains from Eastern Asia, including Peking man, Caucasoid features in the European fossils, and Capoid (Bushman-Hottentots) and Congoid (Negroid) features in some of the ancient remains from different parts of Africa These distinctive geographical patterns are interpreted as indicating separate phyletic descent for at least these five major races of man from the times of Java and Peking man Since the latter are usually included in a different species (*H erectus*) from modern man and some would consider that the differences are so large as to warrant generic status (*Pithecanthropus*), such a view implies extremely extensive parallel evolution.

To account for this parallelism, Coon points out that most of the changes which occurred during the later phases of hominid evolution are, in the evolutionary sense, general improvements, and that, therefore, selection would have favoured them in all environments and every geographical population. In such polyphyletic gradal evolution, however, it is unlikely that the rates of change will be similar in each area, and one would, therefore, expect that some populations are in the biological sense more primitive than others. As evidence of biological primitiveness, Coon points out that the Tasmanians at the time of their discovery were "living in a culture 100,000 years old". He considers, in fact, that some races have only just crossed the *sapiens* species boundary, while others did so some considerable time ago.

It is perhaps this conclusion more than any other which has been responsible for the bitter acrimony with which this book has been received, since it implies that some races of present-day man are biologically inferior to others. However, quite apart from these unfortunate overtones, for which there is no objective scientific evidence, there are a number of objections to Coon's view. The main one, of course, is that all present-day populations of man will naturally interbreed and produce fertile offspring: that they are, in fact, only races of the same species. Had they evolved completely separately from one another, it seems extremely unlikely that the many differences between *erectus* and *sapiens*, even if phenotypically established five times over, would be genetically established in more or less the same way on each occasion.

It seems far more probable that the similarities between present and past populations of the same geographical area, which Coon uses as primary evidence for his polyphyletic theory, represent convergence. It is to be expected that populations living under comparable ecological conditions will evolve similar adaptations. There is, in fact, fairly good objective evidence that such convergence has occurred. Almost certainly it accounts for many of the similarities between African negroids and Melanesians, and between the Eskimos and the people who occupied Europe during the Wurm III glacial period.

This is not to say that Coon's view is completely untenable. In the first place, it is rarely possible in palæoanthropology to prove a hypothesis wrong: the best that can usually be done is to balance the various possibilities in the light of general evolutionary theory. On this score, Coon's hypothesis seems extremely unlikely in that it calls for an unprecedented degree of parallel genetic change. On the other hand, there can be little doubt that hominid evolution is largely a gradal phenomenon and that selective pressures must have been qualitatively similar the world over. The real point at issue is the extent to which past populations were reproductively isolated from one another. Coon acknowledges that gene flow must have occurred at times, but discounts its effects. Yet it seems likely that such gene flow was, in fact, the very agent by which all populations obtained the same genetic basis for their gradal evolution, and it would, of course, act to keep the species intact. Perhaps one of the most important factors determining human evolution has been the widespread movement of populations within, geologically speaking, short periods of time.

The most reasonable view of the terminal phases of human evolution is of the species evolving as a whole the world over. It could well be that there is temporal continuity between the particular ecological adaptations of present populations and those found in ancestral groups, but the important point is that *Homo sapiens* has a monophyletic rather than a polyphyletic origin.

While most anthropologists will find the general thesis of this book unacceptable, everyone must be indebted to Coon for the detailed account he provides of the fossil hominids. Practically all the authenticated remains are fully described, including many remains which, even for palæoanthropologists, have been little more than names. There are errors of fact, perhaps more than one expects in even such an extensive review, and English readers may well find the writing stylistically irritating, but this is a comprehensive up-to-date document of human evolution and an invaluable reference book.

G. AINSWORTH HARRISON

EVOLUTION IN FORAMINIFERA

Evolutionary Trends in Foraminifera

Edited by G. H. R. Von Koenigswald, J. D. Emeis, W. L. Buning, and C. W. Wagner. (A collection of papers dedicated to I. M. Van der Vlerk on the occasion of his 70th Birthday.) Pp. viii + 355. (Amsterdam and London: Elsevier Publishing Company, 1963.) 45 D.fl.; 90*s*.; 50 D.M.

FORAMINIFERA are small in size and occur in most marine sediments. They have been abundant since the Upper Palæozoic and have shown well-marked evolutionary changes since their appearance in the Cambrian, providing unique opportunities for investigations of populations through space and time. An enormous amount of work has been published on this group, stimulated by their stratigraphic importance and consequent economic value in oilfield exploration and development. It might, therefore, be expected that the main lines of evolution would be known and important principles deduced relating to the theory of evolution in general. The publication of this well-produced, well-illustrated book which brings together fourteen papers by different authors enables us to take stock of progress in this field.

Modern classification of Foraminifera leans heavily on the evidence of wall structure, recapitulation and the stratigraphic succession. These papers show that great progress has been made both in the investigation of the basic structure of the shell wall, regarded as a progressive evolutionary character, and also in detailed knowledge of the stratigraphic succession. In general the principle of recapitulation is accepted but not, apparently, by all the authors. Family relationships are now seen to be more complicated than was thought, even a few years ago, with parallel development along many lines. The sheer amount of new knowledge that has accumulated is well shown by Cita-Sironi. This author points out that the few species known in the Globotruncanae before 1940 have now swollen to more than one hundred, while, instead of one genus, *Globotruncana*, there are now twenty-three.

Dunbar outlines very clearly the main trends in the Fusilininae and Schwagerininae. These include development of inner and outer tectoria in the Fusilininae and increase in size and honeycombing of the layered, granular wall in the Schwagerininae. Both sub-families developed folded septa. Dimorphism became emphasized in the Permian with appearance of gigantic, microspheric individuals. These trends are well expressed stratigraphically and the idea of recapitulation is supported by the presence of a flat, initial spire in microspheric forms which is taken to indicate descent from *Endothyra*. Rauser-Chernousova also supposes that evolution in this group is characterized by recapitulation, with descent from the endothyrids, but her evolutionary scheme differs in detail. The value of species and genera in intercontinental correlation is discussed and a distinction drawn between conservative, ancient families and advanced families with genera of short range.

The trend towards accentuated dimorphism also occurs in the Nummulitidae, discussed by Schaub. Again, the shell tended to become larger and adult chambers longer and lower through Palæogene time. Morphological continua are also traced in great detail in the Miogypsinidae (Drooger) and Orbitoidae (MacGillavry) with analysis of equatorial chamber pattern and nepionic reduction. The presence of a gumbeline initial part is confirmed in the Orbitoidae which throws an unexpected light on their possible ancestry. Phyletic relationships in the Miliolidea are also discussed (Smout).

Doubt is thrown on the principle of recapitulation so far as the Lagenidae are concerned, by Brotzen, who derives *Lenticulina*, *Frondicularia* and *Nodosaria* independently from layered, granular palæonodosarids allied to *Nodosinella*, through granular and fibrous mesonodosarid intermediaries in the Triassic and Liassic. The Rotaliidea and Buliminidea are considered to be derived through the Triassic Duostominidae from either the Endothyridea or the Trochamminidae. The possibly independent origin of *Globigerina* from arenaceous ancestors is also suggested. This stimulating paper will undoubtedly cause controversy. Evolution in the Lagenidae is also considered by Barnard, who shows that an explosive burst of evolution occurred in the Lower Jurassic with variation in general morphology. Variation in ornament occurs later and in *Lingulina* and *Frondicularia* progressive evolution of ornament is of stratigraphical value. Rather similar variation in ornamented species of *Bolivina* is of value in Upper Cretaceous stratigraphy (Hiltermann).

This new work supports the idea that the Lituolidea, Endothyridea and Miliolidea are well-defined groups (Glaessner). Relationships in the Rotaliidea s l and Buliminidea are, however, complex and investigations have not gone far enough for the main evolutionary lines to be made clear.

Although Rat relates the architecture of the Orbitoline test to sedimentary milieu and Hottinger relates the shape of the Alveoline test to changes in surface/volume ratio of the endoplasm, little is known of the adaptive significance of most morphological trends. This is largely due to lack of work on living Foraminifera, consequent in part on the difficulty of making observations with the ordinary microscope. The extended use of electron microscopy may be expected to throw a flood of light on the structure and adaptation of modern forms.

Biologists as well as palæontologists should read this book. We may then expect an equally impressive symposium on Dr Van der Vlerk's eightieth birthday.

J HAYNES

HABITATS OF THE PAST

Principles of Paleoecology

An Introduction to the Study of How and Where Animals and Plants Lived in the Past. By Derek V Ager. Pp xi+371. International Series in the Earth Sciences (New York McGraw-Hill Book Company, Inc , London McGraw-Hill Publishing Company, Ltd , 1963) 83*s* 6*d*

IN the past twenty-five years or so a vast number of papers on various aspects of palæoecology have been published. In view of this interest it is surprising that a text-book on the subject written in the English language has not hitherto appeared. Dr Ager is to be congratulated on providing such a book.

Principles of Paleoecology is a compact volume divided into three parts. The first introduces the subject briefly and explains the principles involved and the relationships to kindred subjects, the second describes in some detail methods of determining the ecology of fossil taxa, the third discusses the ecology of fossil communities. After the niceties of the introduction are dispensed with the meat of the book begins. In the second part (termed 'paleoautoecology') the author reviews various methods of interpretation such as comparative anatomy and physiology, comparative habit and habitat, the problems involved in the interpretation of extinct groups, shell orientation and its significance, organic associates and trace fossils. Discussion of fossils and the sedimentary environment is treated both in this and in the subsequent part, but the author feels that a discussion of the sediments themselves has no place in the book.

In the third section, 'paleosynecology', topics include density and diversity of fossil assemblages, isotope investigations, species interrelationships including symbiosis and antagonism, lateral and vertical changes in fossil assemblages, zoogeography of fossils, and a final chapter on palæoecological synthesis.

The framework of the book is comprehensive and carefully thought out, and the examples quoted in the subsections give fair coverage of the methods used. Bias towards Mesozoic and Tertiary examples and the brachiopods in particular is evident and is disarmingly admitted by the author. Less explicable is the haphazard presence or absence of commentary on some of the examples. Ma's work on the distribution of fossil corals and their bearing on continental drift, for example, is described, but no warning on the shortcomings of this work is provided for the inexperienced reader. The author stresses the need for a statistical approach to palæontology, yet the record of the orientation of one shell of *Cyrtodonta* in a shale is quoted without comment as evidence of an anchored mode of life.

The importance of transported fossils in palæoecology is minimized and the subject dismissed as being more akin to sedimentology. If the end is merely sedimentological then Dr Ager is right, but it is well to remember that transported fossil assemblages are common and have had a palæoecological history. Sedimentological analysis ought, therefore, to precede palæoecological synthesis and not be an end in itself.

Throughout the book there is an underlying appreciation of the influence that palæoecology must have on systematic palæontology and the species concept, but nowhere does this surface as a major theme. Palæoecologist and systematic palæontologist are in a position to help each other and present a more enlightened image of palæontology. If Dr Ager had developed this theme explicitly he might have more surely strengthened the links between —as he puts it—the impractical theorizers and the old-fashioned museum palæontologists.

The book is well illustrated and attractively presented. Apart from two or three obvious spelling mistakes (Americanized spelling excepted) the only error noted was that of the drawing in Fig 7 3 purporting to represent the trace fossil *Corophioides*. Unusually the book is written in the first person singular (and occasionally plural) which at times intrudes egotistically into the narrative.

Principles of Paleoecology contains much of interest for the specialist and yet can be understood by students with a year's training in geology. Even if the book had not been the first in the field it would have deserved commendation. As it is, Dr Ager is to be congratulated on the successful compilation and condensation of such an enormous field of investigation.

G Y CRAIG

MODERN MICROBIOLOGY

Modern Microbiology

By Wayne W Umbreit. Pp viii+507 (photos and diagrams) (San Francisco and London. W H Freeman and Co , 1962) 48*s*

THIS latest book by Prof Umbreit is a text-book of microbiology which seems to be designed for university students specializing in microbiology—although at no point is this stated by the author. In addition to sections on the structure and physiology of micro-organisms in general there are chapters on pathogenic microbiology (*sic*), brewing, food microbiology, the microbiology of milk products and the use of micro-organisms in the industrial production of solvents, antibiotics and pharmacologically active compounds The treatment of the subject is rather unbalanced in places, for example, the theory, technique and uses of electron microscopy are dealt with in about 300 words along with 2 figures, and bacterial growth factors in about the same space, whereas there are considerable direct quotations from original papers by Gram, Fleming and Florey, together with a long account of the Pasteur/Liebig controversy about fermentation

In a field where good text-books already exist, the value of a new book depends on whether it has a valuable, new approach to the subject or is significantly more up-to date than its predecessors and whether it is accurate Although this book does contain some accounts of recent work, and perhaps has greater emphasis on industrial processes than previous books, these advantages are far outweighed by the many misleading statements, inaccuracies and omissions It is possible to give only a few examples The book contains no account of the glyoxylate cycle while referring readers to reviews (1951–52 vintage) which support the presence of a dicarboxylic acid cycle in bacteria The sections on transformation, transduction and mating (called recombination by the author) are very misleading, and neither the concept of the episome nor the process of lysogenic conversion is mentioned Similarly, the account of bacterial cell walls is completely inaccurate No mention is made of the presence in walls of D-amino-acids, nor of *N*-acetyl glucosamine *N*-acetyl muramic acid is stated to account for all the amino sugar of the wall and is given a structure (p 69) which is completely incorrect In the section on antibiotics no mention is made of the cephalosporins, and most penicillin-resistant staphylococci are incorrectly stated to be resistant by reason of some unknown mechanism rather than by the possession of penicillinase

The book is full of confusing argument—for example " . any present evidence for a nucleus in bacteria must of necessity be somewhat indirect First, bacteria possess DNA Second, bacteria do possess hereditary character Hence, even though we may be unable to see a nucleus there is no doubt but that a nucleus does exist" The style of the book is repetitious and chatty, and large passages in the book give the impression of being direct transcriptions of tape-recorded lectures

M H RICHMOND

BIOLOGICAL EFFECTS OF IONIZING RADIATIONS

The Initial Effects of Ionizing Radiations on Cells

A Symposium held in Moscow, October, 1960, supported by UNESCO and the IAEA, and sponsored by the Academy of Sciences of the U S S R Edited by R J C Harris Pp xii+367 (London Academic Press, Inc (London), Ltd , New York Academic Press, Inc , 1961) 75*s*

THIS volume is a report of a symposium held in Moscow in 1960, and was published in 1961 That it is reviewed at this late date is due mainly to the timeliness of the subject matter

Radiobiology has in its brief existence already reached the stage at which the volume of literature produced, the inter-disciplinary nature of the subject, and the single-mindedness of its research workers make it essential that attention should be directed again to work which has already been done and to theories which have already been postulated In the collected papers of the symposium, beginning and ending with stimulating papers by Alexander and Bacq, ideas are put forward on the role of nuclear as opposed to cytoplasmic sites for the initial lesion of radiation injury, on the relative roles of injury and repair, on the ubiquitous role of oxygen in radiation lesions, and the systems in which oxygen seems to play little or no part There emerges a pattern of biological response to even smaller doses, especially in relation to neuromuscular response The work on the central nervous system is described by the Soviet workers in more detail and allowing for a better assessment than was possible from the Geneva conference on "The Peaceful Uses of Atomic Energy" in 1958 Enough detail is given here to trigger off a full-scale study of this sort of functional response to radiation damage, which is inevitably going to be a more sensitive indication than morphological changes at present used as the main criterion of damage Increasing investigations of functional derangements in systems by radiation exposure may well lead to a change in our concept of radiation sensitivity

The presentation of the papers and discussion in the volume are good, misprints should be unnecessary in a book which has taken this long to produce An interesting aspect of the discussion is a complete lack of East–West division, even when such controversial subjects as the genetic effects of radiation and their relation to dose and dose-rate, or the role of the nervous system in radiation injury are discussed

Looking through the list of contributors to the volume, one is impressed at the galaxy assembled between the covers These people give evidence of their thoroughness, originality and stimulation in the presentations and discussions—and most of them have maintained their promise since Perhaps it is not a bad thing to review a book several years after its appearance, when it can be seen in perspective as a brick which has helped to build up the subject in subsequent years PATRICIA J LINDOP

A RUSSIAN VIEW OF FERRITES AT MICROWAVE FREQUENCIES

Ferrites at Microwave Frequencies

By A G Gurevich Authorized translation from the Russian by A Tybulewicz Pp viii+332 (London Heywood and Co , Ltd , 1963) 140*s*

A STUDY of ferrites at microwave frequencies was first initiated about thirteen years ago The material treated in this work covers the first nine years of this period, which includes part of a very progressive period between 1954 and 1961 Interest in ferrimagnetics at microwave frequencies can conveniently be divided into two main areas a study of the principles and properties of microwave devices and a study of the physical behaviour of ferrimagnetics The author and his colleagues have made very significant contributions to the latter field and it is, therefore, not surprising that the most informative sections of the book are those concerned with physical processes There is comparatively little information on devices, and the book will have only a slight appeal to the microwave engineer Unfortunately, a complementary Russian work dealing with devices has not so far been translated

The work is in three parts dealing, in effect, with magnetic properties of ferrites, wave-guides containing ferrites and non-linear processes It is assumed in the first section that the reader is already familiar with the origin of magnetism in ferrites The statement that "Ferromagnetic resonance can be explained quite simply on the basis of quantum mechanics" is perhaps somewhat terse By compensation, the treatment of spin waves is very well presented, and included is a useful survey of Walker's theory of magnetostatic modes The title of the first chapter "Isotropic Ferrites Magnetized to Saturation" is perhaps somewhat misleading A magnetized ferrite exhibits an anisotropic permeability, and the author is actually referring to the absence of crystalline anisotropy when he states that the medium is isotropic. The phenomena of ferromagnetic resonance is very adequately discussed and, in this section especially, the author has referred primarily to Russian sources for experimental data.

Although the sections on electromagnetic wave propagation in infinite ferrite media and waveguides containing ferrite have been carefully prepared it is in this area that the book is weakest. The author appears to have overlooked completely any British contributions to this field and there is only one figure showing results for circular waveguides. The rectangular waveguide containing a transversely magnetized ferrite slab has been treated more fully and the author presents the results of his own computations. There is no discussion of the junction circulator configuration which forms the basis of an important class of ferrite components.

As previously mentioned, the book does not describe devices, and it is only likely to prove helpful in this area to the microwave engineer who is already fully conversant with the factors which affect the design of waveguide ferrite components.

In the final section on non-linear processes, the author draws more on work from the United States than elsewhere in the book. A very readable review of work until 1959 is presented. Unfortunately, many very interesting developments have occurred since that time of which the study of longitudinal pump instabilities and magnetoacoustic interactions are, perhaps, the most significant.

In conclusion, the book can be very warmly recommended to those who are interested in studying an approach to microwave ferrimagnetics which is not based primarily on Western literature. The numerous references to Russian results are of particular value since this information is often not readily available to Western readers. The text was published as part of a series on the physics of semiconductors and semiconductor devices, and it is to be hoped that a treatment of Russian studies in this field, after 1959, will become available within the next few years and without such a long delay in translation.

P. J. B. Clarricoats

QUANTUM BIOCHEMISTRY

Quantum Biochemistry

By Prof. Bernard Pullman and Alberte Pullman. Pp. xvi+867. (New York and London: Interscience Publishers, a division of John Wiley and Sons, 1963.) 195s.

"WHEN I use a word", Humpty-Dumpty said in a rather scornful tone, "it means just what I choose it to mean—neither more nor less". The Pullmans have much the same attitude to the words 'quantum biochemistry', by which they evidently mean the calculation, using traditional quantum-mechanical methods, of charge distributions and associated parameters in organic molecules. Most of the book is, in fact, an edited collection of their previous publications, little or no interest being shown in other workers' results, in newer and more sophisticated calculations or in actual measurements, for example, of electron donor and acceptor properties of molecules. Classical experiments on quantum yields in photosynthesis and photodissociation of carbon monoxide complexes with hæm, initiated in Warburg's laboratory thirty years ago and since extended with most valuable results, are not even mentioned. Metal ions and their complexes are referred to only incidentally, and no account is given of experiments carried out by nuclear magnetic resonance and other techniques which have helped to clarify the role of such ions in enzymatic reactions. These contributions, among many others, are important to the development of a quantum biochemistry. The term itself perhaps has a limited usefulness, since it is surely not being too optimistic to believe that all biochemical reactions must in the last analysis be interpreted according to some quantum-mechanical scheme.

Quantum Biochemistry is a personal catechism and can be judged as such. A general introduction outlines quantum mechanics and methods, but it is difficult to imagine that more than a small minority of biochemists will be able to follow the arguments. Those who can, will already be familiar with more acceptable treatments such as are found in Coulson's *Valence*, Kauzmann's *Quantum Chemistry* and Streitweiser's *Molecular Orbital Theory*. The Pullmans' account is often dated: the discussion of bond-lengths and bond-orders, illustrating the value of molecular orbital calculations of the ground-state of aromatic hydrocarbons, is based on data at least ten years old. It is disappointing to find no proper discussion of self-consistent field molecular orbital methods. Biochemists who do follow the introductory chapters may be misled by the discussion of l and m_l quantum numbers (p. 17) and, for example, by the discussion of the bond-angles in water (p. 59), it is now generally accepted that hydrogen–hydrogen repulsions are insufficient to account for the observed bond-angle of 106° or so, the deviation from perfect tetrahedral symmetry being more satisfactorily explained by the use of oxygen hybrid orbitals rather than pure '*p*' orbitals, lone-pair-bonding-pair repulsions decreasing the H—O—H angle by several degrees.

There is no doubt that the compilation of calculations of charge distribution in series of molecules occurring in biological systems is of interest, even if it is clear that they will, in many cases, require considerable revision. Thus, it has already been shown experimentally that the electron-donor properties of chlorpromazine and the electron-accepting behaviour of the tropylium and pyridinium ions are quite different from those anticipated by elementary molecular orbital theory. In reading this book one might not appreciate that one of the major tasks of the next decade will be that of relating the electronic properties of molecules to their biological activity, using more rigorous theoretical methods based on a more complete understanding of the underlying biochemical reaction mechanisms. One should recognize that in many instances the successful application of quantum mechanics to biochemistry is held up not by the lack of theoretical equipment but rather by a complete absence of information on what one should be calculating. The final discussion leads to the conclusion of "the essential importance of molecular systems with mobile electrons, and, therefore, of electronic de-localization, in the phenomena of life": an unobjectionable sentiment perhaps, but one that has often been expressed. At this point the reader is left with a strong *déjà* sensation: as the Walrus said, "It seems a shame to play them such a trick, after we've brought them out so far, and made them trot so quick".

The Pullmans deserve great credit for having been among the first to recognize the possible importance of electron density distributions and π-electrons in biological systems. This book, while usefully summarizing their views, also indicates that they do not appear to recognize consistently the limitations in their approach, even at the theoretical level. Alternative approaches are dismissed far too lightly, and this must be regretted since these calculations do not solve many biochemical problems, rather do they indicate the direction along which lines of enquiry might be profitable. The Pullmans have written some of the first words about the electronic properties of biological molecules—indeed, many of the first words—but what they have to say is certainly not the last word on the subject.

A. C. Allison
R. Mason

Generalised Electrodynamics and the Structure of Matter

By [the late] Prof. S. R. Milner. Edited by Dr. C. W. Kilmister. Pp. ii + 290. (Sheffield: The University Library, 1963.) 42*s*.

SIR MILNER'S posthumous papers contained a rich collection of results of his investigations in three closely allied fields. There was, first, an algebraical technique which arose, for Milner, in his attempts to elucidate Eddington's later work, though it has no necessary connexion with it. This technique made it possible for Milner to operate conveniently with Clifford algebras (for example, quaternions, Dirac matrices) and to seek different criteria for what constitutes a simple system. This was then useful to him in the second kind of investigation—that into the generalized electromagnetic theory. Here Milner used his algebraic tools to suggest an extremely natural and straightforward generalization of Maxwell's equations, which leaves electromagnetic theory exactly the same, away from charge, but modifies the equations in charge in such a way that the classical electron model becomes a possible stable configuration, without additional forces to hold it together. The more detailed investigations of this kind led him on to considering general relativity, and in this field he sought to re-express the existing theory of gravitation in a way that was more directly connected with ideas of mass, energy and force.

These papers have now been collected together and edited by Dr. C. W. Kilmister, King's College, University of London, who has added a number of notes relating the new notations of Milner to more commonly used ones.

Mass and Abundance Tables for Use in Mass Spectroscopy

By Dr. J. H. Beynon and A. E. Williams. Pp. xxi + 570. (Amsterdam and London: Elsevier Publishing Company, 1963.) 80*s*.

THERE is little one can say in a review of this set of tables except that it is a must for all workers interested in the determination of molecular weights and molecular structure of organic compounds. This is especially true of those who are working in the field of high-resolution mass spectrometry, in which area a molecular weight determination leads directly to the molecular formula. After an introduction in English, German, French and Russian, the book lists under the appropriate mass numbers (*a*) the atomic composition, (*b*) the molecular mass (^{12}C = 12·000000), (*c*) 100 P_{m+1}/P_m, (*d*) 1,000 P_{m+2}/P_m, (*e*) P_{m+1}/P_{m+2}, where P_{m+i} is the height of the (parent + *i*) isotopic peak. On the carbon-12 scale, some of the molecular weights fall below the mass number, and this must be remembered when using the tables. Thus under mass 319, there is the entry for $C_{14}HN_5O_5$, 0·997764. This refers to 318·997764.

As has been said before, this book will be invaluable, especially for workers in the relatively new field of the high-resolution mass spectrometry of organic compounds.

A. MACCOLL

Flows in Networks

By L. R. Ford, jun., and D. R. Fulkerson. Pp. xii + 194. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1963.) 48*s*. net.

THE most familiar example of a network flow problem is that defined by Kirchoff's laws for the flow of an electric current in a network of resistors. In this problem a certain quadratic function of the current representing the heat dissipated has to be minimized subject to linear constraints. This is an example of the kind of problem more complicated than that considered in *Flows in Networks*, where the attention is limited to corresponding problems in which linear functions are minimized, and the further restriction is adopted that the assumption of integral data provides the existence of an integral solution. It is, then, an introduction to the wide and rapidly developing field loosely classified under the name of linear programming, which has come to the fore in recent times.

It should not be thought, however, that the application of the ideas of the book is in some narrowly restricted field concerned primarily with the transport of petrol across deserts. The subjects discussed by the authors range from such practical ones as the way across deserts, to the extremely theoretical questions in graph theory, the theory of partially ordered sets, and the theory defined by matrices, the elements of which are 0 and 1. In all cases a great deal of attention is directed to finding effective procedure which will enable the actual calculation of a solution to be carried out. The book is divided into four chapters, in which the first forms a necessary introduction to each of the others. These last three discuss, respectively, combinatorial applications, minimal cost flow problems, and multiterminal maximal flows.

There is a great deal in the book which is not available elsewhere except in the original papers, and much of the work is in fact due to the authors themselves in the past few years. The book is well produced, and should provide a means of linking workers in the (at present) separated fields of operations research, communications engineering, and combinatorial mathematics.

C. W. KILMISTER

Recent Advances in Allergy Research

Edited by F. Hahn, P. Kallós and G. B. West. (Transactions of the Collegium Internationale Allergologicum, Fifth Symposium in Freiburg i. Br., October 1962.) Published simultaneously as Vol. 22, Nos. 2, 3, 4, 5 and 6 (1963), *International Archives of Allergy and Applied Immunology*. Pp. iv + 355. (Basle and New York: S. Karger, 1963.) 55 Sw. francs; 55 D.M.

THIS book contains 34 of the 39 scientific papers given at the fifth symposium of the Collegium Internationale Allergologicum in Freiburg. Because of the wide range of topics covered and the fact that the papers were given by many different groups of research workers, such as physiologists, pharmacologists, biochemists, allergists and clinicians, the reader is given a very good insight into the present trends of research into allergy. As we still do not understand the underlying causes of the symptoms seen in allergic reactions many papers are devoted to the compounds which may produce these symptoms. At one time the symptoms of allergy were thought to be due to histamine, but recently many other substances have become associated with allergy. This aspect of research is reflected in the many papers dealing with the origin, storage, metabolism and role of histamine, together with papers on some of the other substances believed to be involved including the kinins and a much newer set of substances, the neuraminyl glycosides.

Several papers deal with the diagnostic aspects of the causes of allergy. It is interesting to read that the skin-sensitivity test can give erroneous results and that a bronchial test for asthmatic allergy is more informative. Another group of papers deals with the relationships of collagen diseases and allergy. There is a good review of the dermatological aspect of the collagen diseases and also a paper on the similarity between collagen diseases and the effects of experimentally implanted fibrin in connective tissues. There is a good historical review of the changes which have occurred in the medical concepts of allergy, and this includes an excellent bibliography. Among the many other subjects discussed are anaphylotoxin, penicillin allergy, precipitins to moulds in asthmatic sera and allergic reactions in blood.

A few papers tend to be rather brief, but on the whole this book keeps the reader who is interested in a better understanding of the causes of allergy abreast of the latest trends in allergy research.

N. G. WATON

THE 'M.R.C.' FIFTY YEARS AGO AND NOW

By DR. P. B. MEDAWAR, F.R.S.
Director, National Institute for Medical Research, London, N.W.7

IN Great Britain the support of medical research on a national scale began just fifty years ago, as a by-product of the National Health Insurance Scheme which Lloyd George had launched two years beforehand, in 1911. The regulations which brought a Medical Research Fund into being called for the setting up of a small independent Medical Research Committee, consisting mainly but not entirely of 'scientific experts', to advise the Minister in charge of national insurance on how the Fund should be spent. The size of the Fund itself was determined by a capitation fee of one penny for each insured person in the United Kingdom, and the sum available to the Committee in its first year was £55,000—the equivalent of perhaps £300,000 to-day.

The arrangement made sense, for its sponsors had urged that the Fund, itself a form of insurance, would ultimately lighten the nation's burden of care for the sick and disabled. In practice it turned out to be a bad bargain, for when war broke out in 1914 and insured persons entered the armed forces they ceased to be classified as such. The sum allotted to medical research thus inevitably declined—a fact to which the Committee directed attention in language more temperate ("The Committee venture to hope . . .", etc.) than might be used in the same circumstances to-day. Inflation made matters worse, and by the time it came to write its fifth annual report the Committee declared that the funds available to it were inadequate to support the research it had already put in hand.

After the War the Government's support of medical research was put on a new administrative basis. All the departments and boards which had to do with matters of health were brought together under a single Ministry of Health, but in a famous memorandum Dr. Christopher Addison, M.P., then speaking as Minister of Reconstruction, urged that it would be unwise for the Committee to come under the new Minister's charge. (Addison himself later became the first Minister of Health.) In 1920, therefore, the Committee became the Medical Research Council, with its own Charter, and with the right to determine its own policy and to dispose of the funds allotted to it as it thought best. Administratively, the Council is not a Government Department (and its employees are not Civil Servants), it is under a ministerial committee of the Privy Council whose chairman, now the Minister for Science, answers to Parliament for the Council's work. Though the business it now transacts has multiplied enormously, the Council is still a small body: its Constitution limits it to twelve members, of whom nine are scientists or clinicians and two others are members of, respectively, the House of Commons and the House of Lords. The Council receives an annual grant from the Treasury, to-day of about six million pounds—and unlike the exiguous funds administered by the old Committee, it shows no signs of diminishing year by year.

The Committee's metamorphosis into a Council was in no sense a criticism of its work. On the contrary, the transformation was intended to make sure that its independence and power to take the initiative were not overborne by departmental pressures in the difficult and harassing days of reconstruction. The Committee was, in fact, a brilliant success, and now that we can weigh up their consequences its actions and intentions seem quite remarkable in their prescience and good sense. At this distance of time it is scarcely possible to distinguish individual contributions to its counsels, but though Addison must be given most of the credit for the Committee's highly effective administrative structure we may suspect that Walter Morley Fletcher, the first secretary—there have been only three in all—had most to do with devising the Committee's scientific policy.

The Committee intended from the outset to support research, both centrally, in a research institute manned by its own scientific staff, and peripherally, by way of grants to help research workers in universities and elsewhere. Anyone might be supported whose work gave promise "of being co-ordinated within a general scheme for the organized advancement of medical knowledge". The Committee soon found it necessary to set up "special committees for special purposes", by this means bringing together scientists who in those days worked very much more on their own than they do now.

In its fifth report at the end of the First World War, the Committee took stock of its progress and of the likely future of medical research. In its first report it had spoken almost with awe of "the duty laid upon them of wielding the new and potent instrument" which had been "placed in their hands by the State", but what it had to say now was peremptory and to the point. "Our countrymen must decide whether they are to encourage and maintain" a medical research service or not. If so, it would no longer do for medical research to be pursued as a private hobby by the well-to-do or in time stolen with difficulty from routine duties. Its practitioners must enjoy "reasonable financial security, given directly for research work and not for something else". Above all, the foundations of medical research must be laid in science: "the important attainments of new knowledge come from disinterested enquiry followed under the spur of free intellectual impulse, rather than from frontal attacks made in a utilitarian spirit upon problems of immediate practical interest". Indeed, "there is . . . a real danger lest the insistent calls given by disease and suffering for immediate relief should actually delay progress by attracting scientific effort along the wrong paths". This was a very weighty declaration: at the end of an utterly destructive War during which it had to face the agony of insistent and immediate medical demands, the Committee was better placed than anybody before or since to put a just value on the merits of the pragmatic or directly utilitarian approach to medical science. At all events, the declaration became and remained one of the canons of the Council's policy—became part, indeed, of the great unwritten charter of academic freedom. It is a charter which we should not come to think of as beyond all criticism in all its clauses: some yielding to social, economic and rudely utilitarian pressures there must be, but no one has yet succeeded in drafting a clause in suitably abstract language which gives them their due, and not more than their due, weight.

The Committee's Central Research Institute remained for five years a purely notional body, for war broke out before it could take up residence in Mount Vernon Hospital in Hampstead. (Mount Vernon remained a hospital, eventually a hospital for the Royal Flying Corps.) With the end of the war the building was regained and occupied by what (with the grant of a charter) could now be called a National Institute for Medical Research, and it still houses two large and important divisions of the Institute. By the mid-'thirties it became clear that the Institute would have to be housed in larger premises, which the Council decided to build anew on an estate already in its possession at Mill Hill. Unfortunately, the attempt to house the Institute in suitable quarters was again held up by a World War: the building was taken over by the Admiralty, and final occupation was delayed until 1950.

The original design of the Central Research Institute provided for five divisions under five heads of great distinction bacteriology, under Almroth Wright, biochemistry and pharmacology under Henry Dale (who became first director of the National Institute and first won it an international reputation), applied physiology, under Leonard Hill, a department of medical statistics, under John Brownlee, and a department of clinical research, under Thomas Lewis, which can be thought of as the larval form of the Council's future Clinical Research Centre at Northwick Park Among its extra-mural enterprises, it is noteworthy that biological standardization, originally under Georges Dreyer at Oxford, was one of the Council's very earliest concerns To-day the standardization and control of biological agents is one of the Council's statutory responsibilities, and it has long been discharged within the National Institute itself The Institute has from the outset been a many-sided organization, as the great variety of its activities makes clear At this moment the matters under investigation extend from the adaptation of human beings under thermal stress to the nature of a volatile hormone manufactured by queen bees, and from the growth of leprosy bacilli to the measurement of the concentration of alcohol in the blood The consolidation of the Institute into one genuinely organic whole was above all the work of its second director, C. R Harington The Institute's resident scientific staff now numbers about 120, and it receives many guests from overseas who stay for a year or more

With the growth and deployment of medical science no one institute could hope to accommodate all its practitioners, and from time to time the Council has therefore set up research units—groups of scientists brought together for a more particular common purpose than the advancement of basic medical research as a whole There are about 80 research units and groups, some large and world famous, but some—a kindly observer might say—merely world famous A list of these units and groups is given at the end of this article Among the largest are the Units for Radiobiological Research at Harwell and for Molecular Biology at Cambridge—both founded in 1947 during the tenure of office of the second secretary, Edward Mellanby, who unfortunately did not live to see how rich was to be the scientific harvest of the biophysical research which he took such pains to cultivate A rough (and to some small extent overlapping) classification of the activities of the groups and units shows that about a dozen are predominantly concerned with clinical problems, about a dozen with problems of psychology and of mental illness, and no less than ten (counting part of the Radiobiology Unit) with one form or another of genetics (No one could say now what one far-seeing critic said in 1914—that the Committee gave no recognition to the study of heredity) Environmental, social and industrial medicine account for 10, radiobiology in all its aspects for 7, microbiology for 8, special senses for 5, tropical medicine for 5, nutritional matters for 4 There is no simple interpretation of these figures the distribution of subjects depends partly on their intrinsic scientific value, partly on social urgency, and partly on the extent to which they are in need of special care and protection—itself a function of the degree to which they may have been neglected by the universities The Council aims primarily at the support of men and ideas, many of the groups and units have grown up around and on the initiative of individual scientists of great ability, and often dissolve when their head retires Only the largest units housed in their own premises are permanently established With limited funds it is clear that no research council would have room to manœuvre if it were to commit its entire income to permanently recurrent subsidies The Council's total staff now numbers 2,931, of whom 788 are scientifically qualified It is a sign of the times that, of these 788, only about 30 per cent have medical degrees

The support of groups and units is not all the Council does to subsidize medical research, for its funds contribute largely to the maintenance of the Institute of Cancer Research (including the Chester Beatty Institute) and the Strangeways Research Laboratory The Council maintains an external staff, appoints research fellows and scholars, and supports many promising research schemes by monetary grants Beyond all these enterprises, the Council has in very recent years undertaken the complex and immensely important task of "promoting and co-ordinating medical research in the tropics, in so far as this directly or indirectly concerns this country" (the qualifying clause is interpreted in a wide and humane sense) By and large, then, the Council's activities span the whole spectrum of modern medical research The Council's task has been growing in size and complexity, but in terms of any of the 'objective' standards which can be applied on these occasions it is one that has been successfully discharged The records of the research it has sponsored show that the M R C has indeed created situations conducive to the growth and development of medical science

M R C RESEARCH ESTABLISHMENTS

Date of establishment	Title of Unit	Address	Director
(1915)	National Institute for Medical Research	The Ridgeway, Mill Hill, London, N W 7	Dr P B Medawar, F R S
(Projected)	Clinical Research Centre	c/o M R C Clinical Research Centre Planning Office, Medical School, The University, Birmingham 15	Prof J R Squire
(1958)	Unit for Research on Occupational Aspects of Ageing	University Department of Psychology, Abercromby Square, Liverpool 7	Prof L S Hearnshaw (*honorary*)
(1955)	Air Pollution Research Unit	The Medical College of St Bartholomew's Hospital, Charterhouse Square, London, E C 1	Dr P J Lawther
(1962)	Atheroma Research Unit	Western Infirmary, Glasgow, W 1	Dr B Bronte-Stewart
(1955)	Unit for the Experimental Investigation of Behaviour	Department of Psychology, University College, Gower Street, London W C 1	Prof G C Drew (*honorary*)
(1947)	Biophysics Research Unit	26–29 Drury Lane, London, W C 2	Sir John Randall, F R S
(1959)	Blood Coagulation Research Unit	Churchill Hospital, Oxford	Dr R G Macfarlane, F R S
(*)	Blood Group Reference Laboratory	Gatliff Rd , off Ebury Bridge Rd , London, S W 1	Dr A E Mourant
(1946)	Blood Group Research Unit	Lister Institute, Chelsea Bridge Rd , London, S W 1	Dr R R Race, F R S
(1954)	Body Temperature Research Unit	Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford	Sir George Pickering, F R S (*honorary*)
(1959)	Bone-seeking Isotopes Research Unit	Churchill Hospital, Oxford	Dame Janet Vaughan (*honorary*)
(1956)	Carcinogenic Substances Research Unit	Washington Singer Laboratories, The University, Exeter	Sir James Cook, F R S (*honorary*)
(1945)	Cell Metabolism Research Unit	Department of Biochemistry, Oxford	Sir Hans Krebs, F R S (*honorary*)

*Administered since April 1950 by the Council for the Ministry of Health

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M R C Research Establishments

Date of establishment	Title of Unit	Address	Director
(1963)	Cellular Immunology Research Unit	Sir William Dunn School of Pathology, University of Oxford	Prof J L Gowans (*honorary*)
(1962)	Chemotherapy Research Unit	Molteno Institute, Downing Street, Cambridge	Dr Ann Bishop, F R S
(1919)	Department of Clinical Research	University College Hospital Medical School, London, W C 1	Dr E E Pochin
(1962)	Cyclotron Unit	Hammersmith Hospital, Ducane Rd , London, W 12	D D Vonberg
(1949)	Wernher Research Unit on Deafness	King's College Hospital Medical School, Denmark Hill, London, S E 5	Dr T S Littler
(1961)	Dental Research Unit	Dental School, Lower Maudlin Street, Bristol 1	Prof A I Darling (*honorary*)
(1946)	Clinical Endocrinology Research Unit	2 Forrest Road, Edinburgh 1	Dr J A Loraine
(1962)	Unit for the Study of Environmental Factors in Mental and Physical Illness	London School of Economics, Aldwych, London, W C 2	Dr J W B Douglas
(1962)	Epidemiological Research Unit (Jamaica)	University of the West Indies, Mona, Kingston 7, Jamaica	Dr W E Miall
(1960)	Epidemiological Research Unit (South Wales)	4 Richmond Road, Cardiff	Prof A L Cochrane (*honorary*)
(1945)	Department of Experimental Medicine	Tennis Court Rd , and 5 Shaftesbury Rd , Cambridge	Prof R A McCance F R S
(1953)	M R C Laboratories, Gambia	Fajara, near Bathurst, Gambia, British West Africa	Dr I A McGregor
(1961)	Gastroenterology Research Unit	Central Middlesex Hospital, Park Royal, London, N W 10	Dr E N Rowlands
(1957)	Clinical Genetics Research Unit	Institute of Child Health, Hospital for Sick Children, Great Ormond Street, London, W C 1	Dr J A Fraser Roberts, F R S
(1955)	Experimental Genetics Research Unit	University College, Gower Street, London, W C 1	Prof H Gruneberg, F R S (*honorary*)
(1962)	Human Biochemical Genetics Research Unit	Department of Biochemistry, King's College, Strand, London, W C 2	Prof H Harris (*honorary*)
(1957)	Microbial Genetics Research Unit	Hammersmith Hospital, Ducane Rd , London, W 12	Dr W Hayes
(1958)	Population Genetics Research Unit	Old Road, Oxford	Dr A C Stevenson
(1959)	Psychiatric Genetics Research Unit	Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London, S E 5	Dr E T O Slater
(1946)	Experimental Hæmatology Research Unit	Wright-Fleming Institute of Microbiology, St Mary's Hospital Medical School, London, W 2	Prof P L Mollison
(1963)	Abnormal Hæmoglobin Research Unit	Department of Chemical Pathology, St Bartholomew's Hospital, E C 1	Dr H Lehmann (*honorary*)
(1952)	Industrial Injuries and Burns Research Unit	Birmingham Accident Hospital, Bath Row, Birmingham 15	Dr J P Bull
(1947)	Laboratory Animals Centre	M R C Laboratories, Woodmansterne Road, Carshalton, Surrey	Dr W Lane-Petter
(1953)	Infantile Malnutrition Research Unit	Mulago Hospital, Kampala, Uganda	Prof R F A Dean
(1960)	Unit for Research on the Chemical Pathology of Mental Disorders	Department of Physiology, The Medical School, Birmingham 15	Prof I E Bush (*honorary*)
(1957)	Metabolic Disturbances in Surgery Research Unit	Department of Urology, The General Infirmary, Leeds 1	Prof L N Pyrah (*honorary*)
(From October 1964)	Metabolic Reactions Research Unit	Imperial College of Science and Technology, London, S W 7	Prof E B Chain, F R S (*honorary*)
(1947)	Laboratory of Molecular Biology	University Postgraduate Medical School, Hills Road, Cambridge	Dr M F Perutz, F R S
(1958)	Mutagenesis Research Unit	Institute of Animal Genetics, West Mains Road, Edinburgh	Dr Charlotte Auerbach, F R S (*honorary*)
(1962)	Neuroendocrinology Research Unit	Department of Human Anatomy, South Parks Road, Oxford	Prof G W Harris, F R S (*honorary*)
(1958)	Neuropharmacology Research Unit	Department of Experimental Neuropharmacology, The Medical School, Birmingham 15	Dr P B Bradley (*honorary*)
(1957)	Neuropsychiatric Research Unit	M R C Laboratories, Woodmansterne Rd , Carshalton, Surrey	Dr D Richter
(1944)	Human Nutrition Research Unit	Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, N W 7	Prof B S Platt
(1926)	Dunn Nutritional Laboratory	Milton Road, Cambridge	Dr E Kodicek
(1955)	Obstetric Medicine Research Unit	University of Aberdeen Medical School	Sir Dugald Baird (*honorary*)
(1954)	Wernher Research Unit on Ophthalmological Genetics	Royal College of Surgeons of England, Lincoln's Inn Fields, London, W C 2	Prof A Sorsby (*honorary*)
(1944)	Otological Research Unit	National Hospital for Nervous Diseases, Queen Square, London, W C 1	Dr C S Hallpike, F R S
(1948)	Environmental Physiology Research Unit	London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London, W C 1	Dr J S Weiner
(1945)	Pneumoconiosis Research Unit	Llandough Hospital, Penarth, Glamorgan	Dr J C Gilson
(1960)	Unit for Research on the Epidemiology of Psychiatric Illness	Department of Psychological Medicine, University of Edinburgh	Prof G M Carstairs (*honorary*)

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M R C Research Establishments

Date of establishment	Title of Unit	Address	Director
(1957)	Clinical Psychiatry Research Unit	Graylingwell Hospital, Chichester, Sussex	Dr P Sainsbury
(1948)	Social Psychiatry Research Unit	Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London, S E 5	Sir Aubrey Lewis (*honorary*)
(1963)	Psycholinguistics Research Unit	Institute of Experimental Psychology, Oxford	Prof R C Oldfield (*honorary*)
(1944)	Applied Psychology Research Unit	15 Chaucer Road, Cambridge	D E Broadbent
(1918)	Industrial Psychology Research Unit	University College, Gower Street, London, W C 1	Prof G C Drew (*honorary*)
(1956)	Clinical Effects of Radiation Research Unit	Department of Radiotherapy, Western General Hospital, Edinburgh 4	Dr W M Court Brown
(1959)	Environmental Radiation Research Unit	Department of Medical Physics, General Infirmary, Leeds 1	Prof F W Spiers (*honorary*)
(1947)	Radiobiological Research Unit	Harwell, Didcot, Berks	Dr J F Loutit, F R S
(1953)	Radiological Protection Service	Clifton Avenue, Belmont, Sutton, Surrey	W Binks
(1953)	Experimental Radiopathology Research Unit	Hammersmith Hospital, Ducane Road, London, W 12	Miss Tikvah Alper
(1958)	Rheumatism Research Unit	Canadian Red Cross Memorial Hospital, Taplow, Maidenhead	Prof E G L Bywaters (*honorary*)
(1952)	Unit for Research on the Experimental Pathology of the Skin	The Medical School, Birmingham 15	Dr C N D Cruickshank
(1948)	Social Medicine Research Unit	The London Hospital Research Laboratories, Ashfield Street, London, E 1	Prof J N Morris
(1926)	Statistical Research Unit	University College Hospital Medical School, 115 Gower Street, London, W C 1	Dr W R S Doll
(1947)	Toxicology Research Unit	M R C Laboratories, Woodmansterne Road, Carshalton, Surrey	Dr J M Barnes
(1956)	Trachoma Research Unit	Lister Institute of Preventive Medicine, London, S W 1, and M R C Laboratories, Gambia	Dr L H Collier (*honorary*)
(1955)	Tropical Metabolism Research Unit	University of the West Indies, Mona, Kingston 7, Jamaica	Prof J C Waterlow
(1948)	Tuberculosis Research Unit	M R C Laboratories, Holly Hill, Hampstead, London, N W 3	Dr P M D'Arcy Hart
(1956)	Unit for Research on Drug Sensitivity in Tuberculosis	Postgraduate Medical School of London, Ducane Road, London, W 12	D A Mitchison (*honorary*)
(1955)	Virus Research Unit	M R C Laboratories, Woodmansterne Road, Carshalton, Surrey	Dr F Kingsley Sanders
(1959)	Experimental Virus Research Unit	Institute of Virology, Church St, Glasgow, W 1	Prof M G P Stoker (*honorary*)
(1962)	Vision Research Unit	Institute of Ophthalmology, Judd St, London, W C 1	Dr H J A Dartnall

M R C Research Groups

Date of establishment	Title of Group	Address	Director
(1960)	Research Group in Enzymology	Department of Chemical Pathology, St Mary's Hospital Medical School, London, W 2	Prof A Neuberger, F R S
(1960)	Research Group on Adrenergic Mechanisms	University Laboratory of Physiology, Oxford	Sir Lindor Brown, F R S
(1960)	Clinical Immunology Research Group	Institute of Diseases of the Chest, Brompton Hospital, London, S W 3	Dr J Pepys
(1961)	Research Group on Experimental and Clinical Problems of Transplantation	Department of Surgical Science, University of Edinburgh	Prof M F A Woodruff
(1961)	Research Group on the Organization of Central Mechanisms Subserving Vision	Department of Physiology, University Medical School, Edinburgh	Prof D Whitteridge, F R S
(1961)	Cardiovascular Research Group	Postgraduate Medical School of London, Ducane Road, London, W 12	Dr J P Shillingford
(1961)	Research Group on Demyelinating Diseases	13 Framlington Place, Newcastle upon Tyne 2	Dr E J Field
(1962)	Research Group on the Relation of Functional to Organic Psychiatric Illness	Department of Psychological Medicine, Queen Victoria Road, Newcastle upon Tyne 1	Prof M Roth
(1962)	Epigenetics Research Group	Institute of Animal Genetics, University of Edinburgh	Prof C H Waddington, F R S
(1963)	Research Group on Respiration and Energy Metabolism in the Newborn	London Hospital Medical College, Turner Street, London, E 1	Prof K W Cross
(1963)	Biomechanics Research Group	Royal College of Science and Technology, George Street, Glasgow C 1	Prof R M Kenedi
(1963)	Research Group on the Immunological Aspects of Dermatology	Institute of Dermatology, Lisle Street, London, W C 2	Dr J L Turk
(1963)	Research Group on Genetic Problems in Orthopædic Disease	Department of Orthopædic Surgery, University Medical School, Edinburgh	Prof J I P James
(1963)	Research Group for the Study of Megaloblastic Anæmias	Postgraduate Medical School of London, Ducane Road, London, W 12	Dr D L Mollin

PROGRESS OF CANCER RESEARCH

THE fortieth annual (1962) report of the British Empire Cancer Campaign* is a weighty volume of nearly 700 pages, the titles of papers published by the research workers financed wholly or in part by the Campaign, or who work in centres affiliated to the Campaign, numbered nearly 900 in 1962 (almost a third of this huge total came from the Institute of Cancer Research, London) As in previous reviews, the material of the report will be divided into three main groups and treated by three reviewers "Carcinogenesis and the Biology of Cancer Generally" (I H), "Radiobiology, Radiotherapeutics and Physics" (P A) and the "Clinico-Pathology of Cancer" (A L L)

It should scarcely be necessary to say that only a fraction of the many interesting research topics can be dealt with, and many must be omitted

Haddow *et al* have continued their investigation of the carcinogenic potency of compounds containing iron, those showing activity were saccharated iron-oxide, ferric-sodium gluconate, ferrous glutamate and iron hydrogenate dextran The interest and importance of these investigations are, of course, that these compounds, or at least substances similar to them, are being used in medical practice as anti-anæmia preparations, and on a large scale

The Fahmys have continued their work on mutagens as carcinogens, in *Drosophila*, they find that calf thymus histone (basic protein) and poly-L-glutamic acid (acid protein) produce small chromosome deletions, but other macromolecules were also effective, for example, polymethacrylic acid (molecular weight about one million)

The failure to show resistance by normal tissue to invasion by cancer tissue has been explained by Green as due to a loss of immunological identity factors, and this idea is given support by the discovery that tumour tissue is lacking in certain of its protein components, the work of the Millers and others on protein changes in azo-dye carcinogenesis in rat livers lends support to Green's hypothesis The main feature of the deletion theory of carcinogenesis, according to Green, is that the transformation consists in the loss of certain specific immunological factors (iso-antigens) which determine the relationship of cells to one another, normal cells are supposed to exhibit identifying iso-antigens, the carcinogen combines with these antigens, the normal tissues react by attempting to destroy the cells presenting the new antigen complex, these cells respond by losing the new antigen which carries with it the identifying iso-antigen, the resulting cells are now free from this antigen and can now invade tissue without being recognized According to this view the cancer cell is deficient in antigenic factors, but fortunately for the outlook for therapy on immunological or chemotherapeutic lines, evidence is now coming to light that cancer cells do in fact contain new antigens not present in normal tissue, and although it would be going too far to say that there are grounds for much optimism, pessimism no longer seems entirely justified In the early years of organized cancer research in the first decade or two of the century, it was found that some immunity to transplantable tumours in mice could be induced by first inoculating the animal with embryonic tissue or with skin But when pure strains of mice became available, this immunity was shown to have been brought about by strain differences rather than by any immunological differentiation between normal and neoplastic tissues Nevertheless, attempts to make use of immunity as an anti-cancer technique has been the aim of countless experiments ever since transplantable tumours became available in the laboratory.

Work at the Westminster Hospital, London, on tissue proteins by electrophoretic analysis has shown that in cancer the composition of the liver is changed, the more basic components are decreased and the more acid components increased Baldwin at Nottingham, in immunological investigations of rat liver cell antigens during azo-dye carcinogenesis, states "Further confirmation of deletion of normal rat liver cell sap antigens from DMAB-induced liver tumour has been obtained from the analysis of rabbit anti-normal liver cell sap antisera pre-absorbed with tumour cell sap Immunoelectrophoresis of absorbed antisera has permitted further resolution of cell sap antigens showing that out of sixteen components, six normal liver antigens were deleted from tumour Following exhaustive absorption of anti-tumour cell sap antiserum with normal liver cell sap, three antigenic components reacting with the tumour fraction were still detectable Hence it is concluded that DMAB-induced liver tumour contains at least three abnormal cell sap antigens which do not cross-react with normal liver . Microsome fractions from DMAB-induced tumour have also been shown to contain abnormal antigens Thus four antigenic components were detectable following agar-gel immunodiffusion analysis of deoxycholate solubilized microsome fractions from liver tumour with anti-tumour microsome antisera, and at least one of these antigens did not cross-react with normal liver microsome fractions Further confirmation that DMAB-induced liver tumour contains abnormal microsomal antigens was obtained from the analysis of anti-tumour microsome antisera pre-absorbed with normal microsomes"

Schild and Dale at University College, London, refer to some of their work as "Anaphylaxis and the Investigation of Specific Tumour Antigens"

Woodruff, at the University of Edinburgh, from an examination of *A* strain mice bearing spontaneous or transplanted mammary carcinoma, concludes that this tumour possesses one or more antigens not present in normal *A* strain tissues, and retains these after transplantation. He now states "Further work (Woodruff and Symes, 1962*b*) has shown that after a certain number of transplant generations, which differs for different tumours, the capacity to evoke splenomegaly is lost and this is attributed to deletion of tumour-specific antigen It is suggested that such deletion may occur also in tumours which are not transplanted but remain sufficiently long in the animal in which they arise

"If this hypothesis is correct it would seem to provide an important clue to the understanding of the behaviour of malignant tumours not only in experimental animals but also in man During the phase of antigenic difference a tumour may conceivably be held in check, or even destroyed, by immunological mechanisms, whereas after deletion of all tumour specific antigens the natural immunological defences have no *point d'appui* and any restraining effect they may have is lost "

Tissue culture has rapidly become an important laboratory technique in cancer more than twenty different investigations using the *in vitro* method are described in the report, for example, cultures of tumour cells are being used as test-tube material for assessing the cytostatic effect of drugs used in the chemotherapy of cancer Wylie and Easty state "The most consistent success has been obtained in growing various gliomata, including astrocytomata, medulloblastomata and ependymomata.

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* British Empire Cancer Campaign Fortieth Annual Report covering the year 1962 Part 1 The Chairman's Statement and the Accounts of the Central Organization Pp xxxviii Part 2 The Scientific Report of the Researches undertaken by the Central Organization and its Autonomous Councils in the United Kingdom and by some of its Affiliated Organizations Overseas Pp xxxix–lx + 698 (London British Empire Cancer Campaign, 1963)

Thirty-seven of these tumours have been grown, twelve of them for periods of 4 days or more in the presence of various chemotherapeutic agents, including melphalan, chlorambucil, thioTEPA, methotrexate and mannitol myleran. Methotrexate proved wholly inactive in this system, possibly on account of the composition of the medium. The results of these studies were assessed by two parameters: the LD50, or dose of a drug (*in vitro*) found to kill 50 per cent of the cells present on the first day of the test, and the Tissue Culture Therapeutic Index which is the ratio between the LD50 of the drug *in vitro* and the dose required clinically to suppress the neutrophil leucocyte count by 95 per cent.

"The pattern of results obtained in the brain tumours was fairly consistent irrespective of histological type. Initially, i.e. in the first 48 hours, melphalan appeared to be the most toxic drug as expressed by the LD50 but was surpassed in most cases after 4 days by thioTEPA. The Tissue Culture Therapeutic Index, however, always favoured mannitol myleran on account of the non-toxicity of the latter drug *in vivo*."

Easty and Yarnell write: "The concept of rapidly growing tumour cells as being 'nitrogen traps' has led to the suggestion that plasma and other proteins may be ingested intact by tumour cells. The uptake of macromolecules is also of fundamental importance in the process of cell transformation using preparations of DNA or RNA. With this in mind the uptake of proteins from the medium by monolayer cultures of normal and malignant cells has been continued this year. The process has been followed using fluorescent-labelled proteins added directly to the medium, and by immunofluorescent staining with the fluorescent antibody technique. The proteins enter the cells by the process of pinocytosis, initially in the form of spherical vacuoles, which from an electron microscope study of Dr. E. H. Mercer are seen to be enclosed by a membrane originating from the plasma membrane.

"Cultures of twenty-seven different cell types, fifteen of 'normal' origin and twelve of 'tumour' origin, have been examined. The results have confirmed our earlier conclusions. Fibrocytes pinocytose vigorously, and sarcoma cells, presumably derived from connective tissue cells, behave similarly. Normal epithelial cells in primary culture pinocytose vary slightly, if at all, whereas epithelial cells which have been maintained in culture for many generations exhibit moderate pinocytosis, as do some carcinoma cells. Several of the lines of 'normal' epithelial cells are suspected of having undergone transformations, 'malignant' or otherwise. It is, therefore, not surprising that they should show properties similar to those of the carcinoma cells."

Ambrose *et al.* have examined carcinogenesis *in vitro*, that is, the action of the polyoma virus acting on tissue cultures of normal hamster kidney fibroblasts. "Further investigations indicated that this change from normal to tumour cell surface was dependent on an alteration in a sialic acid containing macromolecule (possibly mucoprotein or glycolipid). More experiments have now shown that the heterogeneity of the transformed cells after prolonged culture is due to some inherent instability of the transformed cells. When examined soon after the transforming event, the malignant cells consist of a homogeneous population having an electrophoretic mobility about 25 per cent greater than that of the normal clone. Experiments are in hand to elucidate further the nature of this enhanced surface charge, since this increase appears to be remarkably characteristic of the malignant change, whether carcinogenesis is by viral, hormonal or chemical agents."

At the Strangeways Laboratory in Cambridge, further experimental evidence has been obtained of the transforming effect of simply culturing cells *in vitro* without the addition of any specific carcinogenic agent. "It is a common experience in tissue culture laboratories that after prolonged cultivation cell lines usually become malignant, i.e. when injected into animals they form transplantable tumours.

"During the present investigation of the action of cobalt chloride on cells *in vitro*, two lines of dermal fibroblasts have been developed from the same pooled sample of dorsal skin from fœtal rats. Of the two lines, one (16*C*) has received no cobalt treatment, whereas the other (16*R*) was exposed intermittently to cobalt chloride during the first 8 months *in vitro* and became more tolerant of cobalt than the control (16*C*). The metabolic differences between the two lines after 6 months in culture have been described in a previous report (B.E.C.C. Report, 1959); they included a relatively low respiration and increased glycolytic activity in the cobalt-treated line which also, unlike the untreated line 16*C*, produced much hyaluronic acid. At that time, neither line formed tumours when implanted into rats.

"From the eighth month *in vitro*, the respiration of the control line 16*C* also began to fall, and by the eighteenth month it was similar to that of line 16*R*.

"The cells have now been grown *in vitro* for 3 years, and the effect of the injection of the two altered cell lines into rats has recently been re-investigated. For each line, approximately 10^7 cells were injected intramuscularly into the right thigh of each of ten rats. Both lines gave rise to tumours.

"The tumours produced by both the 16*C* and 16*R* cell lines were poorly differentiated fibrosarcomata with little intercellular material; both lines of tumours were invasive and transplantable."

The antigenic properties of these cells in culture have been investigated also at Cambridge. "An investigation is being made of the antigens of the two lines of rat fibroblasts, 16*C* and 16*R*, which, as mentioned above, have developed the ability to produce tumours when implanted into rats. Preliminary studies on the *B* antigen have shown that both lines consist of a mixed population of cells, some of which possess this antigen. Preliminary chromosomal analyses have revealed the presence, in many cells of each line, of one or more long metacentric chromosomes which are absent from normal rat cells. In both lines there is a wide range of chromosome complement, from less than the diploid to above the tetraploid number."

The discovery of cancer-producing viruses might have suggested that, even if no immunity could be created against the tumour, at least it might be brought about against so different an organism as a virus; moreover, the virus theory of the origin of cancer has been steadily gaining reinforcements since more and more viruses as carcinogenic agents have been discovered, for example, the mammary tumour virus, the leukæmic viruses of Gross, Friend, Graffi and Moloney, the polyoma virus of Stewart and some viruses in cattle.

Salaman finds that centrifugates and cell-free filtrates of thymus and lymph nodes from bovine malignant lymphoma when injected into mice increase the incidence of leukæmia from 0·5 to 9 per cent. He has also been working with the Moloney virus, which induces leukæmia in mice and is conveyed by vertical transmission (*VT*), that is, leukæmia develops in high incidence in the untreated progeny of virus-inoculated animals; *VT* has been reported for four murine leukæmogenic viruses (those of Gross, Graffi, Moloney and Friend). "Transference of virus during lactation was demonstrated by fostering experiments. Females in advanced pregnancy were placed in the upper half of a cage separated from the lower half by ½-in. wire mesh, and a foster-mother with a newborn litter (marked) in the lower half. The litter of the female in the upper half fell through the mesh within minutes of birth. Of thirty-one mice born of MLV-inoculated females and fostered by normal females, observed for 60 weeks, only one became leukæmic, at 49 weeks. Of nineteen mice born of normal females and fostered by MLV-inoculated females, five became leukæmic before the 15th week, and a further thirteen before the 50th week

"These results showed that MLV is transferred from mother to offspring during the lactation period, and no evidence was obtained of transmission from the father, or from the mother during the prenatal period They agree fairly closely with those of Law and Moloney, who used the same virus, though these authors also quote results, which they regard as equivocal, which appear to show that MLV may occasionally cross the placental barrier Gross (1961) found that his passage *A* virus passes from the mother, but not from the father, to the progeny "

The Moloney virus (MLV) will induce leukæmia in rats as well as in mice and is also conveyed by vertical transmission, of ten offspring of MLV-inoculated female rats mated to normal males, nine became leukæmic A further virus has been identified, the plasma lactic dehydrogenase-elevating virus of Riley (RV) which has been found in many standard transplantable mouse tumours with long laboratory histories and contaminates many strains of murine oncogenic viruses RV fails to induce neutralizing antibodies and it has never been shown to be leukæmogenic or oncogenic, the virus is transmitted to the offspring of females (which acquire the infection either naturally or by inoculation) during pregnancy, transmission occurs *in utero* and not via the milk

Workers at the Glasgow Veterinary School find that lymphosarcoma in domestic animals (cattle, the dog, cat and pig) cannot be transmitted to rats and mice, when examined in the electron microscope the tumour shows virus-like particles very similar to those found in mouse leukæmias Bovine papilloma is the commonest transmissible tumour of cattle, and electron micrographs of the virus show a close resemblance to the viruses of rabbit papilloma and to polyoma

Oettlé at the South African Institute for Medical Research reports that *Mastomys*, the Natal rat, which he discovered was very susceptible to spontaneous gastric cancer is now showing a high incidence of skin papillomatosis which occasionally develops into metastasizing carcinoma, Oettlé describes the condition as infectious papillomatosis

Sir Charles Dodds *et al* have been assaying the œstrogen content of poultry which has been treated with hexœstrol in order to improve weight and tenderness of the meat, but "has been regarded as a possible carcinogenic risk to the consumer In our experiments the œstrogenic residues were determined in the livers and flesh of three birds treated with a 25 mg pellet of hexœstrol implanted in the neck for six weeks before slaughter At the same time the tissues of three untreated birds were extracted in the same manner The tissues were completely extracted with ether, and the total phenolic fraction of this extract, which was considered to contain all the œstrogenic matter, was dissolved in arachis oil and assayed for œstrogenic activity by injection into groups of immature female mice

"The carcases of the hexoestrol-treated chickens contained a total œstrogenic activity corresponding to 1 96 μg of hexoestrol and the untreated chickens contained less than 0 04 μg In both cases the livers contained œstrogenic activity corresponding to approximately 0 9 μg hexoestrol" Clearly by the time the birds are eaten the injected œstrogen has been all but eliminated, that is, from 25 mg to less than 2 μg

Orr at Birmingham, working on the chemical induction of breast tumours in mice, finds that the C_{57} strain has a low incidence of spontaneous pseudo-pregnancy and is relatively insusceptible to breast carcinogenesis by methylcholanthrene, but virgin mice of the 1*F* strain and F_1 hybrid have a high incidence of spontaneous pseudopregnancy and are very susceptible to breast tumour induction "Pseudopregnancy attained by mating with vasectomised males causes the greatest increase in susceptibility in all three genetic types of mice, the effect being most noticeable in C57B1.

"In another experiment, F_1 hybrid virgins have had their own ovaries removed and replaced by ovaries from one of the parent strains or from similar hybrids This enables the response of different genetic types of F_1 hybrid breast tissue to tumour induction by MC while under the hormonal influence from ovaries to be investigated

"The results of these experiments may enable us to determine whether the genes controlling the susceptibility of the breast tissue to tumour induction by MC are acting indirectly through their effect on ovarian hormone production, or directly on the breast tissue itself "

Galton *et al* have been working on the Philadelphia chromosome discovered in the leukæmic cells of human subjects "Ten patients with chronic granulocytic leukæmia, untreated, treated and at varying stages of the disease, have all shown the Ph_1 chromosome in every cell in the bone marrow on direct preparation The frequency and significance of the presence of the Ph_1 chromosome and its relation to treatment in these ten patients over a period of 18 months forms the basis of material to be published shortly

"Two children, aged 11½ and 12, with chronic granulocytic leukæmia, both treated with busulphan for 4 and 8 weeks respectively, showed the Ph_1 chromosome in every cell in the bone marrow on direct preparation

"Chromosome studies in direct bone marrow and peripheral blood culture preparations obtained from patients with chronic lymphatic leukæmia are in progress in the hope of detecting differences between those patients with advanced but only very slowly progressive disease and those with more rapidly progressive disease "

As in previous years a very substantial proportion of research work sponsored by the British Empire Cancer Campaign is concerned with radiation research, and important findings are reported at all the different steps which intervene between the exposure to the radiation and the final biological injury

The new technique of flash radiolysis for detecting short-lived chemical species in water irradiated with μsec pulses of intense electron beams has been exploited highly successfully at two centres J P Keene (Christie Hospital, Manchester) first observed a broad band of optical absorption when pure de-aerated water was irradiated, and this proved the presence of a short-lived and highly reactive (particularly with oxygen) species other than OH and H radicals Parallel research at Mount Vernon Hospital, London (J. W Boag *et al*), appears to have established that this entity is a hydrated electron, and the chemical reactivity of this interesting intermediary is now being studied Whether hydrated electrons play a part in initiating biological damage remains to be discovered New techniques (Guy's Hospital Medical School, London) promise to provide direct evidence on the magnitude of the local heating effect along the track of an ionizing particle Rises in temperature of up to 100° C are calculated and it is conceivable that these may bring about the denaturation of protein molecules following irradiation in the solid state It has long been a puzzle why a single ionization anywhere within a protein molecule should result in far-reaching changes in its secondary structure

Following on the various physical processes, a variety of molecules in the irradiated material undergo irreversible chemical changes and some of these reactions constitute the primary lesions for radiation-induced cell death So far there is no clear evidence concerning the nature of the actual cell constituents involved There appears to be no increased radiation damage to DNA in cells rendered more radiosensitive by a variety of procedures (Chester Beatty Research Institute, London), and J Weiss also concludes from chemical investigation that DNA in nucleoprotein is highly protected against radical attack.

If not DNA, what are the sites of the primary lesions? A clue may be derived from the finding by L. H. Gray *et al.* that the permeability of cells is increased by a radiosensitizing treatment. It is also interesting that D. L. Dewey finds it necessary to explain his survival curves with bacteria by postulating the formation of a radiotoxin which is responsible for some of the lethal action of X-rays.

Many studies of factors which alter the radiosensitivity of cells are reported. They are of the greatest importance for mammalian radiobiology and in addition may throw light on the fundamental problems discussed above. Electron spin resonance techniques have made it possible to establish that the mechanism by which sulphydryl compounds protect the relatively simple system of salmon sperm heads against γ-rays involves repair of a radical by the hydrogen atom from the sulphydryl group (P. Alexander, A. Baker and M. G. Ormerod). This restitution reaction is prevented if the radical becomes peroxidized and the net extent of damage involves a competition between the sulphydryl compound and oxygen for the radical. Attempts have been made to correlate the radioresistance of different cell lines with their sulphydryl content. Changes in the physiological state of the cell have long been known to influence radiosensitivity to a marked extent. J. V. Davies and C. Russell now find that the magnitude of the sensitization obtainable by pre-irradiation treatment with nitric oxide depends on the metabolic character, while at Mount Vernon Hospital ageing of ascites cells *in vitro* increased the extent of chromosome damage, which was also enhanced if the cells were pretreated with ATP. The stage in the division cycle at which a cell is irradiated has been known to alter the dose required for killing the cell, and A. J. Bateman has now demonstrated for the mouse that the mutagenicity of X-rays varies in the different stages of spermatogenesis.

Radiosensitization by pyrimidine analogues and by sulphydryl poisons, such as iodoacetamide, of bacteria and mammalian cells *in vitro* has been investigated, but the mechanisms remain obscure. Although these treatments have to be given before irradiation the possibility that sensitization involves post-irradiation recovery processes cannot be excluded (Chester Beatty Research Institute, London). The same group finds that the composition of the growth medium and post-irradiation storage conditions critically alter the lethal effect of X-rays to leukæmia cells. The discovery made fifteen years ago at Oak Ridge that the survival of irradiated bacteria could be increased by storage for a few hours at sub-optimal growth temperature has now been extended to mammalian cells. This observation might be usefully linked to the ingenious suggestion of D. B. Cater *et al.* to heat up selectively by radiowave diathermy badly vascularized tumours. Blood-vessels act as cooling pipes and the cells most distant from blood-vessels will attain the highest temperature. J. Read in New Zealand has shown that yet another post-treatment—namely, exposure to infra-red—which was known to increase the effect of X-rays in plants also increases the lethal action of X-rays for mammalian cells *in vitro*. The dose of radiation needed to kill mammalian cells is clearly capable of being varied in many ways by both pre- and post-irradiation treatments, and it seems quite possible that further work along these lines may well be capable of practical application to radiotherapy.

On moving from the cellular level to irradiation of mammals many complexities are revealed. The treatment of radiation injury by bone-marrow transplants continues to be investigated at many laboratories, and there remain many intricate problems. E. A. Wright *et al.*, by combining protection by cysteamine and hypoxia with treatment with antibiotics and syngeneic bone-marrow, were able to increase the LD_{50} for mice to 8-MeV electrons from 940 to 3,520 rads. Data continue to accumulate concerning the importance of effects at a distance (or abscopal effects), and D. Maor and P. Alexander report marked biochemical alterations in lymphatic organs of rats following irradiation of the head only.

The ætiology of many of the delayed effects of radiation remains quite obscure. S. M. Landers and P. L. T. Ilbery (Sydney) find chromosome abnormalities in the thymus of irradiated mice long before these became leukæmic, and conclude that cells with altered karyotype were present before supervention of malignancy as judged by the criterion of transplantability. At Mount Vernon Hospital, an earlier observation that croton oil promotes skin tumour induction by β-rays has been confirmed.

The life-span shortening effect of whole-body radiation continues to be investigated. D. I. Connell compared the pathologies of irradiated and control animals killed at monthly intervals, and concludes that radiation cannot be considered as a generalized acceleration of normal ageing because the time course of different diseases is affected quite differently by irradiation. At St. Bartholomew's Hospital, London, very extensive investigations have shown a quite different disease pattern for mice sublethally irradiated at 4 weeks and at 30 weeks of age, and the researchers conclude that the cause of life-span shortening depends on the hormonal status of the mice at the time of irradiation. The same group has been quite unable to confirm in a very large-scale experiment an earlier American claim that a single dose of irradiation to male mice reduces longevity of their offspring. 350 r to the fathers even when repeated over three successive generations did not alter the life-span, disease incidence, fertility or growth rate of their progeny.

Amoroso and Stein-Werblowsky report some interesting observations on the association of the liability to cancer and pregnancy in rats. Using the so-called transplantable malignant chorion-epithelioma of Stein-Werblowsky, they found inhibition of tumour growth in the uterine horns of rats which contained post-coital secretion, compared with virgin rats, the tumour inhibition being maximal in fluid which had accumulated several hours post-coitally. In a second experiment tumours which were temporarily placed in the uterine horns of both virgin rats and in post-coital fluid failed to grow on transfer to a second host, and a similar result was obtained when tumours were exposed to post-coital fluid *in vitro*. Post-coital fluid is, of course, a composite, and it was found that by exclusion of spermatozoa (mating with vasectomized males) the resultant fluid had no anti-tumour activity, neither had the fluid in such matings after it had been incubated *in vitro* with spermatozoa. From this result they concluded that the uterus in presence of spermatozoa produces a tumour-inhibiting agent, or that spermatozoa in that environment release the active agent.

There are three reports on lymphangiography and its results; the art of outlining lymphatics is almost as old as the discovery of the lymphatic system, which is a system of blindly ending capillaries present in most parts of the body, the collected lymph being filtered at intermediate lymph nodes *en route* for the innominate veins into which the main lymphatic vessels ultimately empty. It is essentially an absorbative system. Most carcinomas, all the malignant lymphomas and some sarcomas involve the lymphatic system, and the very foundation of contemporary management of such cancers is to remove or otherwise destroy the regional lymphatic drainage of a malignant tumour, in addition to the treatment of a primary lesion. That the various methods attempted have met with some success there can be no doubt, but there are unsatisfactory aspects of the different procedures which need judgment in their use. For example, it is manifestly impossible to remove all the existent nodal lymphatic tissues in the axilla, yet radical mastectomy for carcinoma of the breast aims to do just this, as part of the operation. Removal of the rectal lymphatics can only be performed by removing the rectum and lower colon, with the consequent formation of an artificial anus, a standardized procedure for operable carcinoma of the rectum Irradiation of lymph nodes where for various reasons operation seems to be technically impossible, or fraught with risks to spread, is necessarily a blunderbuss affair, as all tissues in the fields of irradiation, sound or diseased, are severely injured The effectiveness cannot be guaranteed because the tumour cells may prove refractory, and, unlike the surgical procedure where the operation specimen can be examined histologically, it may never be known whether the procedure was deserved or not As may be guessed, the whole business of the management of the lymphatics in cancer is a fertile ground for argument between protagonists of the various procedures, for the good reason that in many fields the value of the different approaches is not conclusively proved

Lymphangiography is performed by injecting a radio-opaque oil into the lymphatics of an extremity and following its progress radiologically through the regional lymphatics and ultimately to remote lymphatics It is possible to distinguish involved nodes from uninvolved nodes where these are inaccessible to palpation (in the latter half of the twentieth century nothing has rivalled manual examination for assessing the state of palpable lymph nodes) The procedure may be unhelpful or even misleading, though, as with axillary lymph nodes, where many of them may be 1 mm in diameter and when eyth do contain a few malignant cells, as is not uncommonly found histologically, this is a situation clearly beyond the resolving power of contemporary radiological equipment On the other hand, it is possible that lymph nodes which are of any size can be accurately outlined and radio-therapeutic fields planned accordingly This seems to be a very worth-while technique, and over the next few years it is to be expected that the results will be forthcoming and its position in medicine and value in the management of malignant disease will be assessed

Malignant disease along with accident are the common causes of death in childhood, and a report from the Children's Tumour Registry at the University of Manchester is an analysis of a group of tumours called neuroblastoma comprising 54 cases, and another group of 14 cases with similar histology but not so designated on the grounds that they appeared to be primary in bone and that their clinical and biochemical behaviour was unlike secondary deposits of neuroblastoma in bone

Neuroblastoma is a tumour arising in the sympathetic nervous system, a fact often demonstrated anatomically or suggested by the histological appearances The special group of 14 cases the authors consider resemble a group delineated by the late James Ewing, after whom it is known eponymously as Ewing's tumour Histologically this group resembles the least differentiated but acceptable neuroblastomas The tables presented show an age of onset later than the first two years of life and show a worse prognosis than in the neuroblastoma group taken as a whole From their figures it is also apparent that the outlook for the group of neuroblastomas which occupy the same age groups as the Ewing's tumour is equally hopeless, but expectation of life in months is somewhat better. The debate on whether Ewing's tumour is an entity has been alive for more than two decades, and it appears that the present work has arrived at an impasse From the data presented it could be argued that Ewing's tumour and poorly differentiated neuroblastoma are variants of a common disease The problem involved does leave one to speculate on some of the limitations of the histological method and its place in the management of malignant disease The difficulty of assessment of the results of treatment and comparison between various methods in the first instance is having the same commodity to compare There is nothing in human tumours so far as standards of identity are concerned remotely comparable with the purity attainable by the chemist, and histological diagnosis is the most universally acceptable diagnostic criterion, since the material in which the diagnosis made is permanent and can always be reviewed

Since a histological opinion is ultimately a matter of subjective impression, clearly there must be some common ground or Babel would result and no useful communication between pathologists would be possible What is usually the situation is something like this—in the case of the rare, well-defined tumour there is universal agreement in diagnosis With regard to common malignant tumours, histological diagnosis would depend on how much of the specimen has been examined, for it is a frequent experience that the more tissue submitted to histological examination the wider the range of patterns will extend Patterns to the histologist are a reflexion of differentiation on the part of the tissue, progressive loss of the capacity to form the normal micro-anatomical appearances connoting anaplasia Ultimately with extreme anaplasia all tumours whatever their origins must look alike, and are only modified by local factors such as the presence, density and nature of any supporting stroma, the rates of cell division and death, and the local blood supply In this sort of situation the histologist only reporting on what he sees cannot really point to the anatomical site of origin of an undifferentiated tumour He could indicate perhaps certain lines of investigation which might lead to it, but by and large the problem of the undifferentiated tumour is a clinical and anatomical one Clearly, if an undifferentiated tumour arises in bronchus, cervix or bladder as it is likely to do, then it is respectively a carcinoma of the bronchus, cervix or bladder, irrespective of the fact that the three tissues are quite indistinguishable from each other histologically

The problem over Ewing's tumour is that the histological method has probably been pushed beyond its limits and that poorly differentiated neuroblastoma cannot really be differentiated from other similar anaplastic tumours The justification for splitting it off from the main group of diseases rests entirely on anatomical and clinical grounds It is reasonable to call the histological appearances undifferentiated tumour, thus leaving the question of its histogenesis open, but allowing the clinician to decide on its nosological status.

P Alexander
I Hieger
A L Levene

MEDIEVAL PREPARATIONS OF URINARY STEROID HORMONES

By Dr LU GWEI-DJEN and Dr JOSEPH NEEDHAM, F.R.S.
Gonville and Caius College, Cambridge

KNOWLEDGE of the physiology and biochemistry of the steroid sex hormones, both androgens and œstrogens, is a characteristic achievement of modern science stemming from nineteenth-century transplantation experiments and twentieth-century examination of the unsaponifiable lipins It would scarcely be expected, therefore, that in any phase of ancient or medieval science it should have been possible to make preparations which possessed activity of this kind

Recently, however, we have come across a corpus of material which indicates that just this was accomplished by the Chinese iatro-chemists between the tenth and the sixteenth centuries A.D. Guided by theories of traditional Chinese type, not of course the same as those of modern science, and using urine in large quantities (up to 300 gallons) as their starting-point, they succeeded in preparing mixtures of androgens and œstrogens in relatively purified form, and utilizing them in medicine for many hypogonadic conditions. The classical discovery of Aschheim and Zondek in 1927 that pregnancy urine contains large quantities of certain steroid sex hormones[1], and the subsequent recovery of these and others from other urinary sources, was thus strangely anticipated.

All the methods (we have translated six of them) start with large amounts of urine (as if on a modern pharmaceutical manufacturing scale), from adults or adolescents of each sex. The simplest, possibly the oldest, took the whole mass obtained by evaporating to dryness, including the steroid glucuronides and sulphates with much else, but most of the methods embodied various precipitations to obtain a colourless and odourless product. One used calcium sulphate, which would have assisted protein precipitation and therefore that of the conjugates also. Another made use of the saponins from the soap-bean tree, *Gleditschia sinensis*[2], the bean juice being added to the urine at the rate of one bowlful for every tub. The precipitate was afterwards extracted with boiling water, so that some steroids carried down on the protein were probably released as it denatured, while those combined with the saponins remained insoluble. Without *ad hoc* experiments, which it would indeed be interesting to carry out, one cannot say which steroid hormones could have been thus separated; but the quasi-empirical anticipation of the classical discovery of the digitonin precipitation of cholesterol by Windaus in 1909 is extraordinary[3]. Besides the vegetable protein added in the soap-bean juice, it is highly probable that other proteins were usually present, since in medieval times proteinuria would be expected among some of the numerous donors, and this would aid the precipitation of the steroid conjugates.

In nearly all the methods the process ends by a sublimation. At first this seemed difficult to understand, but in fact the urinary steroid sex-hormones do sublime unchanged in air between about 140° C and 280° C, there being considerable differences in sublimation temperature between them[4]. The description of the sublimation vessels in the texts is quite clear and unmistakable. Small earthenware pots were used with carefully luted covers, and great attention was paid to the degree and duration of heating. Chinese alchemists had been perfecting sublimation and distillation techniques since the second century B.C.; the iatro-chemists could therefore now prepare a rather purified mixture, as is indicated by the descriptions of the material as white, crystalline, glittering and lustrous or pearly like translucent jade. Finally, the sublimate was often emulsified with milk fat. Of course the end-products were complex mixtures of many different compounds, depending on the fractionation procedures used and on the original source material, but their pharmaceutical efficacy is vouched for by the descriptions of the conditions for which they were prescribed.

The six methods mentioned date from the eleventh to the sixteenth centuries A.D., and are preserved in Chapter 52 of the great Pharmacopœia of 1596 (*Pên Tshao Kang Mu*) by Li Shih-Chen from books no longer extant, or extremely rare. The oldest is the *Ching Yen Fang* (Tried and Tested Prescriptions) by Chang Shêng-Tao, dating from 1025, and quoted already in the *Chêng Lei Pên Tshao* of 1249. Then from the early years of the twelfth century A.D. we have two further descriptions in the *Shui Yun Lu* (Water and Clouds Record), written by a famous scholar, Yeh Mêng-Tê (1077–1148). Another book of the same period, the *So Sui Lu*, by an unknown author, theorizes about these preparations. The rest are in three books of the Ming period. If one asks how these medieval iatro-chemists could have been led to their urinary preparations, the answer surely is that classical Chinese medical and physiological theory envisaged a constant interaction among the organs of the body mediated through the circulating blood. Since the urine was considered as "of the same category as" (*thung lei*) the blood, then the virtues emanating from the organs might perhaps be found in it also.

The full material will appear in *Medical History*. For helpful discussions in its evaluation we thank Dr. Roger Short, Prof. H. Breuer and Dr. Hal Dixon.

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[1] *Klin. Wochenschr.*, 6, 1322 (1927).

[2] See Needham and Lu, *J. Hist. Med. and Allied Sci.*, 17, 429 (1962) (abridged in *Health Educ. J.*, 17, 170, 1959).

[3] *Ber. deutsch. Chem. Gesellsch.*, 42, 238 (1909).

[4] Breuer, H., and Kassau, E., *Proc. First Intern. Congr. Endocrin.*, Sect. 11d, No. 561, Copenhagen (1960). Kassau, E., *Deutsche Apotheker Zeitung*, 100, 1102 (1960).

OBITUARY

Dr. William C. Hayes

DR. WILLIAM CHRISTOPHER HAYES, curator since 1952 of the Egyptian Department of the U.S. Metropolitan Museum of Art, who died suddenly on July 10, was one of the outstanding Egyptologists of his generation, being equally celebrated as a historian, philologist and archæologist.

He was born at Hempstead, Long Island, on March 21, 1903, of an English father and of a mother who came of a prominent Philadelphia family. He was educated at St. George's School, Newport, where he won a medal for classics and graduated in 1924 at Princeton University. Here, two years later, he took his master's degree, having won a Carnegie scholarship for the study of medieval and Byzantine art, an interest which is reflected in his early paper on an engraved glass bowl in the Vatican Library. On being appointed to the staff of the Metropolitan Museum Egyptian Expedition in 1928, however, he henceforth devoted his talents to the study and elucidation of Ancient Egypt.

Hayes came comparatively late to his subject, and the only formal training in Ancient Egyptian that he received was with Sir Alan Gardiner at Oxford, where on occasions he stayed on his way home after the season's excavations. Nevertheless, he produced in 1935 his *Royal Sarcophagi of the XVIIIth Dynasty*, a remarkable and unique work in which his early discipline as an art-historian as well as his Egyptological learning was brought to bear on the study of a whole group of monuments, and succeeded among other things in solving some long-standing problems concerning the history of the Dynasty. This model publication, for which he received a Ph.D. from Princeton, was the first of a number of books and articles all characterized by the impact of a well-balanced and clear-sighted judgment and written by a master of English prose. His publication of a hieratic papyrus in the Brooklyn Museum, which he assembled from many hundreds of fragments, showed how an unpromising text could be transformed in his hands into a wealth of information on late Middle Kingdom social life and administration. His most recent works were four fascicles of the revised *Cambridge Ancient History*.

In Egypt he had largely been concerned with the reconstruction of the smashed statues of Queen Hatshepsut found by the Americans scattered in a quarry near her great temple at Deir el-Bahri and over a number of European museums He also, with Lansing, excavated the tomb of the parents of Sennemut, but when the Expedition closed its operations in Egypt in 1936, he returned to New York as an assistant curator in the Metropolitan Museum He had spent much of his spare time messing about in boats off the New England coast, and when the U S called up reserves in 1941 he joined the Navy, rising to the rank of lieutenant-commander and being decorated for gallantry in the Pacific theatre of the War He returned to the Museum in 1945 as associate curator. In 1959 he was appointed American representative on the International Committee for the Preservation of the Nubian Monuments He has left a widow and two sons.

A man of great integrity, quiet strength and a penetrating wit, Bill Hayes earned the respect and indeed the affection of all his colleagues on both sides of the Atlantic The loss to Egyptology by his unexpected death with much of his work still in draft form is a grievous one

C. Aldred

NEWS and VIEWS

The Royal Society: Officers for 1964

At the Anniversary Meeting held on November 30, Sir Howard Florey, provost of the Queen's College, Oxford, and formerly professor of pathology in the University of Oxford, was re-elected president of the Royal Society.

The other officers re-elected for the ensuing year were *Treasurer*, Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd , *Physical Secretary*, Sir William Hodge, Master of Pembroke College and Lowndean professor of astronomy and geometry in the University of Cambridge; *Foreign Secretary*, Sir Patrick Linstead, rector of the Imperial College of Science and Technology Prof A A Miles, director of the Lister Institute and professor of experimental pathology in the University of London, was elected biological secretary

The other members of Council elected (or re-elected, marked *) were Prof P M S Blackett, professor of physics in the Imperial College of Science and Technology, Prof A R Clapham, professor of botany in the University of Sheffield, Prof A H Cottrell, Goldsmiths' professor of metallurgy in the University of Cambridge, Prof C A. Coulson, Rouse Ball professor of mathematics in the University of Oxford, *Prof E F Gale, professor of chemical microbiology in the University of Cambridge, *Prof E L Hirst, Forbes professor of organic chemistry in the University of Edinburgh, Prof B Katz, professor of biophysics in University College (University of London), *Prof D Lewis, Quain professor of botany in University College (University of London), Sir Bernard Lovell, professor of radio astronomy in the University of Manchester and director of the Nuffield Radio Astronomy Laboratories, Jodrell Bank, *Dr P B Medawar, director of the National Institute for Medical Research, *Prof W T J. Morgan, deputy director of the Lister Institute and professor of biochemistry in the University of London, Prof A. B Pippard, John Humphrey Plummer professor of physics in the University of Cambridge, Prof J W S Pringle, Linacre professor of zoology in the University of Oxford; Sir Ewart Smith, formerly a deputy chairman of Imperial Chemical Industries, Ltd , Prof J H Taylor, professor of geology in King's College (University of London), *Dr H W Thompson, university reader in infra-red spectroscopy, Oxford

(Publication of the presidential address and award of medals at the anniversary meeting of the Royal Society has been deferred until the issue of December 28)

Chief of Electrical Instruments Section at the National Bureau of Standards: Mr F. L. Hermach

Mr F L Hermach has been appointed chief of the Electrical Instruments Section in the Electricity Division at the National Bureau of Standards, U S Department of Commerce Mr Hermach replaces Dr F M Defandorf, who died in August Born in Bridgeport, Connecticut, in 1917, Mr Hermach has been with the Bureau since 1939 He received a B E E in 1943 from George Washington University In 1954, he was the recipient of the Commerce Department's Silver Medal for Meritorious Service for his contribution to the standardization of electrical measurements in the development of a highly accurate transfer instrument for measurements In that same year, Mr Hermach received a Superior Performance Award from the National Bureau of Standards As chief of the Electrical Instruments Section, Mr Hermach will be concerned with the dissemination of values for a c and d c standards for use in measuring electric current, voltage, power, and energy from very low to very high values He will also be responsible for miscellaneous electrical measurements needed for engineering purposes.

Chemical Engineering at Monash University: Prof. O. E. Potter

Dr O E Potter, at present reader-in-charge of the Department of Chemical Engineering at the University of Melbourne, has been appointed to the foundation chair of chemical engineering at Monash University After graduating with honours in applied science from Queensland University, and completing a Master's degree, Dr Potter proceeded on a Queensland Foundation travelling scholarship to University College, London, where he read history and philosophy of science Later he became a lecturer in chemical engineering at the Manchester College of Science and Technology in the University of Manchester, where he also completed a Ph D on a number of fundamental aspects of liquid–liquid reactions In 1960, Dr Potter was appointed reader-in-charge of the Department of Chemical Engineering in the University of Melbourne and head of the Department in the Royal Melbourne Institute of Technology At that time the Institute had a very limited staff, while there was no permanent University staff Through Dr Potter's very considerable personal efforts, staff was obtained for both Departments, laboratories were vitalized and student numbers began to increase Postgraduate research was started, so that after only three years there are twelve full-time students for higher degrees Dr Potter has wide research interests in fluidization investigations, mass transfer phenomena and technical kinetics He has published a number of scientific papers in these fields and has contributed the section on technical kinetics in the *Chemical Engineering Practice* series

Physiology in the University of Malaya, Kuala Lumpur: Prof G. Barber-Riley

Dr. G Barber-Riley, lecturer in physiology in the University of Cape Town and formerly lecturer in physiology in the University of Liverpool, has been appointed the first professor of physiology in the University of Malaya, Kuala Lumpur Dr Barber-Riley was born in 1920 He was educated at Wallasey Grammar School. Between 1941 and 1946 he served with the R A F V R, holding the rank of warrant officer, with the duties of observer, and later navigator and wireless operator He made many sorties over Europe, including low-level flights in *Mosquitoes* After the War he first studied in the University of Manchester (1946–48) and then became a medical student in the University of Liverpool, graduating M B, Ch B in 1958 He had intercalated a year of study for the B.Sc honours degree in physiology, which he obtained in 1955. He was an assistant lecturer in the Department of Physiology of the University of Liverpool during 1958–60, when he obtained the degree of M D. He was then appointed to the post of lecturer in physiology in the University of Cape Town He has published a number of papers, alone or in collaboration with others, mainly on clinical and experimental aspects of liver function

University of Keele. Statistical Research Unit in Sociology

MR B DE VOS has been appointed the first director of the new Research Unit in Sociology of the University of Keele He will take up the appointment from January 1, 1964 Mr De Vos has been a director of the Gallup Poll for the past five years He will remain a director of that organization As director of the Unit, he will be responsible for developing new sociological studies based primarily on sample survey techniques and also for reinforcing the strong links already existing between the different departments and disciplines at Keele Since its foundation in 1949, one of Keele's special features has been the maintenance of close links between different disciplines and the avoidance of over-specialization within departments Up until now, there has not been a separate Department of Sociology, although considerable emphasis has been placed on the sociological aspects of studies in the Departments of Economics, Education, Geography and Politics The formation of the Research Unit has been made possible by a gift of £50,000 from the Nuffield Foundation The University is also responsible for running the *Sociological Review*

Transport Investigations at the Imperial College of Science and Technology

THE Nuffield Foundation has granted £45,500 to the Imperial College of Science and Technology to enable Prof C Buchanan to continue the investigations on which he has recently been engaged in the Ministry of Transport Mr David Crompton and Mrs Ann MacEwen, who worked with Prof Buchanan in the Ministry, have been appointed Research Fellows at the College The particular topics which it is proposed to study are American transportation survey methods, the use of cost-benefit analysis for urban roads and redevelopment projects, environmental standards in relation to traffic, and alternative movement systems for urban areas The appointment of Prof Buchanan to the newly established chair of transport tenable at the College was announced earlier in the year (*Nature*, **197**, 1153, 1963)

Transportation and Economic Growth

A BOOKLET being distributed by the Battelle Memorial Institute, entitled *Can New Transportation Concepts Contribute to Economic Growth ?*, directs attention to the lack of the integrated knowledge needed to answer some of the basic questions relating to the efficient and economic operation of transport facilities (Pp 8 Columbus, Ohio Battelle Memorial Institute, 1963) Solutions of such problems, it is argued, have frequently been designed to meet the immediate needs of specific and narrowly defined situations, and further research to gain knowledge of the interactions of various parts of the complex would contribute substantially to economic growth

International Bureau of Time Uniform Time and Constant Frequency for the Year 1964

FOLLOWING the decision of the International Committee of Time at its meeting in Berkeley during August 1961, and the General Assembly of the Union Radioscientifique Internationale at its meeting in London during September 1960, and the plenary assembly of the Comité Consultatif International des Radiocommunications at Geneva during January–February 1963, the International Bureau of Time consulted the observatories which have cæsium atomic resonators and compared their astronomical observations It has now calculated the nominal value of the frequency to transmit during the year 1964 as -150×10^{-10} with regard to the scale of time such that the frequency of cæsium has the value $f(\mathrm{Cs}) = 9\ 192\ 631\ 770$ c/s The value indicated will not change during the course of 1964 The International Committee of Time, the Union Radioscientifique Internationale and the Comité Consultatif International des Radiocommunications recommend that the value of the frequency indicated by the International Bureau of Time should be utilized by all organizations wishing to transmit uniform time and constant frequency.

Physical Review and *Physical Review Letters*

IN spite of the number of new periodicals that have been launched in recent years to deal with specialized branches of physics, editors still find it difficult to satisfy the demand for additional space for the publication of papers submitted to them *The Physical Review*, which has until now been issued at fortnightly intervals but which has been considerably expanded during the past few years, is to become a weekly as from January 1964 The fifty-two issues in the calendar year will each be dated on the Monday of the week of issue and will be divided into four volumes of thirteen issues each, with the thirteenth issue consisting of a comprehensive quarterly index The first twelve issues of a volume will be divided into two sections, *A* and *B*, of six issues each, appearing alternately Section *A* will be devoted to atoms, molecules and condensed matter, and Section *B* to the physics of nuclei and elementary particles The quarterly index will be a combined one relating to both Sections *A* and *B* Subscriptions must cover both sections The cost to members of the American Physical Society will be increased by five dollars In a parallel move, *Physical Review Letters* will also be issued weekly and will consist annually of two volumes of twenty issues each There will be no division of subject-matter and there will be no increase in size or subscription

Automata Theory and Information Retrieval

THE expressed purpose of the survey, *The Application of Automata Theory to Problems in Information Retrieval*, by R A Kirsch, is to suggest certain topics in automata theory that may be explored so as to achieve further understanding of the information retrieval problem (National Bureau of Standards NBS Report 7882 Pp ii+70 Washington, D C U S Department of Commerce National Bureau of Standards, 1963) The literature of automata theory is intimately connected with the study of machines, and in early literature the machines studied were intended to appear lifelike However, in the more recent literature the emphasis is on information processing, with many papers on the design of computor-life devices and switching circuits Papers dealing with the early approach and with the design of devices and switching circuits are excluded from the selected bibliography given at the end of the survey Attention is directed more to the abstract area of automata theory On the subject of finite automata, literature concerned with problems in representing languages, rather than with studies of relations between finite state languages and the memory requirements or structural constraints which they impose on the machines which generate or recognize them, are listed in the bibliography Certain references to recursive function theory are included. in addition to some on mechanical theorem proving, especially those which make use of syntactic techniques and a technique based on the expansion theorem of Herbrand The particular applications discussed in the survey are information retrieval systems for processing natural language, dealing first with automata theory as a source of mechanisms for the description of English text; and secondly, with automata theory for the description of other phenomena as languages, artificial languages in information retrieval systems, including a brief consideration of the algorithmic language ALGOL, inference in information retrieval systems, and theoretical feasibility and complexity questions The bibliography consists of 321 items

Research in Space Science

SELECTED papers from the Special Reports in Space Science issued by the Astrophysical Observatory of the Smithsonian Institution during the years 1958–61 are brought together in *Smithsonian Contributions to Astrophysics*, 6, *Research in Space Science* (Pp vii+242 Washington, D C Government Printing Office, 1963. 2 dollars) The Smithsonian Astrophysical Observatory has been among the leaders in several branches of space research—the optical tracking of satellites, the determination of orbits from observation, the use of these orbits for geophysical studies, the development of orbital theory, etc.—and the papers are, therefore, of considerable interest as a record of progress in these subjects Some of them are printed as they originally appeared (including one which was in error and is corrected 15 pages later); but others have been revised in varying degrees The authors include regular members of the Smithsonian Astrophysical Observatory, such as L G Jacchia and I G Izsak, and visiting consultants, such as M Nicolet and Sir Harold Jeffreys This publication is supplemented by *Smithsonian Contributions to Astrophysics*, 8, No 1, *Accurate Drag Determinations for Eight Artificial Satellites, Atmospheric Densities and Temperatures*, by L G Jacchia and J Slowey (Pp ii+99 Washington, D C Government Printing Office, 1963 65 cents) In this report the rate of decrease of orbital period for eight satellites during the years 1958–61 is evaluated from field-reduced observations with Baker–Nunn cameras, and the upper-atmosphere densities and temperatures are then determined The report is notable for its accuracy and wealth of detail, the variations in density and temperatures at heights of 300–700 km, both short-term and year-by-year, being fully displayed.

Chesapeake Bay Bridge–Tunnel

SOME facts about the nearly completed bridge and tunnel crossing from Wise Point, Va, to the Virginia shore 15 miles from downtown Norfolk are of interest to Britain and France in view of the controversy that has raged over the possibility of bridging the English Channel, as opposed to a tunnel link under the Straits of Dover This project overcomes the last and most formidable obstacle on the 998-mile Ocean Highway between New York and Florida It is acclaimed not only as one of the world's modern engineering wonders but also as " man's longest span across navigable ocean water" "The stream of seagoing traffic will inaugurate a spectacular 17 6-mile system of trestles, man-made islands and tunnels that draws the boundary between the Atlantic Ocean and Chesapeake Bay". More than two-thirds of this Highway is 30 ft above water, elevated on 2,600 concrete legs, each 54 in in diameter and embedded 100 ft into the sea-floor The structure is designed to withstand hurricane winds, a 10-ft rise in sea-level, and even 14 5-ft ocean swells The tunnel sections account for two miles of the total span, one being 5,450 ft long under the Chesapeake Channel, the other 5,738 ft under Thimble Shoal—both are main shipping channels Each tunnel, stated to be as wide and high as a three-storey building, was prefabricated, floated to site, then encased in concrete and sunk end-to-end in deep trenches excavated under the shipping lanes The vital statistics of this bridge–tunnel are that it took 1,000 men 3 5 years to construct, used 550,000 cubic yards of concrete, 55,000 tons of steel; 34,000 carloads of rock, and more than 5 million cubic yards of sand-fill Another interesting feature of the design is the use of 14,700 elastic bearing pads the size of "standard desk dictionaries" "Laminated of neoprene synthetic rubber around steel plates . . the pads separate elevated 75-foot, 65-ton road sections from their supports. Thus they prevent concrete grinding and cracking caused by movement from thermal expansion and contraction or wind and water current". An illustrated article entitled "Seagoing Roadway Rides on Rubber", by H E Davis, published in the *Du Pont Magazine* (57, No 4, 13, July–August, 1963) and from which the foregoing data are taken, gives many other technical details of this remarkable achievement

Antibodies

Antibodies is the title of a recent issue of the *British Medical Bulletin*, which contains a series of authoritative articles by recognized experts each dealing with a particular aspect of the study of these substances (19, No 3, September 1963. *Symposium on Antibodies* Pp 169–262+4 plates London The British Council, 1963 30*s*) Concise papers on the nature of antigen–antibody reaction, sensitivity of methods of detecting antibodies, the isolation of antibodies, their chemical structure, γ-globulin metabolism, factors affecting antibody response, origins of immunological competence, as well as the nature of antitoxins, reagins, and auto-antibodies are among topics included In the introduction, Prof A A Miles, the chairman of the planning committee of *Antibodies*, reminds us that the story of antibodies began in 1890 with the demonstration by von Behring and Kitasato of the specific antitoxic property of the serum of animals some weeks after the injection of tetanus toxin Almost three-quarters of a century has passed since then, and during that time not only has detailed knowledge of the subject increased enormously, but also we have a much deeper understanding of the process of antibody production. The editors are to be congratulated on having compressed so much into the space available and yet left us with a lucid and fascinating picture of present-day ideas on this important aspect of immunology

Archæology in Western New South Wales

A RECENT issue of the *Records of the Australian Museum* contains an important article on "The Archæology of Mootwingee, Western New South Wales", by F D McCarthy and N W G MacIntosh (Vol 25, No 13, December 3, 1962 Pp 249–298+plates 19–27 Sydney The Australian Museum, 1962) This describes the paintings and engravings which can be seen in two principal sites, called Main Gallery and Dingo Rock The existence of this art has been known for 100 years, and was probably discovered by a member of the Burke and Wills party in 1860 The art consists of representations of humans, weapons and animals, and is considered in some cases to show incidents in the life of the Aborigines. The Dingo Rock decorations are believed to be at least 300 years old, probably more, on the basis of the fracturing and weathering of the rock following its engraving. The report attempts to interpret some of the scenes and objects in the light of local information, while knowledge of the technique of the engravings has been lost, certain groups can to-day be explained as portrayals of Aboriginal myths In the same area, a series of painted rock shelters were recorded, and excavations made of certain of their floor deposits In no case can the stone implements recovered be related to the series of paintings, and it seems probable that occupation of these shelters ceased well before the decoration was made

Paul Instrument Fund Grants

THE Paul Instrument Fund Committee has made grants as follows £12,800 to A H W Beck, lecturer in engineering, University of Cambridge, for the construction of apparatus for the amplification and generation of extremely high frequency electromagnetic waves (below 1 mm), £2,630 to Dr B V Bowden, principal of the Manchester College of Science and Technology, for the continuation of work on the development of narrow pass band dye filters (for night sky astronomical photography), £1,000, as a supplementary grant, to Dr S Evans, senior assistant in polar research, University of Cambridge, for the development of an instrument for measuring the depth of continental ice sheets by a radar technique, £1,870 to Dr E T Hall, director of the research laboratory for archæology and the history of art, University of Oxford, for the development (*a*) of a fully transistorized differential flux-gate magnetometer, and (*b*) of a miniaturized flux-gate compass, £725, as a supplementary grant, to Dr D W Hill, senior lecturer in physics, Research Department of Anæsthetics, Royal College of Surgeons of England, for the construction of an infra-red gas or vapour analyser using interference filters, £5,250 to Dr J V P Long, assistant director of research in the Department of Mineralogy and Petrology, University of Cambridge, for the development of an ion-probe analyser for mass spectrometric measurements on selected micro-volumes of 1–20μ diameter in polished specimens of minerals and metals, £5,690 to Prof J Ring, Department of Applied Physics, University of Hull, for the construction of apparatus for research into the applications of Moiré fringes as analogue Fourier transformers, £2,300 to Dr T H Wilmshurst, Department of Electronics, University of Southampton, for the construction of an electron spin resonance spectrometer for examining short-lived paramagnetic species produced by a pulsed light source

The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science"

The Indian Botanical Society: Awards

To commemorate the sixtieth birthday of Prof P Maheshwari, University of Delhi, the Indian Botanical Society presented him with an advance copy of the *Maheshwari Commemoration Volume* in recognition of his work of exceptional merit for the advancement of botanical research in India The *Volume* is a compilation of articles contributed by eminent botanists from all over the world and represents almost all disciplines of botany It is expected that it will be published in a month or so

The Society awarded the Birbal Sahni Medal for 1962 to Prof T S Sadasivan, Madras University, for organizing, promoting and fostering botanical studies in India Prof Sadasivan is the sixth recipient of this award

University News: London

The following titles have been conferred *Professor*, Dr J M Alexander (engineering plasticity), in respect of his post at the Imperial College of Science and Technology, Dr. T Barnard (micropalæontology), in respect of his post at University College, Dr F. R Bell (experimental veterinary medicine), in respect of his post at the Royal Veterinary College, Dr L P Mair (applied anthropology), in respect of her post at the London School of Economics, Dr M J Seaton (physics), in respect of his post at University College *Reader*, Dr D E Barton (statistics), in respect of his post at University College, Mr H. Billett (mechanical engineering), in respect of his post at University College, Dr W G Chaloner (botany), in respect of his post at University College, Dr R W Guillery (anatomy), in respect of his post at University College, Dr K J Ives (civil and municipal engineering), in respect of his post at University College, Dr V. H T James (chemical endocrinology), in respect of his post at St Mary's Hospital Medical School, Dr C R Boddington Joyce (psycho-pharmacology), in respect of his post at the London Hospital Medical College, Mr O C J Lippold (physiology), in respect of his post at University College, Dr D H Martin (physics), in respect of his post at Queen Mary College, Dr D V W. Parke (biochemistry), in respect of his post at St Mary's Hospital Medical School, Dr A F Seager (mineralogy), in respect of his post at Birkbeck College, Dr D A Walker (botany), in respect of his post at Queen Mary College The following appointments to readerships have been made: Dr E M. Deuchar (embryology), tenable at University College, Dr B R Rabin (biochemistry), tenable at University College, Dr C W. Rees (organic chemistry), tenable at King's College

Announcements

MISS RACHEL CARSON, author and ecologist, and Prof H Clifford Darby, professor of geography in University College, London, have been awarded the Cullum Geographical Medal and the Charles P Daly Medal, respectively, by the American Geographical Society for their respective contributions in the fields of conservation and geography. Miss Carson, who is well known for her books, *Silent Spring* and *The Sea Around Us*, has been affiliated with the Marine Biological Laboratory, Woods Hole, Mass, since 1929 Prof Darby is known most widely for his work in historical geography, particularly for his book, *The Domesday Geography of England*, of which four volumes have so far been published, earlier this year he was awarded the Patron's Medal of the Royal Geographical Society He was president of the British Institute of Geographers in 1961

AT the tenth general assembly of the International Council of Scientific Unions held recently in Vienna, Dr H W Thompson, of St John's College, Oxford, and university reader in infra-red spectroscopy, was elected president of the Council for the next two years Prof D Blaskovic, vice-chairman of the Czechoslovak Academy of Sciences and director of the Academy's Virological Institute, was elected secretary-general, and Ing Gen. G Laclavère, National Geographic Institute, France, was elected treasurer

THE *Memoirs and Proceedings*, 1962–63, of the Manchester Literary and Philosophical Society (105 Pp 128+xlix Manchester Manchester Literary and Philosophical Society, 1963 31*s* 6*d*) contain M W Thring's Joule Memorial Lecture, "Can Machines make Humans Happier ?", and papers by E Mendoza on the history of the kinetic theory of gases, by F C Toy on universities, research associations and industry, and by S Toulmin on the discovery of time The volume also includes the annual report of the Council, and lists of special lectures, members and members of Council

THE twelfth inter-university geological congress entitled "The Present is the Key to the Past" will be held in the University of Sheffield during December 15–18 Further information can be obtained from H G Davies, Department of Geology, University of Sheffield, Mappin Street, Sheffield, 1

A SYMPOSIUM on "The Use of Redundancy in System Design", arranged by the Systems Engineering Section of the Society of Instrument Technology, will be held at the Northampton College of Advanced Technology on February 14 Topics under discussion will include automatic landing of aircraft, nuclear power systems, techniques of redundancy in logical circuits, reversionary modes in complex systems Further information can be obtained from the Symposium Secretary, Society of Instrument Technology, 20 Peel Street, London, W 8

BRITISH AID TO DEVELOPING COUNTRIES

*Aid to Developing Countries** outlines developments in Britain's economic assistance to developing countries since the White Paper, *Assistance from the United Kingdom for Overseas Development*, was issued in March 1960 (see *Nature*, 186, 501; 1960), and describes briefly Britain's present-day effort. The total flow of resources from the industrialized nations of the Free World to the developing countries is at present running at about £3,000 million a year, one-third of which consists of private investment. Of the £2,000 million from public funds, more than a half comes from the United States; the British Government has provided £1,367 million since the end of the Second World War, and the present contribution is £148 million, compared with £151 million in 1960–61 and £160 million in 1961–62, the decrease being due to the inability of overseas Governments to take full advantage in that year of the funds placed at their disposal, particularly in the way of loans. British private investment, mostly in the more developed countries of the Commonwealth, is running at about £300 million a year, but is tending to level off or decline. The British Government is participating in an examination, in the Development Assistance Committee of the Organization for Economic Co-operation and Development, of the feasibility and desirability of multilateral investment assurance. Moreover, it is supporting the proposal of the International Bank for Reconstruction and Development to set up a centre for conciliation and arbitration on investment disputes as well as an attempt to draw up a convention for protecting foreign investors.

Of the present annual expenditure on overseas aid, about one-sixth is spent on technical assistance, and only part of this is a direct charge on Britain's balance of payments. Since December 1962 something like £15 million of additional aid has been offered from surplus productive capacity. Of bilateral aid totalling £138 million in 1962–63, £60·6 million was to Colonial territories and £62·3 million to independent Commonwealth countries, the proportion of grants to loans being 2 : 1 for the former and 1 : 2 for the latter. After summarizing in some detail the distribution of capital aid, the White Paper summarizes the effort in technical assistance, more particularly since the first progress report of the Department of Technical Co-operation was issued in April 1962, and the White Paper on overseas recruitment policy in May 1962. Expenditure on technical assistance is estimated at £30·3 million in 1963–64, or 25 per cent more than in 1961–62; about half this is for the Overseas Service Aid Scheme, in which 39 Governments now participate and some 15,400 officers are covered, 8,900 on permanent and pensionable terms. In 1962 the Department of Technical Co-operation made 1,042 appointments compared with 880 in 1961; another 187 appointments were made through the British Council, and 408 through the Crown Agents.

Voluntary service is of growing importance: 296 volunteers went out in 1962, 550 are going during 1963, and it is planned that 800 should go in 1964; school-leavers numbered 262 in 1962, 300 in 1963, and 300 are planned for 1964; for graduates these figures are 36, 250 and 500. Besides running the school-leavers scheme, Voluntary Service Overseas is sending more than half the volunteers under the graduate scheme. About £17 million is being spent directly from public funds as British aid in education and training, apart from contributions to international agencies assisting in this field. In 1961–62 659 fully qualified teachers left Britain for non-university posts in developing Commonwealth countries and 87 non-university teachers were recruited for developing foreign countries, while recruitment has since increased. On the advice of a Committee under Sir Charles Morris as chairman (see *Nature*, 198, 919; 1963), the Secretary for Technical Co-operation proposes during 1964 to make up to 25 special Commonwealth awards to eminent British academic persons willing to serve for two or more years in universities in developing Commonwealth countries, and the Inter-University Council continues to find candidates for more than 200 posts a year. The British Institute of Management has been given a grant of £5,000 for clearing-house activities in training for management, and schemes for East Africa and for Asia are being prepared.

Of more than 60,000 full-time overseas students at British universities, training colleges and other institutions, two-thirds are from the developing countries. These also represent a substantial contribution on Britain's part to the educational development of these countries, and some £3 million is expected to be spent in 1963–64 in this way by means of scholarships, fellowships and other awards for study in Britain. The British Council is also administering a Government grant of £3 million towards the cost of providing additional residential places for overseas students.

On research the White Paper gives little information. Part of the £2·15 million provided through the Department of Technical Co-operation in 1963–64 for research into the economic and social problems of developing countries is devoted to the support in Britain of research institutes dealing with tropical problems, such as the Tropical Products Institute, the Anti-Locust Research Centre and the Tropical Divisions of the Road Research Laboratory and the Building Research Centre. Significant financial support is given to the Tropical Metabolism Unit of the Medical Research Council in Jamaica, to the Tropical Fish Culture Research Institute in Malaya and to various Regional Research Centres in the tropics, while the specialist advisory staff of the Department of Technical Co-operation are constantly assisting overseas Governments in the investigation of particular problems. No real idea of the scale or extent of the contribution to research can, however, be gained from the White Paper, but there is some further reference to assistance in the medical field, in agriculture and in surveys.

In medicine, the Government has accepted most of the recommendations of the Working Party under the chairmanship of Sir Arthur Porritt on medical aid to developing countries (see *Nature*, 198, 719; 1963), including the creation of 25 postgraduate bursaries at British universities and the establishment of 12 senior and 6 junior lectureships at the London and Liverpool Schools of Hygiene and Tropical Medicine. In agriculture the Government has agreed to contribute £52,000 a year for three years towards the cost of the Graduate School of Tropical Agriculture in the University of the West Indies in Trinidad and will also provide up to 20 studentships a year for British agricultural graduates to take this course in preparation for overseas service. Grants are also being made to expand facilities at the Royal Dick Veterinary College in Edinburgh for training in tropical veterinary medicine and to the Commonwealth Forestry Institute at Oxford for a similar purpose. Through the Directorate of Overseas (Geodetic and Topographical) Surveys, the Department of Technical Co-operation is providing the basic topographical mapping for more than 30 countries, and since 1949 one million square miles have been mapped. The Directorate also undertakes the preparation of medium-scale maps required for individual development projects, and assistance is also provided in geology and related matters, including advice on the development of mineral resources and the recruitment and training of geologists for employment overseas.

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* H.M. Treasury. *Aid to Developing Countries*. Pp. 51. (Cmnd. 2137.) (London: H.M.S.O., 1963.) 3s. net.

The White Paper concludes with a brief review of Britain's contribution to multilateral aid, including technical assistance, which is given mainly through the International Bank for Reconstruction and Development, the International Development Association and the International Finance Corporation. To the United Nations Expanded Programme of Technical Assistance and the Special Fund, Britain contributed in 1963 just more than £3·5 million, an increase of 25 per cent on 1962. In mid-1962 there were 431 British experts in the field under United Nations programmes—more than those of any other single nationality—and in 1962–63 we provided study facilities for 751 fellowships financed by the United Nations. Besides a current contribution of £350,000 to the United Nations International Children's Emergency Fund, which has now taken on functions in health, nutrition, education and family service, Britain contributes to the annual programme of aid to the developing members of the Organization for Economic Co-operation and Development, Britain's proportion of the 1963 allocation of £657,000 being 16·3 per cent, through the general budget of the Organization.

THE BRITISH BROADCASTING CORPORATION

THE annual report for 1962–63 of the British Broadcasting Corporation* is concerned largely with the plans for the Corporation's second television programme, which it is hoped to open in the London area in April 1964 with some 25 h of broadcasting a week. This should be extended to the Midlands about a year later and, by the end of 1966, about two-thirds of the population should be covered. Equipment has been ordered, plans for the siting of new transmitters have been discussed with the Post Office and with the Independent Television Authority, and recruitment for the new service has been commenced. The staff employed on March 31, 1963, numbered 17,930 full-time and 1,010 part-time, compared with 17,125 and 887, respectively, on March 31, 1962: the higher rate of recruitment and higher proportion of engineers to technical assistants noted last year is likely to continue during 1963–64. A number of the 170 engineers and 450 technical assistants and operators required for the new television programme have already been recruited.

Introduction of colour television is at present planned for 1965, and, in view of the desirability of the whole of Europe, if at all possible, working on the same system, the Corporation arranged a series of colour demonstrations in London in July 1963 to which delegations from the European broadcasting organizations, including the U.S.S.R. and Eastern Europe, were invited. It is hoped that as a result of these and other demonstrations the European Broadcasting Union will be able to forward an agreed recommendation to the International Consultative Radio Committee in time for a final decision on a European standard to be reached in February or March 1964. All the Corporation's very-high-frequency transmitters will be equipped to transmit colour. There have also been discussions with other European broadcasting organizations in stereophony, but, while further experiments have been made, any decision on a regular service of stereophony in the United Kingdom is likely to be some years distant.

The Corporation's Television has been reorganized to meet the needs of a two-channel situation by creating two new posts of Chief Controller, under the overall control of the Controller of Programmes. The Post Office has set up a joint working group with both the Corporation and the Independent Television Authority to consider engineering matters concerned with the problems of the move to ultra-high-frequencies, and the starting of a second programme on 625 lines. While the Corporation regrets the Government's postponement of a decision to introduce local sound broadcasting, for which there was strong support in local government and university circles, it welcomes the new opportunities opened up for educational programmes of all kinds. An Adult Education Liaison Committee has been set up and a television series of adult education programmes was approved to start in the autumn of 1963. The Corporation is prepared to run a special educational programme to supplement the more general programmes, if needed and if the necessary finance is forthcoming, but it is strongly opposed to the idea of a separate educational authority to run such a programme.

Concern as to the financing of these developments continues. The report points out that unless the Government meets the situation created by its retention for a further six months of part of the proceeds of the £4 licence, a £6 licence will become necessary by April 1, 1965. In the meantime, some of the plans for expansion for which Government approval was given in June 1962 must be deferred. Arrangements are being pressed ahead for providing separate television coverage for Wales, and the best means of filling gaps in the existing coverage in Scotland are being considered. Some disappointment is expressed at the failure to make full use of the extensive very-high-frequency sound coverage: sales of very-high-frequency sets are declining, and the radio industry's plans for dual-standard television sets do not include provision for very-high-frequency sound.

On overseas broadcasting, the report observes that the year saw the thirtieth anniversary of the Corporation's External Services, and while competition is increasing the reputation of the Corporation remains high. Since 1955, the number of radio sets in the world has risen from 237 million to about 400 million, and, as last year, the Corporation ranks fifth in output; the U.S.S.R., the Satellite countries, the Voice of America and China, have increased their lead and in the same order. Audience surveys have revealed that the Corporation's programmes have particular appeal to younger people: half the listeners in the U.S.S.R., where ages have been established, are under 30, and two-thirds of those in France are under 20. Apart from the research and development in connexion with stereophonic broadcasting, the introduction of the 625-line standard and ultra-high-frequency, a continuous programme of work is in progress in connexion with long-distance propagation, which bears on the problems arising when transmitters in different parts of Britain and in the neighbouring countries of Europe operate in the same or adjacent frequency channels.

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* Annual Report and Accounts of the British Broadcasting Corporation 1962–63. Pp. 188+16 photographs. (Cmnd. 2160.) (London: H.M.S.O., 1963.) 11s. 6d. net.

Further information on future developments is given in No 17 of *The B B C Record* (February 1963), which emphasizes the value of the two television services Moreover, it outlines the first year's proposals for adult education in television, and in the Sixth Bishop Bell Memorial Lecture, "Future Prospects in Broadcasting", delivered by H Carleton Greene, the director-general of the Corporation, on April 30, and now published as a pamphlet, Mr Greene stresses the complementary functions of the Corporation and the importance of concerning itself with the whole of the national life, with mass interests and with minority interests He suggests that if broadcasting planners do their jobs properly they can create and stimulate cultural and educational demands as well as meet them He directs attention to the use of television programmes in teaching and to the introduction of experimental programmes for use in technical colleges and colleges of further education Furthermore, he urges that it would not be right to make an educational channel a charge on licence revenue it should receive a separate grant-in-aid, as do the External Services.

SOILS AND LAND USE IN NORTH WALES

A SURVEY of the soils of North Wales was started by the late Prof G. W Robinson and his colleagues, who published several reports and maps in the *Welsh Journal of Agriculture*. The work has been completed recently with a memoir, and accompanying maps, which covers an area of 240 sq miles, stretching from Pentraeth, in Eastern Anglesey, and Llandudno in the north to Waenfawr and Bettws-y-Coed in the south* The Conway valley and most of the high land of Snowdonia are included

The climate is maritime and its most important influence in soil development is the rainfall, which increases steeply from some 30 in on the coast to 120 in on the mountains in the south. The parent materials of the soils include acid and basic igneous rocks, schist and shale and their derived drifts, non-calcareous clay and limestone, peat and various mixed drifts from igneous and sedimentary rocks The soil mapping and classification follow the custom of the Soil Survey and profile description includes information on site, drainage, parent material, horizons, colour, texture and structure Soils of five major soil groups have been recognized, namely, brown earths, calcareous, gley, podzolized and organic soils, but there is also a considerable area of immature soils occurring on youthful sands and alluvium Each is described and sub-divided into sub-groups and soil series, and again grouped into lowland, marginal and mountain soil zones

The larger part of the memoir is naturally taken up with detailed descriptions of the soil series and with appendixes on particular profiles and analytical data, but there is a chapter on land use, compiled with the assistance of G Edwards, H E Roberts and W A Lindsay-Smith This opens with a historical review of the area from the time of the disappearance of the valley glaciers, with inferences to be drawn from studies on pollen grains and dating by radioactive carbon, on changes in climate, flora and human settlement Recent agricultural statistics show the variation in size of holdings and the changes in crops and livestock between 1935 and 1955 An interesting aspect is the continuous rise in sheep production, but there has also been a pattern of technical improvement and change in farm economy to meet the seasonal fluctuations in demand This is discussed in relation to the five regions of the area There are about 12,000 acres of woodland, about half planted by the Forestry Commission—largely conifer high forest Many factors, of course, determine tree growth, but it seems clear that good oak and elm are to be found on freely drained slopes of soils derived from mixed drift although falling short of conifers in yield production

Numerous figures and tables are of great assistance in assimilating the information presented, and several excellent photographs illustrate the outstanding features of the area in question

A. M SMITH

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* Agricultural Research Council Memoirs of the Soil Survey of Great Britain—England and Wales *The Soils and Land Use of the District Around Bangor and Beaumaris* (Sheets 94 and 106) By D F. Ball Pp ix+182+13 plates. (London H.M S O , 1963) 35*s* net

COMPARATIVE BIOCHEMISTRY OF THE LEGUMINOSAE

A SYMPOSIUM on "The Comparative Biochemistry of the Leguminosae", organized by the Plant Phenolics Group, was held at the John Innes Institute, Bayfordbury, Hertford, during September 10–11 The aim of the meeting was to bring together workers in diverse scientific fields but sharing a common interest in this group of plants The Leguminosae, which contains some 550 genera and 12,000 species, is, in economic importance, second only to the Gramineae, and members of the family contain a remarkably large number of interesting and unusual chemical constituents In his opening paper, N W Simmonds (John Innes Institute) reviewed the systematics of the family and summarized the many uses legumes are put to as foods, forages, timbers, gums, resins and tannins Dr R Cooper (Rothamsted Experimental Station), in dealing with root nodulation, pointed out that 90 per cent of the fixed nitrogen in the world had come from leguminous root nodules His review of the conditions required for successful nodulation by *Rhizobium* showed that many factors, including the occurrence of the correct host-symbiont specificities and the presence of the right kind of polysaccharide in the host roots, were involved

Chemistry may well be, in the future, of considerable value to the systematists dealing with legumes Its present contribution is slight, mainly because very few taxa have been thoroughly surveyed chemically Dr R E Alston and his colleagues in the University of Texas are therefore concentrating their efforts on a single genus, *Baptisia* Dr Alston illustrated his talk on this genus with pictures of the phenolic spots obtained on two dimensional chromatograms of leaf and flower extracts The patterns so obtained can be used not only to distinguish most of the eighteen or so species but also many of the interspecific hybrids Compounds which, at the moment, show more promise as taxonomic markers than the phenols are the free amino-acids Besides having the common protein amino-acids, legume seeds contain a range of unusual ninhydrin positive substances, such as canavanine, homoarginine, γ-hydroxyhomoarginine and lathyrine Dr A E Bell (King's College, London), in the next paper, showed that most species of *Lathyrus* could be divided into four groups, depending on their characteristic amino-acid patterns, species of *Vicia* could be divided in a similar manner

Few systematic surveys of alkaloids have been carried out in the legumes, so that no taxonomic inferences can yet be drawn from their distribution. However, three series of characteristic bases (the quinolizidines, the pyrollizidines and the *Erythrina* indoles) are present and, according to Dr. M. F. Grundon (University of Belfast), who provided the next paper, they could all arise from the same two precursors, Δ^1-piperideine and α-ketoglutaric acid, by similar biosynthetic pathways. In the last paper on nitrogenous constituents, Dr. D. Boulter (University of Liverpool) described recent progress on the separation of legume proteins. By using acrylamide-gel electrophoresis, he had been able to separate the albumin fraction of the field pea into more than twenty distinct components.

The second day of the meeting was devoted to phenolic constituents. One group of phenols, the isoflavonoids, occurs far more abundantly in the Leguminosae than in any other family, so that it was fitting that the first paper dealt with chemical studies on some recently discovered members of this group. Prof. W. D. Ollis (University of Sheffield) provided a fascinating account of the use of nuclear magnetic resonance spectroscopy for elucidating the structures of the complex phenols present in *Derris robusta*, *Piscidia erythrina* and species of *Dalbergia*. Dr. H. E. Nursten (University of Leeds) then described the hydrolysable and condensed tannins present in such plants as *Acacia melanoxylon* and *Robinia pseudacacia*. The legumes are an extremely rich source of flavonoids with unusual substitution patterns, and Dr. J. B. Harborne (John Innes Institute), in the next paper, summarized the various structural types present. He then referred to variability in glycosidic pattern, making the point that this sometimes showed correlation with systematics, as illustrated by surveys of the anthocyanins and flavonols of *Lathyrus*, *Pisum* and *Vicia*. Chromatographic patterns of phenolics in flower petals are also of interest at the variety level, Miss J. J. Corner and Dr. D. G. Rowlands (Unilever, Bedford) have found that broad bean varieties can be divided into two groups on the basis of their flavonol content. Beans are rich sources of oligosaccharides of the raffinose series, and Miss M. Walters (Royal Holloway College, London), in the last paper, described work on the biosynthesis of raffinose from uridine diphosphogalactose and sucrose in *Vicia faba*.

The meeting ended with a summary of the chemical aspects by Dr. E. C. Bate-Smith (Low Temperature Station, Cambridge) and of the taxonomic aspects by Dr. B. L. Turner (University of Texas). There was much lively discussion of these and other papers presented at the symposium. This was the first meeting of the Plant Phenolics Group to be held since the group decided to extend its scope to cover other plant constituents besides phenols, and its success underlined the fact that there is a very real need in Britain for an organization broadly devoted to phytochemistry.

J. B. HARBORNE

THE MOON'S PHOTOMETRIC FUNCTION

By DR. ROBERT L. WILDEY

Division of Geological Sciences, California Institute of Technology and Mount Wilson and
Palomar Observatories, Carnegie Institution of Washington and
California Institute of Technology

In the general case, for a flat diffuse-reflecting surface of a given normal albedo (ratio of true brightness to that expected from a non-absorbing Lambert scattering surface under the condition that the direction of illumination and observation are both perpendicular to the surface), the specification of surface brightness, B_λ, in units of the power of the illuminator is obtained with the knowledge of the three remaining degrees of freedom in the problem. These are the azimuthal angle between the illuminator and the observer, the angle of incidence, and the angle of observation, shown in Fig. 1 as μ, i and e respectively. In the case of the Moon an equally valid set of parameters would be the phase angle, α, the angle of elevation of the Sun, $A_\odot$, and the elevation angle of the Earth, $A_\oplus$, which are also shown in Fig. 1.

It follows that all lunar photometric data, when plotted in the four-dimensional space (B_λ, α, $A_\odot$, $A_\oplus$), should, within the observational error, define a 'surface'. Any tendency to abnormally large scatter about a 'surface' in such a plot could be due only to a non-unique scattering law (photometric function) for the lunar surface, or one which was 'polarized'. The latter possibility would derive from an anisotropic surface, that is, the scattering law would not be invariant to a rotational transformation of co-ordinates about the μ axis, and one additional degree of freedom would be introduced.

That the Moon's photometric function is fairly unique is suggested by previous photographic photometry, however, that ray material is a somewhat better backscatterer than most of the rest of the Moon is well known.

This article presents an additional simple observational test, in the case of the inner slopes of crater rims, of the double assumption that (1) the general photometric function of the lunar surface applies to the inner walls of crater rims, (2) the functional form of the photometric function which has been fitted to previous photographic data is correct.

In a review paper, Minnaert[1] has discussed comprehensively the history of photometric observations of the Moon. Except for photoelectric investigations of the integrated light (whole Moon), by Rougier[2] and Bullrich[3], the most widely accepted observations have been by pure photographic photometry, the susceptibility to systematic error of which leaves their reputation open to question, regardless of the skill of the observers involved.

It has been universally concluded that the strongest source of variation of the brightness of a given lunar feature is the variation in phase angle, which conclusion is not seriously questionable. It has also been concluded, however, that the form of the residual dependence on $A_\odot$ and $A_\oplus$ is in the form of a dependence on $h = f(\alpha, A_\odot, A_\oplus)$, and in particular that h is an angle defined locally on the lunar surface as the projection of angle e on the plane containing the vectors pointing toward the Sun and the Earth. h is shown in Fig. 1. This assumption follows from the conclusion by Minnaert[1] and Fessenkov[4] that, making allowances for differences in normal albedo, isophotes tend to coincide with lunar meridians. For a smooth spherical Moon, when the selenographic latitudes of the sub-solar and sub-Earth points are both zero, h equals the selenographic longitude of the particular point on the lunar surface. Hence the name 'brightness longitude' for h, even though h will vary with local strike and dip, regardless of the constancy of selenographic co-ordinates.

Eimer[5] has determined the Moon's photometric function, assuming it to depend only on a and h, from the photographic data of Fedoretz[6]. The result is a function, which, for a given phase angle other than zero, is a monotonic function of h over the range given

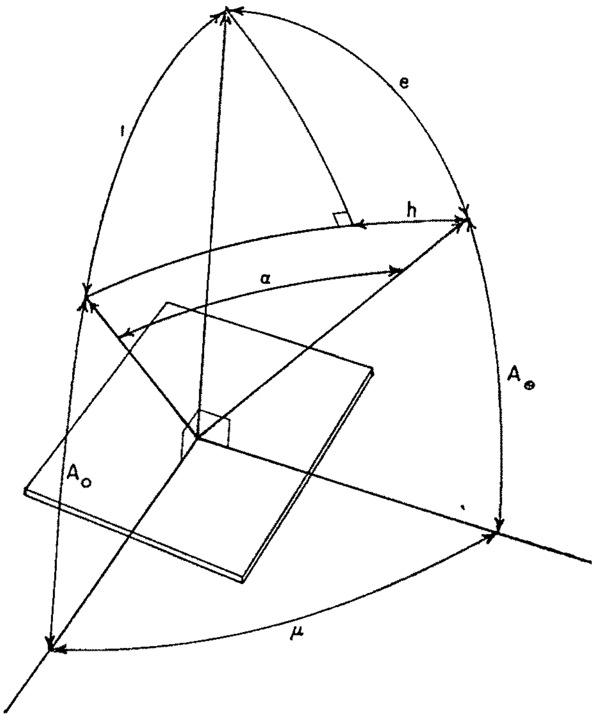

Fig 1 Diagram of angles orienting an element of lunar surface and the directions to the Sun and Earth. i is the angle of incidence, $A_\odot$, the elevation angle of the Sun, e, the angle of observation, $A_\oplus$, the elevation angle of the Earth, α, the phase angle, and μ, the azimuth difference between the Sun and Earth

When such a photometric function is applied to the illuminated portion of the inner slope of a crater rim which is casting a slight shadow on the crater floor, an interesting simple result is predicted If the vector pointed toward the Earth makes a small angle, σ, with the vector which points directly away from the centre of the Moon (normal to the hypothetical lunar surface of a perfectly spherical Moon), then the brightest point on the inner slope of the crater rim should lie nearly opposite the point on the rim which casts maximum shadow On the other hand, if σ is large, and unless α is very small, the point casting maximum shadow and the point of maximum brightness are connected by a lesser chord than a diameter As σ approaches $\pi/2$ the angular distance around the crater rim through which the point of maximum brightness travels, from the point directly opposite maximum shadow, is given by $\cos^{-1}$ ($\cot\kappa$ $\cot\alpha$), where κ is like σ except defined for the Sun, that is, the angle of incidence with respect to the local gravitational vertical rather than the normal to the local terrain Notice that this maximum angular travel is always an obtuse angle for phase angles less than $\pi/2$

We thus predict that shadowed craters at high latitudes should have their point of maximum brightness more nearly adjacent to the shadow, whereas craters near the equator should have shadow and maximum brightness on opposite sides There is a simple corollary which may be applied to a random collection of reasonably good photographs on which the resolution is good enough to make out some detail on the crater rims and for which the range of brightness thereon falls in the monotonic portion of the H and D curve It is that, assuming all craters to be round, for shadowed craters which appear round and for those which appear oval in which the shadow is symmetric about the minor axis of the oval, the rim point showing maximum brightness should be opposite the rim point casting maximum shadow, however, the greater the departure from both these conditions, the closer together on the rim the points casting maximum shadow and showing maximum brilliance tend to lie

This criterion has been applied to a group of photographs of the Moon including some printed from plates by Pease, some from the Moore–Chappel series, and some recent photographs taken by Herbig under extraordinary seeing conditions with the 120-in Lick reflector The results are shown in Fig 2 The outlines of many craters have been traced The line passing through each trace has been drawn through. (1) the point of maximum brightness, (2) the centre of gravity of the shadow image

Few extreme cases are shown, but nearly all those which should give slight departure from perfect bisection do, in weak support of an (α, h) dependent photometric function If one were trying to substantiate an (α, i) dependent photometric function one would look for a line which definitely cuts across the minor axis of the crater trace, but nevertheless appears nearly to bisect the crater. Crater 3 tends to show this slightly Crater 21 shows it a bit better and crater 30 is a good example of such an exception. The present simple method should not be regarded as having established the photometric function of the exceptions noted, but it has quickly revealed them as apt candidates for special, more quantitative investigation

One might, out of belief that all lunar slopes are gentle, conclude that shadowed craters are necessarily so near the terminator that the photometric function in their vicinity must have the limiting form, f (α) cos i, which Minnaert[1] has shown to be a corollary of the second law of thermodynamics and the observation that lunar luminance near the bright limb is nearly constant However, it has been shown[7,8] that the slopes in question are by no means gentle enough for this criticism to be levied

In conclusion, it seems reasonable to say that in the majority of cases where differences in the predictions of an (α, h) and (α, i) dependent photometric function are small, the observations favour an (α, h) dependent function However, two exceptions are rather strong in their deviations. It is thus suggested that either (1) most inner crater rims have an (α, h) dependent photometric function, while a few cases have (α, i) dependence, or (2) all crater

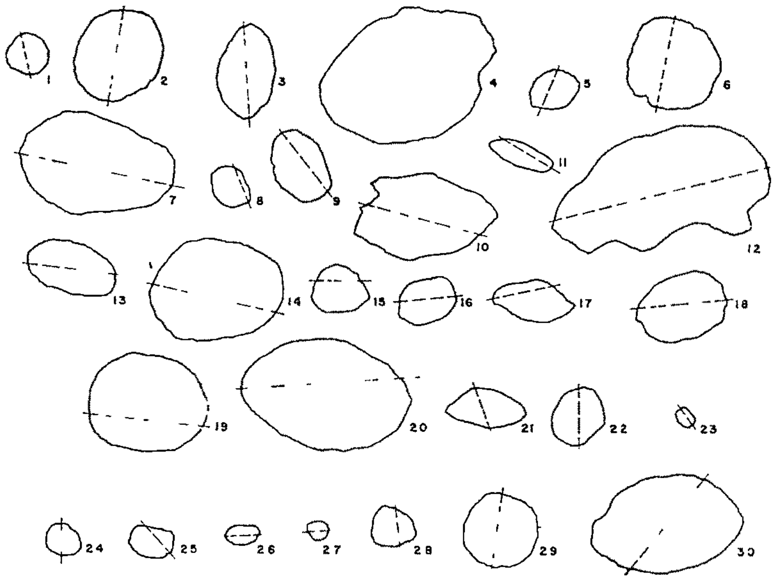

Fig 2 Traces of the projected outlines of craters Dashed lines pass through, (1) the centre of gravity of the inner shadow, (2) the point on the crater rim's inner slope which is brightest

rims have a non-degenerate (α, i, e) dependent photometric function which we do not yet know, or (3) perhaps only one or two cases showing fairly strong deviation from that predicted by an (α, h) dependence are significant and the more minor departures represent the 'noise-level' of the present method The third possibility would be compatible with the second possibility or vice versa of the first

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USE OF GAS CHROMATOGRAPHY FOR THE DETECTION OF LIFE ON MARS

By VANCE I OYAMA

Life Detection Systems Branch, Exobiology Division, Ames Research Center,
National Aeronautics and Space Administration, Moffett Field, California

THE technique of gas chromatography as applied to the identification of pyrolysis products of biological material has been advanced earlier as a means of life detection on Mars[1,2] Preliminary investigations as submitted here confirm in part the feasibility of this concept

The premise on which this concept is based originated from the fact that terrestrial life is an aggregate of carbonaceous macromolecular structures as well as a lesser mass of monomeric organic substances, the latter arise as intermediates in the syntheses of the macromolecules or by-products of metabolic processes The proteins, polysaccharides, and nucleic acids which represent macromolecular structure are composed of basic building blocks such as the amino-acids, monosaccharides, lipids, purine and pyrimidine bases These constituent substances are distributed universally among living materials, and the essential components of the protoplasm of life require that critical proportions of these basic building blocks be maintained either by assimilation from the environment or manufactured by the organism

The simple technique of thermal degradation has been applied to the analyses of chemical structure (The literature is replete with references too numerous to iterate) However, in a complex organic aggregate such as life, the pyrolysates from thermal degradation present a relatively difficult task of determining the original contributing structures Nevertheless, it is possible to present a chromatographic picture (such as has been described in paper chromatography in the two-dimensional display of peptides for the elucidation of protein structure) which could similarly describe the presence of life

Freshly isolated, but unidentified, micro-organisms were grown in trypticase soy broth under aerobic conditions at 30° C These were gathered during log phase of growth, washed with distilled water several times, and lyophilized to dryness

A single strain of micro-organism was used for determining the optimum temperature of pyrolysis which would reveal the major amount of volatile components of thermal breakdown as determined by a hydrogen flame detection device from gas chromatography column separations Since organic substances were of particular interest, this type of detector, incorporated in the Perkin–Elmer model 800, was used for this purpose 0 12 in × 12 ft column of 15 per cent '*SE* 30' on 60/80 mesh chromasorb *W* was maintained isothermally at 200° C The flow of gas was 80 ml /min

The pyrolysis chamber was a stainless-steel loop attached on to a linear 6-way valve, paired with another linear valve, which permitted, initially, evacuation of the sample loop, filling of the pyrolysis chamber with inert helium atmosphere, pyrolysis under closed system, and sweeping of the pyrolysis chamber into the flowing gas system directed into the columns The valving and tubing between the pyrolysis chamber and column were maintained at 125° C during the course of analysis Mass of the components appearing at the detector was measured by an Infotronics digital 'readout' system A weighed sample of material (10–200 µg) was funnelled into the sample loop and a heat pretreated fibre-glass cordage was inserted into the sample loop to prevent particulate matter from entering the critical valving area A pair of heated nickel plates, the temperature of which was regulated by a temperature controller and held together by spring action, was slipped over the loop making direct thermal conductive contact with the stainless steel sample loop

Pre-selected periods of time for various temperatures were utilized to determine the efficacy of residence time and temperature to give the greatest number of products Fig 1 shows that the total integrated products sensed at the detector are a function of these two parameters

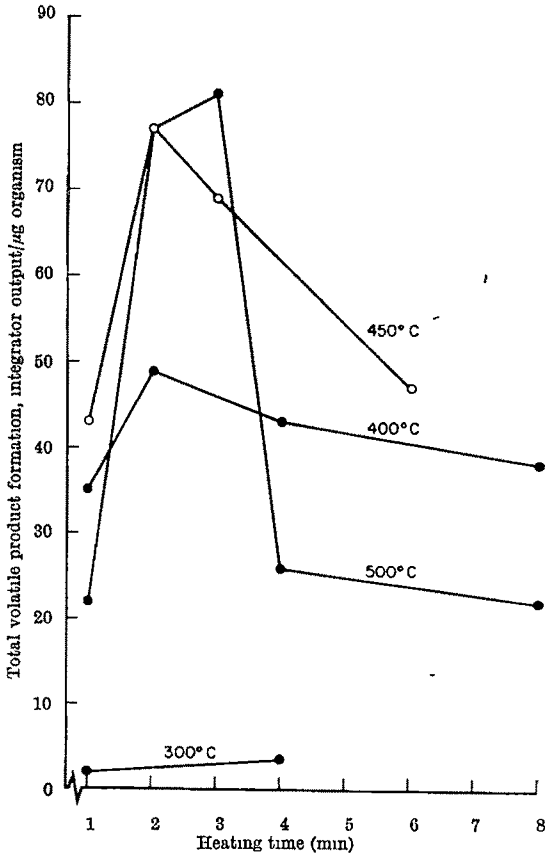

Fig 1 Comparative mass of volatile constituents formed at 4 temperatures for various times

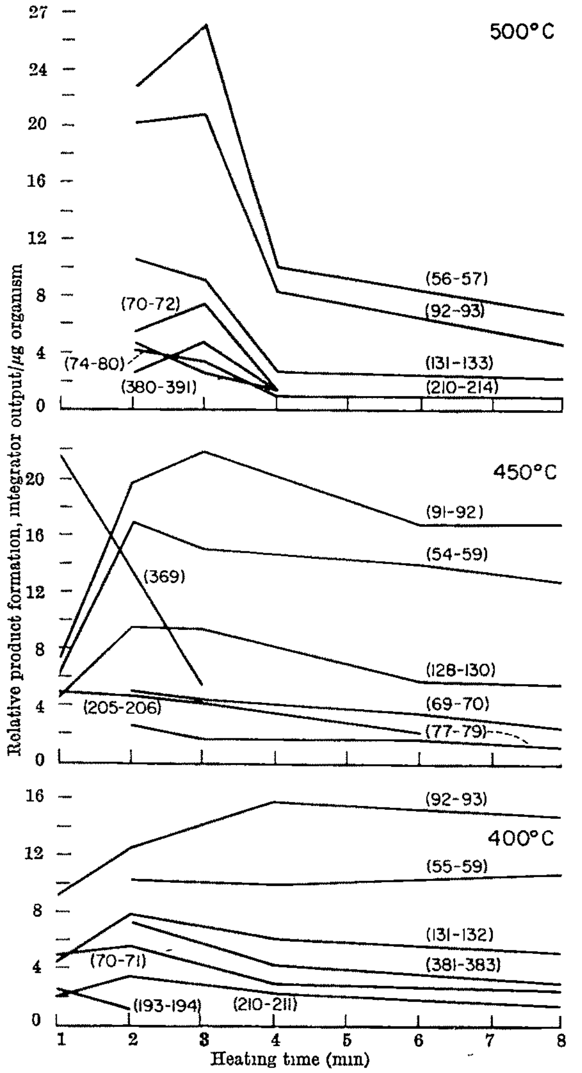

Fig 2 Products formation from dried micro-organisms as a function of temperature and residence time In brackets, retention time of integrated product (sec), range, 3 samples

No significant products are produced at temperatures of 300° C Exposure for 4–8 min will tend to decrease the total mass of substances Optimum temperature and residence time were 450° C or 500° C for 2 min It is not indicated, but to be noted, that the temperature within the stainless steel tubing took approximately 45 sec to attain 450° C

Fig 2 shows that the volatile substances produced by thermal degradation tend in turn to be degraded further Components of thermal degradation as determined by retention times show peak production at various times, for example, a component of retention time 56–57 sec (uppermost curve) is produced maximally at 3 min residence time for the specified temperature of 500° C The amount of product so produced will decrease as time of residence is increased It is to be assumed that synthesis of a thermal product is concurrently in progress with its degradation. The accumulated products of thermal decomposition represent only the thermal product which is measurable at the instant that the vapours are swept into the column The figure also shows that certain products are decomposed rapidly in time, while others reach a maximum at 4 min and appear to be stable at these temperatures Others, on the other hand, reach a maximum yield and degrade relatively slowly The figure shows the importance of reproducing the exact conditions of pyrolysis so that repeatability in terms of relative peak heights may be obtained

In order to ascertain as many of the products of thermal decomposition as is possible with a single system, a 0 010-in capillary column 300 ft long, coated with diethylene glycol succinate polyester, was temperature programmed for the analyses of some micro-organisms isolated from soils Typical results are indicated in Fig 3*A* It can be seen that under these reproducible conditions during linear temperature programming, the chromatographic peaks descriptive of the retention products occur with a periodicity common to all the organisms There are differences in peak-height relative to adjacent peaks, but the chromatograms indicate that thermal decomposition products of micro-organisms can be displayed in a fashion to show equivalence The relative peak-heights in each separate chromatogram show differences and may indicate differences in the relative amounts of precursor organic substance

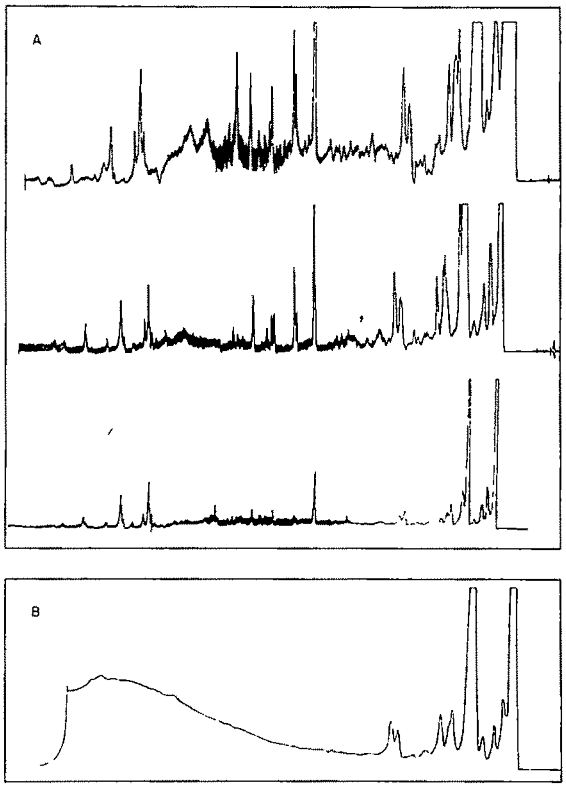

Fig 3 Comparative chromatograms of (*A*) three isolated soil micro-organisms, (*B*) of crystalline bovine albumin

Fig 3*B* shows the pyrolysate chromatogram of crystalline bovine albumin under similar conditions It is to be noted that similar patterns corresponding to early appearing chromatographic retention times of the micro-organisms are reproduced It is not altogether surprising, considering the original premise, to find similar patterns from proteins whether they be of plant or animal origin.

Further work is in progress to determine what these pyrolysate products are, the determination of what precursor organic substances are involved, as well as comparative chromatograms of micro-organisms as an approach to taxonomic differentiation

I thank Mr George Hotz for his contribution toward the design of the linear valving, as well as the pyrolysis heater systems, and Mr Frank Morrelli for his contribution of microbial inoculation sources

Work was sponsored under N A S A. Contract *NAS*–7–100 at the Jet Propulsion Laboratory, California Institute of Technology

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POLYPHASE FOLDING IN THE CALEDONIDES SOUTH OF THE SCOTTISH HIGHLANDS

By D. G. HELM, B. ROBERTS and Dr. A. SIMPSON

Department of Geology, Birkbeck College, University of London

THE high degree of structural complexity displayed by the Highland schists has long been known. Since the brilliant observations by C. T. Clough[1] in Cowal and the classic investigations by Sir Edward Bailey in the south-west and central Highlands, it has been apparent that the Caledonian architecture of this terrain has resulted from the impress of several, successive phases of orogenic movement.

During the past decade, detailed work by many workers[2-6] in various parts of the Moine and Dalradian Series has served to emphasize the polyphase nature of their Caledonian tectonics. Early acute or isoclinal flexures, often recumbent, have been re-folded on all scales by later generations of folds, usually with a more open profile, resulting from subsequent orogenic pulses. Each fold-system is generally accompanied by the development of a syngenetic, axial-plane foliation. In the earliest acute to isoclinal folds the latter has the character of a slaty cleavage or schistosity in pelite. On this primary foliation are successively impressed the strain-slip cleavages which accompany the later generations of flexures. This polyphase sequence of folds and cleavages is not confined to the more highly metamorphosed strata but is equally well developed in the mildly altered Dalradian sediments of the Highland border and Loch Awe region.

In contrast to the lengthy tectonic chronology of repeated folding displayed in the Scottish Highlands, the considerable volume of literature dealing with the remainder of the Caledonides to the south has suggested a much simpler orogenic programme. In such regions of deformed Lower Palæozoic strata as the Southern Uplands, Lake District, Wales and south-east Ireland, only a single phase of severe Caledonian folding, accompanied by an axial-plane cleavage, has been described. This fold-system, and the related cleavage, is steeply inclined with a predominant Caledonoid trend. The metamorphic alteration which accompanies the deformation of these fossiliferous strata is, of course, slight.

More or less centrally placed in the Irish Sea region, and surrounded by the above spreads of Lower Palæozoic strata, lies the Isle of Man possessing as its geological foundation the thick Cambrian pile of the Manx Slate Series. Within these strata Simpson[7] has recently demonstrated the existence of Caledonian polyphase tectonics which attain a scale and complexity comparable with parts of the Scottish Highlands. Three distinct phases of tectonic movement (F_1, F_2, F_3) each formed large-scale folds, a suite of congruous minor flexures and an axial-plane cleavage (S_1, S_2, S_3). The major and minor structures formed during the later phases deform those which originated earlier.

The F_1 folds are acute in profile and the accompanying S_1 is a flow or slaty cleavage in pelite. The early axial planes and S_1 are steeply inclined with a Caledonoid trend and for the most part the flexures plunge to the north-eastern and south-western quarters. The later F_2 and F_3 folds possess a more open, right-angled style and the accompanying S_2 and S_3 are strain-slip cleavages which were successively impressed on the complex pile. The F_2 flexures usually display Caledonoid plunges, their axial planes and S_2 are characteristically flat-lying or gently inclined. The axial planes of the F_3 folds and S_3 dip steeply to the north-eastern quarter, these latest plications always plunge steeply. Large-scale Caledonoid flexures of F_1 and F_2 age embrace the whole Island.

Following the recognition by Simpson of Caledonian polyphase folding and cleavage in the Isle of Man, research by us in the Lake District and North Wales has shown that comparable deformation is also developed in Lower Palæozoic strata of the mainland.

North Wales. Throughout North Wales the predominant fold-system resulting from the Caledonian orogeny possesses steep axial planes and is accompanied by a syngenetic axial-plane cleavage. In his tectonic summary of North Wales, Shackleton[8] has emphasized that, while displaying some variation in orientation, these structures are generally steeply inclined towards the north or west. This southerly-to-easterly vergence of the cleavage and the parent folds reflects the direction of translation of the higher layers in the sedimentary pile relative to those below during the formation of the fold-system. The intensity of these structures is greatest in the north-west towards Anglesey, towards Shropshire in the south-east the degree of deformation decreases.

During a volcanological examination of the Caradocian ignimbrite sequence in the Llwyd Mawr plateau, south-east Caernarvonshire, it became apparent to Roberts that the associated sediments occasionally show the impress of more than one phase of Caledonian deformation. In this area, as elsewhere in North Wales, the dominant, earliest (F_1) folds are steeply inclined to the north-western quarter and the accompanying axial-plane cleavage (S_1) usually has the character of a flow cleavage in pelite.

Sporadically, however, two subsequent generations of more open flexures (F_2 and F_3) deform the earlier S_1 and are accompanied by axial-plane strain-slip cleavages (S_2 and S_3). The F_2 folds display a low Caledonoid plunge and their axial planes, together with the related S_2, possess a gentle inclination, usually to the south-east. The axial planes of the later F_3 folds, and the accompanying S_3, dip steeply to the east-north-east and the flexures possess a steep to vertical plunge trending at 300°–320°.

The F_2 and F_3 folds are only mildly and sporadically developed in the Llwyd Mawr area. They are mesoscopic structures usually only a few inches across, though larger F_2 flexures with amplitudes up to 3 ft. have been observed on the western slopes of Mynydd Tal-y-mignedd. The later structures tend to be confined to pelite. For example, they have been observed in Cambrian (Ffestiniog) slates, in the Maesgwm Slates (Llanvirn) and in the Pennant Slates of Cwm Pennant (? Arenig).

Observations made by Roberts and Simpson at many other widespread localities in North Wales have shown that, while the steep F_1 folding and cleavage (S_1) remain pre-eminently responsible for the deformation of the Lower Palæozoic strata, the later F_2 and F_3 structures are sporadically developed in numerous places within Caernarvonshire and Denbighshire.

Consideration of the available evidence suggests that the sporadic F_2 folding in North Wales forms only a minor element of the Caledonian architecture and does not assume major proportions. The existence of large-scale F_2 folds would be reflected in the existence of broad Caledonoid belts, measured in miles across, within which S_1 would display a rotation through the vertical to a south-easterly inclination. Such belts would represent the short limbs of major F_2 fold-pairs Throughout North Wales, as Shackleton[6] has emphasized, S_1 retains a predominant steep northerly to westerly dip and reversals of the type described do not appear to be extensively developed

A possible exception is in north Denbighshire, where Boswell[9] has recorded the occurrence of a 'cleavage-fan' in S_1 extending eastwards from the Conway valley to the Clwydian Range Along a line lying 6–13 miles from the coast S_1 passes rapidly through the vertical and assumes a southerly dip which is retained for several miles farther north It seems possible to us that this 'fanning' may be due to large-scale F_2 flexuring, the question requires further investigation

As has been pointed out by Shackleton[6], one of the most obvious tectonic features in North Wales is the arcuation through 90°, convex to the north-west, in the trend of S_1 and the parent fold-system This deflexion embraces the whole of North Wales It takes place round a north-west–south-east axial trace running from the Menai Strait to Long Mountain in Shropshire The only interruption in the continuity of this open hinge is a sinistral offset of several miles apparently due to transcurrent movement along the Bala fault which is clearly portrayed on Shackleton's cleavage map[6] In the west (south Caernarvonshire) and south-west (Harlech region) the F_1 structures are aligned due north or a little east of north, to the north-east and east in Denbighshire the trend has swung round to the east or a little south of east

The arcuation is sharpest on the north side of the Berwyns and is clearly outlined on the 'ten-mile' geological map by the deflexion of the various Lower Palæozoic formations and major F_1 fold axial traces For example, on entering the deflexion, the core of the Llandderfel syncline (northward continuation of the Central Wales syncline) curves rapidly through 90° from its north-north-east trend and, continuing as the Llangollen syncline, preserves a trend a little south of east until it plunges below the Lower Carboniferous escarpment Five miles to the south-east, the complementary Berwyn anticline undergoes a comparable right-angled deflexion Farther north[6] the regional arcuation of the F_1 structures between south Caernarvonshire and Denbighshire is more gradual

The regional deflexion of the Caledonian structural pattern in North Wales has previously been interpreted as an arcuation of the orogenic belt presumably formed at the same time as the dominant (F_1) fold-system

Four miles south-west of Corwen, around Llandrillo, the deflexion of bedding and S_1 through 90° occurs rapidly and can be followed out within a few miles We have recently examined this tract, in the hinge of the arcuation, and have observed the presence of a strong S_3 in the slates which intersects and lineates both bedding and S_1 This strain-slip cleavage dips steeply to the north-eastern quarter and its north-westerly trend is parallel to the axial trace of the regional arcuation in the earlier structural elements The F_3 lineation and occasional small-scale, congruous F_3 crumples have a steep north-westerly plunge These F_3 structures tend to die out to the east and south-west away from the hinge of the arcuation and are clearly related to it in origin Farther north where the regional deflexion is more open, comparable F_3 structures are sporadically developed in Caernarvonshire and Denbighshire, but are especially noticeable around Conway, which also lies close to the crest of the deflexion

We conclude that the regional arcuation of the Caledonides in North Wales was not developed synchronously with the severe F_1 movement-phase but outlines a large-scale flexure of F_3 age This *North Wales antiform* has a steep-to-vertical axial plane and its north-westerly plunge is reflected by the convexity of the arcuation it has produced in the earlier structural elements Its style and orientation are identical to the related minor F_3 folds on its flanks and hinge S_3 has an axial-planar relationship to the parent flexure

The style and disposition of the North Wales antiform are similar to the several large-scale F_3 flexures recently described by Simpson[7] in the Isle of Man In particular, the resemblance to the Sulby antiform, the largest of the Manx structures, is especially striking The latter embraces the northern part of the Isle of Man and causes an identical arcuation to the east or east-south-east in the Caledonoid trend of the pre-existing linear and planar structures as its Welsh counterpart The upwarp of the core of the Caledonoid F_1 Isle of Man syncline by the Sulby antiform has caused a conspicuous plunge culmination in the early structures

The flexing of the F_1 Llandderfel–Llangollen syncline by the North Wales antiform has resulted in an identical reversal in the plunge of the early system Immediately west of the F_3 hinge the Llandderfel syncline has an overall plunge to the south-west On the opposite flank of the antiform the Llangollen syncline retains an easterly plunge until it disappears below the overlying and unconformable Carboniferous Limestone

The Taconic (end-Ordovician) folding of the Lower Palæozoic sediments in Shropshire dies out rapidly to the north-west and, as Shackleton[8] had emphasized, the pronounced deformation by folding and cleavage in North Wales must be assigned to the late Silurian–early Devonian interval

The observations made by us have shown that the orogenic chronology displayed by the Caledonides in North Wales is more complex than previously envisaged Not merely one but three phases of folding, each accompanied by cleavage-generation, can be distinguished This polyphase tectonic history can be summarized as follows

(1) *First movement-phase* (F_1) The F_1 fold-system, accompanied by the axial-plane S_1, was formed The large-scale structures produced have been admirably described by Shackleton[6] The F_1 structures pervade the Lower Palæozoic pile from the Cambrian at the base to the highest Ludlow (Dinas Bran) beds preserved in the core of the Llangollen syncline This was the most severe episode of Caledonian deformation in North Wales, though its imprint gradually declines towards Shropshire

We believe that the F_1 system originally possessed a Caledonoid (north-east–south-west) trend and a south-easterly vergence reflecting the direction of relative translation of the higher layers in the pile during folding

(2) *Second movement-phase* (F_2) The effect of this phase was relatively mild with the sporadic generation of mesoscopic folds accompanied by the gently inclined, axial-plane S_2 Major F_2 folding appears to be absent, though the 'cleavage-fan' in S_1 of north Denbighshire may represent F_2 flexuring on a larger scale

(3) *Third movement-phase* (F_3) Compression was applied to the folded pile along a Caledonoid line The large-scale North Wales antiform was produced as a cross-fold together with the sporadic generation of congruous minor F_3 folds and the steep axial-plane S_3 From their pre-existing Caledonoid (north-east–south-west) trend, the earlier structural elements (predominantly F_1) were flexed by the antiform into the conspicuous arcuation which is so obvious over the whole of North Wales The North Wales antiform is by far the largest F_3 flexure recognized by us so far in the Irish Sea region

Lake District A structural investigation being carried out by Simpson in the main tract of the Skiddaw Slate Series has shown the polyphase nature of the Caledonian deformation to which these Arenig sediments have been subjected The sequence of structures impressed is identical to that already described[7] in the Isle of Man The same three episodes (F_1, F_2, F_3) can easily be distinguished and the successive generations of mesoscopic structures are similar in style and orientation to their Manx contemporaries

The earliest F_1 folds are acute and display a Caledonoid trend, their axial planes, and the accompanying S_1, are steeply inclined Two subsequent pulses of movement produced the more open, right-angled F_2 and F_3 fold-systems with their related axial-plane strain-slip cleavages The F_2 structures retain their characteristic flat-lying to gently inclined disposition and Caledonoid axial plunges The F_3 flexures form a suite of cross-folds, their axial planes, and accompanying S_3, dip steeply to the north-eastern quarter

This sequence of repeated folding and cleavage-formation is clearly evident throughout the broad expanse of the Skiddaw Slates, though the phenomenon has received no mention in the previous literature For example, the superimposition of F_1, F_2, and F_3 structures can be clearly seen in the crags above Whinlatter Pass and on the flanks of Skiddaw itself

In the south-western corner of the Lake District the Skiddaw Slates crop out from below the overlying Borrowdale Volcanic Series to form a large inlier centred on the mountain of Black Combe In the course of a detailed stratigraphical and structural study of these strata, Helm has also recognized a comparable tripartite (F_1, F_2, F_3) programme of Caledonian deformation In addition he has shown that the Caledonian architecture of Black Combe differs in certain respects from that displayed in the main Skiddaw outcrop twenty miles farther north

The trend of the steep F_1 folds, and related S_1, is north-south and their original attitude was vertical Moreover, the F_2 flexures are usually more acute, their limbs enclosing an angle of 40°–60° The strong axial-plane S_2 dips consistently at 20°–40° north-west in contrast to the rather variable azimuth of this gentle structure farther north As a result of the pre-existing northerly grain of the F_1 system, the F_2 folds and second linear structures plunge at 30°–40° to the north-western quarter Minor F_3 folds, and accompanying S_3, deform the structures of the earlier movement-phases and retain the usual steep north-easterly disposition

Conclusion. It will be clear from the foregoing account of our observations in the Isle of Man, North Wales and the Lake District that the Caledonian tectonic chronology of these areas is more complex than previous workers have envisaged The detailed results of our work on the mainland will be published later The purpose of the present contribution is to emphasize that polyphase Caledonian folding with its accompanying sequence of superimposed cleavages is by no means confined to the metamorphic terrain of the Scottish Highlands forming the northern part of the Caledonides It seems inevitable to us that subsequent research will reveal a comparable sequence of folding and cleavage in other parts of the Irish Sea region

It may be emphasized that in each of the three areas we have examined the sequence of movement-phases, together with the style and orientation of the resultant structures, is similar Acute, steep folding (F_1) with a Caledonoid trend (locally veering to the north in Black Combe) forms a tectonic basis which is successively deformed by more open flexures round gently inclined, Caledonoid axial planes (F_2) and by steep cross-flexuring (F_3) with a north-westerly trend

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EFFECT OF SMALL PROTRUSIONS ON MASS TRANSPORT TO A ROTATING-DISK ELECTRODE

By G. T. ROGERS and K. J TAYLOR

Isotope Research Division, Wantage Research Laboratory, U.K Atomic Energy Authority

THE rotating-disk electrode is increasingly being used to examine electrode reactions at known rates of transport of reactants to the electrode surface. An essential result of Levich's[1] theoretical treatment of transport to the electrode is that, for a reaction where the rate is purely transport-controlled, reaction conditions are uniform over the area of the disk Several workers, when using the electrode to study metal deposition[2,3] and dissolution[4–6] reactions, have found spiral patterns on their disks The presence of these patterns shows that, in practice, conditions may not be uniform over the disk The causes of spirals and their effects on the rate of transport of reactants to the electrode surface are therefore of interest This article describes an investigation of these aspects of spiral patterns in a study of metal deposition on disks used at Reynolds numbers of about 10^4 The results indicate that the spirals are traces of the wakes behind small protrusions, such as burrs or gas bubbles, on the disk There is no evidence of boundary layer separation or vortex formation in the wakes The formation of vortices would result in rates of transport greater than those calculated from the Levich theory Experiments on the transport-controlled reduction of coumarin during nickel electrodeposition have shown that the overall rate of transport to a disk with many spirals did not differ significantly from the rate to a disk which showed no spirals

Spirals produced by hydrogen evolution A rotating disk electrode has been used to study the effects of coumarin on the electrodeposition of nickel[2]. Deposits plated from a Watts-type nickel plating solution, which contains no coumarin, had a uniform matt appearance Deposits plated under identical conditions, but from Watts's solution containing coumarin showed spiral patterns on the electrode Fig 1 shows a typical pattern These patterns were made up from fully bright, darker areas, and hazy areas of deposit superimposed on a slight pattern in relief There was a minute hole in the deposit at the origin of each spiral and a microscopic defect in the original disk surface at each origin Coulomb balances for the cathodic reactions showed that coumarin markedly increased the amount of hydrogen evolved With coumarin present bubbles of hydrogen could be seen to be flung off the disk during deposition No bubbles could be seen during deposition without coumarin Coumarin thus appears to favour the evolution of hydrogen from defects in the surface As the spirals are formed under conditions where the rate of coumarin reduction is transport-controlled they reflect local changes in the transport rate to the electrode The appearance of the spirals suggested that they might be traces of the wakes of hydrogen bubbles growing at these points, the bubbles acting as protrusions attached to the disk To test this the patterns produced by known protrusions have been investigated.

Spirals produced by burrs Nickel sheet was found to contain less defects than the nickel rod used to prepare electrode disks for earlier work Using disks of about 2-cm diameter made from this sheet, deposits showing only

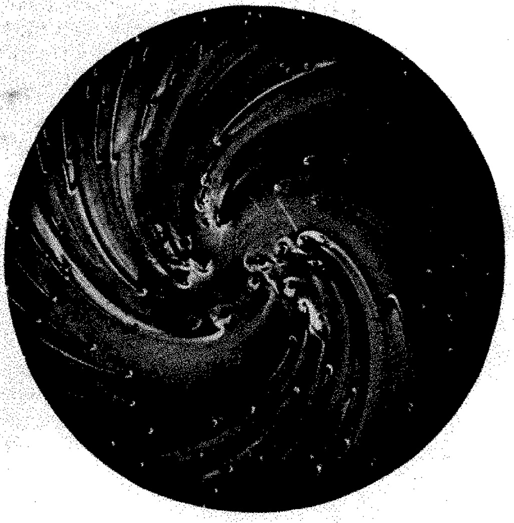

Fig. 1. Photograph of a typical pattern of spirals nickel plated from a Watts solution containing coumarin. Dark areas correspond to fully bright plate (× c. 3·3)

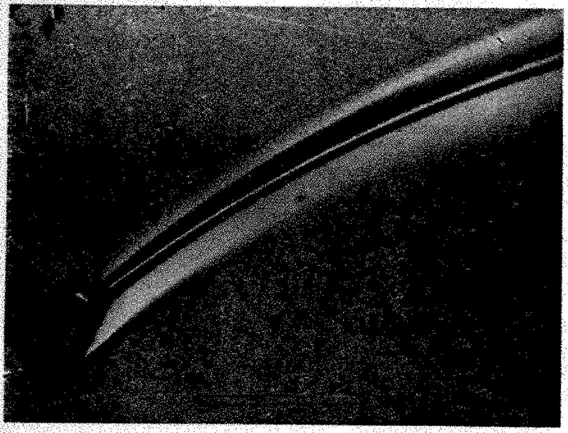

Fig. 2. Photograph of burr and its spiral

1 or 2 spirals could be obtained. Protrusions such as a 400-μ diameter insulating bead stuck on the disk or a flush hole of about 350-μ diameter with a bubble of hydrogen maintained in it gave spirals generally similar to, but much less well defined than, those obtained on smooth disks. Smaller protrusions gave better-defined spirals. The most satisfactory protrusions for further study were the burrs raised by pressing a sharp scriber point against the disk. Disks with burrs were plated using Watts's solution containing coumarin. A disk with 56 burrs was prepared and plated. There was a spiral associated with each burr and a few spirals similar to those in Fig. 1 resulting from hydrogen bubbles. Fig. 2 shows a typical burr and its spiral. No indication of vortex formation or turbulence was found in any of the spirals examined. The heights of burrs were estimated by focusing a microscope on the peaks of burrs and on the deposit near their bases using a microscope with a calibrated fine focus.

Suppose that a protrusion of height h produces a spiral which is the trace of the wake produced by a protrusion in the fluid at height h above the disk. This height can be deduced from the curvature of the spiral. Cochran[7] has described the flow near the disk using co-ordinates r, θ in the plane of the disk and $\zeta = \left(\frac{\omega}{\nu}\right)^{\frac{1}{2}} z$ normal to the disk. ω is the angular velocity of the disk and ν the kinematic viscosity of the fluid. The radial, angular and normal components of velocity u, v, w are given by:

$$u = r\,\omega\,F \quad v = r\,\omega\,G \quad w = (\nu\omega)^{\frac{1}{2}}H$$

where F, G, H are functions of ζ tabulated by Cochran. If u', v', w' are the equivalent components relative to the disk:

$$u' = u,\ v' = r\omega(1-G),\ w' = w$$

for values of $\zeta < 1{\cdot}0$ and $r > 0{\cdot}2$ cm w' is small compared with u' and v' so that the path of the fluid in the plane

$$\zeta = \left(\frac{\omega}{\nu}\right)^{\frac{1}{2}} h$$

can be considered as projected on to the disk. The path is given by $r = r_0 \exp \frac{(F)}{(1-G)} \cdot \theta$ the ratio $\frac{F}{(1-G)}$ being determined by ζ. Paths for different values of ζ were drawn on tracing paper and fitted to the spirals on an enlarged photograph of the disk. The mean height of 8 burrs which had clearly defined spirals, measured microscopically, was $10 \pm 1\mu$. Their spirals corresponded with $\zeta \leq 0{\cdot}1$. One larger burr was $21 \pm 1\mu$ high and had a spiral with ζ slightly less than 0·2. The disk was rotated at 980 r.p.m. and $\nu = 1{\cdot}1 \times 10^{-2}$ strokes, so that $\zeta = 0{\cdot}1$ and 0·2 correspond to heights of 10 and 20μ respectively.

The resultant velocity relative to the disk of fluid in a plane ζ at distance r from its centre is:

$$V(\zeta) = r\omega[F^2 + (1-G)^2]^{\frac{1}{2}}$$

If the height of a protrusion is taken also as a characteristic length for it a Reynolds number for flow near a protrusion is:

$$R = \left(\frac{\omega}{\nu}\right)^{\frac{1}{2}} \zeta r[F^2 + (1-G)^2]^{\frac{1}{2}}$$

For a 10-μ high protrusion on a disk rotating at 980 r.p.m. $\zeta \doteqdot 0{\cdot}1$. Then $R = 6$ for a protrusion 0·8 cm from the centre.

The incorporation of carbon from coumarin in deposits which showed bubble spirals and on disks with burrs has been investigated autoradiographically on deposits from solutions containing coumarin labelled with carbon-14. Fig. 3 is an autoradiograph of the pattern shown in Fig. 1. The lighter areas correspond with lower incorporation of carbon. A comparison of Figs. 1 and 3 shows that incorporation is lower within the spirals. Spirals produced by burrs showed a similar lower incorporation but without the very small areas of higher incorporation at their origins.

Conclusions. The spirals indicate that burrs and gas bubbles produce a disturbance of the flow near them which is stable and has stream lines which are very close to those of the theoretical laminar flow near the disk. There

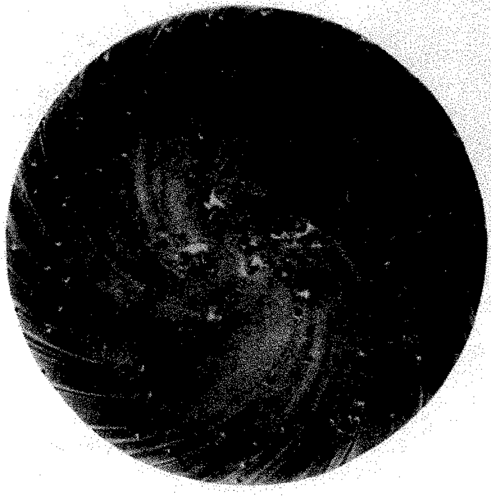

Fig. 3. Autoradiograph of the disk shown in Fig. 1. The coumarin used was labelled with carbon-14. Radioactive areas appear dark (× c. 3·3)

is no sign of vortex formation in the spirals and the Reynolds number evaluated here is an order of magnitude less than that for transition from laminar flow to a vortex street round a circular cylinder[8] or past a protrusion[9]. It seems most likely that the incorporation, in a deposit, of carbon from coumarin will increase as the rate of coumarin transport to the electrode increases. Hence the autoradiographic results indicate a lower rate within spirals than on the uniform surface. This is to be expected if the flow around protrusions was laminar and is the reverse of that to be expected if a vortex with its axis parallel to the disk were present. The flow around the protrusions, therefore, appears to be completely laminar with conditions far from those which would produce vortices. Under these conditions, the difference between the total rate of transport to a disk with spirals and a uniform disk should not be greater than and is probably less than the difference between the uniform areas on the two disks. Any relief in the spirals will slightly increase the available area of the electrode and hence the total rate of transport. This will be counteracted by the lower rate within spirals. The magnitude of the error in a measurement of the rate of a transport controlled reaction due to the presence of spirals is shown by comparing the rates of coumarin reduction at the disk with 56 burrs and one without burrs under conditions where the rate is known to be transport-controlled[2]. A difference of more than 5–10 per cent in rate would have been significant. There was no significant difference in the two rates, which were in agreement with rates calculated from the Levich theory.

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ELECTRICAL PROPERTIES OF PYRITE-TYPE AND RELATED COMPOUNDS WITH ZERO SPIN MOMENT

By DR. F. HULLIGER
Cyanamid European Research Institute, Cologny, Geneva

A LARGE number of transition element compounds of composition TX_2 crystallize in the pyrite, the marcasite or the arsenopyrite structure. In these structures the anions are tetrahedrally surrounded by one anion and three cations, whereas the cations are located in half the octahedral holes formed by the anion sublattice. If, therefore, X and Y denote di- and tri-valent anions, respectively, then non-metallic polycompounds are possible with ionic formulæ $T^{2+}(X_2)^{2-}$, $T^{3+}(XY)^{3-}$ or $T^{4+}(Y_2)^{4-}$. The occurrence of non-metallic properties requires that the chemical bonds be saturated and that all d electrons not involved in σ bonds be localized. These extra electrons not only influence the electrical properties but also the crystal structure. In the pyrite and cobaltite structure the octahedral holes occupied by cations are essentially regular, whereas the marcasite and the related arsenopyrite lattice readily allow for strong distortions and the formation of additional cation–cation bonds. As a consequence, cations with high-symmetry d configurations, such as $d\varepsilon^3 d\gamma^2$, $d\varepsilon^6$, $d\varepsilon^6 d^1_{\text{metallic}}$ and $d\varepsilon^6 d\gamma^2$, are encountered in the pyrite and in a few cases, for example FeS_2, $FeSe_2$, in an ideal marcasite structure. Cations with asymmetrically filled $d\varepsilon$ sub-shells occur only in the strongly distorted marcasite (Loellingite type) and arsenopyrite lattice. No compounds such as MnP_2 or CrAsS with cation configurations $d\varepsilon^3$ are known[1]. The cation configuration most favourable for the occurrence of the pyrite structure is $d\varepsilon^6$. Moreover, this non-magnetic configuration also favours semiconductivity, but does not guarantee it, as will be seen here. The prototype of the $d\varepsilon^6$ pyrites is FeS_2, and its semiconducting properties are well known. Since replacement of the 3d by either a 4d or a 5d cation normally enhances the non-metallic character[2,3] we expected the isomorphous ruthenium and osmium dichalcogenides to be semiconductors as well. This expectation was substantiated by measurements of the electrical resistivity. In agreement with the postulated $d\varepsilon^6$ cation configuration, all compounds proved to be diamagnetic. My data are collected in Table 1.

Semiconductivity in $T^{3+}(XY)^{3-}$ compounds of the cobaltite type has recently been discussed[4], so that we need not consider it here.

To get the configuration $d\varepsilon^6$ in a phosphorous-group compound TY_2, we have to choose the cation T from the nickel group. Indeed, $PdAs_2$, $PdSb_2$, PtP_2, $PtAs_2$, $PtSb_2$ and α-$PtBi_2$ are known to crystallize in the pyrite structure. I have found the following additional mixed pyrite phases:

PdAsSb a = 6·265 Å
PtPAs a = 5·842 Å
PtSbBi a = 6·570 Å

According to the rules[2,3] holding for transition element compounds, we expect non-metallic properties above all in the compound with the heaviest cation and the lightest anion, that is, in PtP_2. As seen from my resistivity measurements, all the compounds down to $PtSb_2$ turned out to be non-metallic (see Table 2).

In PtSbBi the energy gap might just have vanished. This compound reveals a metallic or semimetallic behaviour (the increase of resistivity of our sample between 20° and 400° C was 20 per cent). While the pyrite modification of $PtBi_2$ is metallic but not superconducting[6], the hexagonal[7] high-temperature modification β-$PtBi_2$ is superconducting below 0·15° K (ref. 8).

In the nickel and palladium analogues we expect the transition to metallic properties to occur already for a lighter anion. Because of the difficulty in preparing stoichiometric samples the data for the marcasite-type

Table 1. Preliminary data for the energy gap ΔE, the thermoelectric power α and the magnetic susceptibility χ of ruthenium and osmium dichalcogenides. The energy gap was derived either from diffuse reflectance measurements (*opt.*) or from the temperature dependence of the electrical resistivity (*el.*), assuming $\rho \sim \exp(\Delta E/2kT)$

	ΔE (eV)	$\alpha_{300°\text{K}}$ (μV/°C)	$10^6\chi_m$(C.G.S./mol) Room temp.	Liquid N_2 temp.
RuS_2	~1·8 (*opt.*)		−64	
$RuSe_2$	⩾0·6 (*el.*) ~1 (*opt.*)	120 (*n*)	−52	
RuSeTe	0·35 (*el.*)	300 (*n*)		
$RuTe_2$	0·25 (*el.*)	200 (*n*)	−78	−64
OsS_2	~2 (*opt.*)		−59	−57
$OsSe_2$		80 (*n*)	−69	−59
OsSeTe	>0·2 (*el.*)	150 (*n*)		
$OsTe_2$	>0·2 (*el.*)	100 (*n*)	−44	

Table 2. Survey on the electrical properties of TY_2 compounds. The values for the energy gap ΔE were derived from resistivity measurements. Since the samples were not especially purified, all figures should be regarded as approximate only. α and β refer to different samples

	Structure type	ΔE (eV)	$\alpha_{300^\circ K}$ (μV/°C)	$10^6\chi_m$(C.G.S./mol) Room temp.	Liquid N_2 temp.
NiP_2	NiP_2	0·5	100 (n)		
$NiAs_2$	C18	~0·05 (ref. 1)			
$NiSb_2$	C18	Metallic (ref. 1)		~ −30 (ref. 5)	~ −30 (ref. 5)
PdP_2	NiP_2	0·6–0·7	100 (n)	α −6 β +5	
PdPAs	NiP_2	0·45	240 (n)		
$PdAs_2$	C2	Metallic	(p)	−45	−50
PdAsSb	C2	Metallic	(p)	−44	−50
$PdSb_2$	C2	Metallic	(p)	α −16 β −27	−15 −27
α-$PdBi_2$	Monoclinic	Metallic			
β-$PdBi_2$	$C11_b$	Metallic			
PtP_2	C2	⩾0·6	100 (p)	−27	(+32)
PtPAs	C2	⩾0·4	~10 (n,p)	−62	−59
$PtAs_2$	C2	0·5	200 (n)	−48	−45
$PtSb_2$	C2	⩾0·05	80 (p)	−56	−52
PtSbBi	C2	Metallic	1 (p)		
α-$PtBi_2$	C2	Metallic			
β-$PtBi_2$	Hexagonal	Metallic			

compounds $NiAs_2$ and $NiSb_2$ are perhaps not as yet conclusive, but PdP_2 is definitely semiconducting while $PdAs_2$ is metallic. Moreover, I have synthesized PdPAs and found that it still is non-metallic. It is isomorphous to PdP_2 and its lattice constants are: a = 6·88 Å, b = 6·03 Å, c = 6·41 Å, β = 126·1 deg. As seen from Table 2, the transition semiconductor → metal thus coincides with the change of crystal structure: The non-metallic palladium compounds crystallize in the monoclinic NiP_2 type of structure[9] the atomic positions of which have not yet been determined. However, because of their non-metallic behaviour NiP_2, PdP_2 and PdPAs must be polycompounds, that is, the co-ordination of their anions and cations must be essentially the same as in pyrite and marcasite.

It is remarkable that though palladium can adopt the configuration $d\varepsilon^6$ all pyrite-type palladium compounds are metallic and $PdSb_2$ is even superconducting below 1·25° K (ref. 10). $PdBi_2$ shows two modifications: monoclinic α-$PdBi_2$, which is superconducting below 1·75° K (ref. 11), and tetragonal β-$PdBi_2$ with a transition temperature of 4·25° K (ref. 11). The high-temperature modification β-$PdBi_2$ crystallizes in a strongly elongated $MoSi_2$ type of structure which still reflects the tendency of the anion to form a polycompound.

It would thus appear that in the metallic palladium and platinum compounds the $d\gamma$-levels dip into the $d\varepsilon$ band or into the valence band, thus giving rise to a high density of states favourable for the occurrence of superconductivity. For studying the factors which influence the conduction character, the pyrite-type compounds appear of special interest because the palladium compounds have almost the same lattice spacings as the platinum analogues: superconducting $PdSb_2$: $a = 6·452$ Å (ref. 12); semiconducting $PtSb_2$: $a = 6·441$ Å (ref. 12). Unfortunately, I was unable to prepare mixed crystals (Pd, Pt) Sb_2 either by melting or by sintering at 500° C. This behaviour seems to be significant because $PdSb_2$ readily forms mixed crystals with the superconductor $AuSb_2$ (a = 6·657 Å (ref. 12)) whereas the semiconducting $PtSb_2$ again does not.

I thank Prof. G. Busch, Swiss Federal Institute of Technology, Zürich, for kind permission to use his Gouy balance, and Dr. P. Giesecke, Cyanamid Stamford Laboratories, for some diffuse-reflectance curves, as well as Dr. E. Mooser and Dr. W. B. Pearson for valuable comments and discussions.

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FORCED CONVECTIVE MASS TRANSFER IN ABSORPTION AND DESORPTION

By P. NORDON

C.S.I.R.O. Division of Textile Physics, Wool Research Laboratories, Ryde, Sydney

WHEN a stream of moist air is forced through a uniform bed of hygroscopic fibres such as wool, water is transferred from the air to the fibres or vice versa depending on the relative humidity of the air and the concentration of water already present in the fibres (regain). Simultaneous heat transfer occurs causing temperature changes in the air and the fibre bed.

It was shown by Cassie[1] that such a process is characterized by the propagation through the bed of two temperature fronts, one fast and one slow. Due to his assumptions of infinitely fast heat and mass transfer rates, Cassie's theory predicted that the shapes of these fronts (with respect to time) were the same in the fibre bed as at the boundary. Later work by MacMahon and Downes[2] elaborated the theory to take into account a finite rate of mass transfer the validity of which had been established[3,4] since the publication of Cassie's original work. In solving the differential equations pertaining to the problem, MacMahon and Downes assumed solutions in the form of fronts travelling through the bed at constant velocity. These assumptions necessarily limited the range of the solutions to cases where stable fronts are formed and to times when the steady state has been established.

Recent work[5] on heat transfer in beds of non-hygroscopic fibres has led to a re-examination of the more general problem of combined heat and mass transfer. For purposes of analysis a theoretical model was formulated also based on a finite rate of mass transfer but differing slightly from that introduced by MacMahon and Downes. Provisions were made in the model to allow for the non-linear isotherm of the wool–water system and for the heat of sorption as a function of regain. Introduction of these factors led to non-linear differential equations which were solved numerically by the method of finite differences with the aid of a computor (*Silliac* digital computor, Basser Computing Laboratory, University of Sydney). Only the main concentration profiles were calculated and their associated temperature changes (second fronts). A number of conclusions were deduced from this model which will be reported elsewhere together with the details of the calculations.

An interesting feature of the calculations is that some insight is gained into the vastly different behaviour of beds of hygroscopic fibres in absorption and desorption. The case examined is that of a wool bed of bulk density 0·1 g cm^{-3} and initially at 20° C through which air is forced, also at 20° C, at the rate of 15 cm^3 sec^{-1}/cm^2 bed cross-section. For absorption, the bed is initially dry and the air saturated at the initial temperature, while for desorption the wool is at saturation regain (33 per cent) and the air dry. Otherwise all conditions are identical, sorption hysteresis being neglected.

As shown in Fig. 1, there is a pronounced difference between the overall sorption and desorption curves calculated with the aid of the model. The rate of desorption is constant over most of the process and finally tapers off. Absorption is initially slightly faster than desorption; however, the rate decreases continually and soon becomes slower than desorption. Experiments with real wool beds yield similar results although the difference between the two cases is not as pronounced as in Fig. 1.

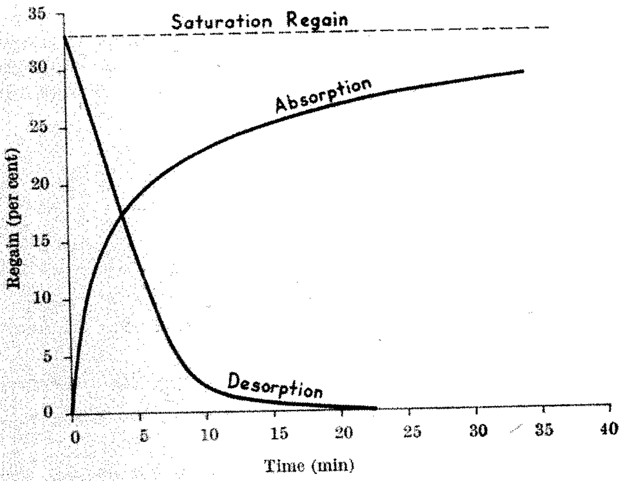

Fig. 1. Overall regain change for a wool bed, 1·5 cm deep during absorption and desorption

A more fundamental difference is revealed when the predicted temperature-time relationships for various bed positions are examined. It becomes apparent that a stable, progressive temperature front is formed in the case of desorption (Fig. 3) but not in the case of absorption (Fig. 2). The general shapes of the curves shown in Figs. 2 and 3 have been verified experimentally.

The explanation for these differences is interesting. For a given small amount of water transferred in the bed, the heat liberated (for absorption) or absorbed (for desorption) is the same and hence the rise or fall in temperature of both wool and air is the same for each case. If it is assumed that the rate of transfer is a function of the difference between the water vapour pressure in the air and the equilibrium vapour pressure of the water in the wool, then the change of temperature which occurs will cause a reduction in the rate of transfer, acting in both cases to reduce the rate. However, due to the exponential relation between the saturation vapour pressure of water and temperature, equal and opposite changes in temperature will not have the same effect in lowering or raising the equilibrium vapour pressure of water in the wool, the effect of an increase in temperature being always greater than that for a decrease. Hence absorption is slowed relatively more than desorption. The effect is cumulative, giving a slower overall absorption rate except at the beginning of the process.

The consequence of this behaviour on the bed as a whole is as follows:

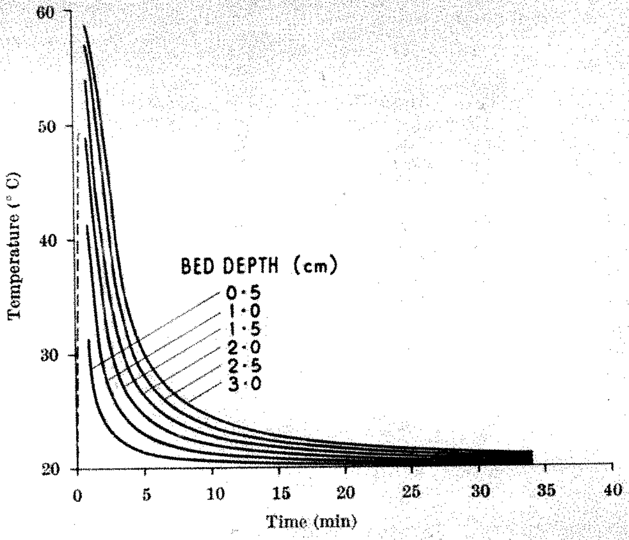

Fig. 2. Temperature at various depths in wool bed during absorption. – – –, approximate course, first front; ——, second front

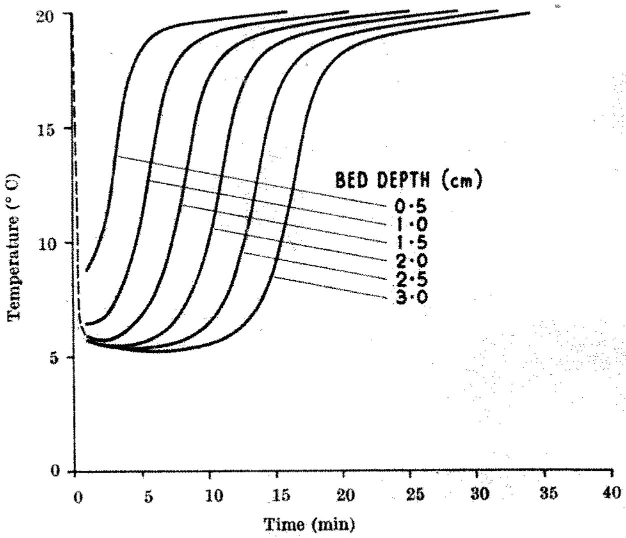

Fig. 3. Temperature at various depths in wool bed during desorption. – – –, approximate course, first front; —— second front

In desorption, so long as the concentration front is contained within the bed, air will emerge from it at an essentially constant temperature, slightly lower than the temperature of adiabatic saturation. Hence during this period the overall rate of desorption remains constant. The emergence of the front from the down-stream side of the bed is an indication that saturation is no longer achieved and the overall rate of desorption becomes smaller. The absorption curve is more complex and a full explanation will not be attempted here. To obtain a constant overall rate, all the moist air entering the bed has to be dried and this requires a much longer bed than that (3 cm) for which the calculation was made. If, therefore, a constant rate is observed at the beginning of absorption, it will last for a much smaller period than the constant rate of desorption for the same bed.

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PHARMACOLOGICAL ACTION OF AJMALINE, THE POSSIBLE MECHANISM OF ITS ANTIARRHYTHMIC ACTION, AND ITS THERAPEUTIC POSSIBILITIES

By PROF. M. L. CHATTERJEE and M. S. DE
Department of Pharmacology, School of Tropical Medicine, Calcutta

AJMALINE is a major indoline alkaloid of *Rauwolfia serpentina* Benth. There are some conflicting reports on its pharmacological action, but gaps still exist in the knowledge, especially concerning its action on peripheral tissues, for example, plain, cardiac and skeletal muscles, and also about its behaviour towards neurotransmitter agents like acetylcholine.

Drugs which inhibit the action of acetylcholine on isolated muscles—smooth, skeletal and cardiac—have, according to Dawes[1], quinidine-like effects on heart. Vane[2] showed that paludrine, which has antiarrhythmic action, satisfies the findings of Dawes[1], and possesses, in addition, properties for potentiating the action of acetylcholine on some isolated tissues in small doses, while it itself shows cholinergic action in higher doses. Burn[3] indicated the role of such drugs on cholinergic receptors.

Antiarrhythmic property was discovered in ajmaline during routine screening by Arora and Madan[4] and this was corroborated by Schmitt and Schmitt later[5]; but the mechanism of its production is not yet known.

There is no information in the literature concerning the effect of ajmaline on the action of the neurotransmitter agents such as acetylcholine, adrenaline, noradrenaline, etc., to enable one to examine the foregoing hypothesis of Dawes[1] or Burn[3]. With the object of examining their views, effects of ajmaline on the action of acetylcholine, adrenaline and noradrenaline have been examined and the results are presented here. A comparison has also been made between these results with ajmaline and that of paludrine, as observed by Vane[2] and Burn and Vane[6], on the action of acetylcholine on isolated muscles.

The results presented here were obtained from the examination on isolated tissue preparations. Isolated guinea pig ileum was prepared and the dose of ajmaline which causes 50 per cent inhibition (ED_{50}) of the contraction due to acetylcholine in this tissue was estimated as suggested by Burn[7]. Seminal vesicles of guinea pigs were isolated after the method of Stone and Loew[8]. Other isolated preparations such as rectus abdominis muscle of frog (*Rana trigrana*), the heart of frog (for perfusion with frog Ringer solution through the sinus venosus) and the auricles of heart of guinea pig were prepared according to the method of Burn[7]. The effect of ajmaline in modifying the normal actions of acetylcholine, adrenaline, noradrenaline, etc., on these tissues was noted.

Ajmaline was dissolved in ethanol to make a 1 per cent solution, which was then suitably diluted with normal saline before use. Ethanol similarly diluted was always tested for control and its effect taken into account to get the true effect of the alkaloid. The neurotransmitter agents tested against were acetylcholine chloride (Hoff-

Table 1. ACTION OF AJMALINE ON SKELETAL, CARDIAC AND SMOOTH MUSCLES

Tissue examined	Conc. of ajmaline (g/ml.)	Effect of ajmaline alone	Effect of ajmaline on the action of acetylcholine (Ach)
Skeletal muscle of frog	10^{-6}	Nil (11/11)	Enhancement of contraction by Ach. Average 24·6 ± 6·38 per cent (6/6)
	10^{-5}	Nil (6/6)	Inhibition of Ach. Average 3·3 ± 2·0 per cent (6/6)
	10^{-4}	Contraction (6/6)	Inhibition of Ach. Average 8·0 ± 3·03 per cent (6/6)
Cardiac muscle (isolated auricles of guinea pigs)	10^{-6}	Rate decreased (11/18)	Rate: inhibition of depression by Ach. (3/4). Average 13·75 ± 6·3 per cent
		Amp. increased (13/18)	Amp.: inhibition of depression by Ach. (3/4). Average 11·75 ± 7·3 per cent
	10^{-5}	Rate decreased (14/18)	Rate: inhibition of depression by Ach. (3/4). Average 38·5 ± 21·85 per cent
		Amp. decreased (9/18)	Amp.: inhibition of depression by Ach. (4/4). Average 30·5 ± 13·3 per cent
Smooth muscle	10^{-6}	Slight increase in tone (24/24)	Enhancement of contraction by Ach. Average 14·0 ± 3·14 per cent (6/6)
	10^{-5}	Contraction (24/24)	Inhibition of contraction by Ach. Average 24·1 ± 5·79 per cent (6/6)
	10^{-4}	Contraction (27/27)	Inhibition of contraction by Ach. Average 42·0 ± 7·4 per cent (6/6)

Figures in brackets indicate the number of times the effect was seen (numerator) out of a total number of observations made (denominator).

Table 2. COMPARATIVE ACTIONS OF PALUDRINE (REF. 2) AND AJMALINE AND THEIR RESPECTIVE EFFECTS ON THE ACTIONS OF ACETYLCHOLINE ON DIFFERENT TISSUES

Tissue examined	Drug	Drug alone		Drug plus acetylcholine			
		Effect	Dose (g/ml.) for the effect	Effect (No. 1)	Dose (g/ml.) for effect (No. 1)	Effect (No. 2)	Dose (g/ml.) for effect (No. 2)
Skeletal muscle (rectus abdominis of *Rana trigrana*)	Paludrine	Contraction	10^{-4}	Enhancement of acetylcholine action	5×10^{-7}	Inhibition of acetylcholine action	10^{-6} to 10^{-4}
	Ajmaline	,,	,,	,,	10^{-6}	,,	10^{-5} to 10^{-4}
Cardiac muscle (on rate and amplitude of beat of isolated auricles)	Paludrine	Inhibition	8×10^{-5}	Antagonization of acetylcholine action	8×10^{-5}	No information on the nicotinic action of acetylcholine	No information
	Ajmaline	,, (Note: amplitude increase at	10^{-5} and above 10^{-6})	,,	10^{-5}	Antagonization of the nicotinic action of acetylcholine	10^{-5}
Smooth muscle of guinea pig ileum	Paludrine	No information	No information	No information	No information	Inhibition of acetylcholine action	No information
	Ajmaline	Contraction	10^{-5} and above	Enhancement of acetylcholine action	10^{-6}	,,	10^{-5} to 10^{-4}

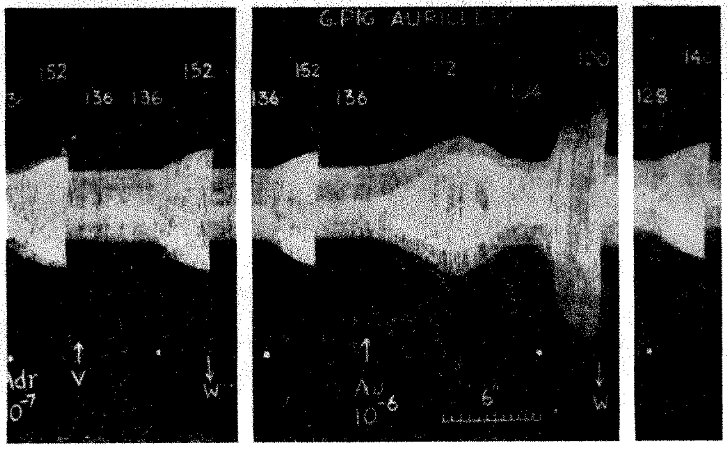

Fig. 1. Action of ajmaline alone and its modifying influence on the action of adrenaline on isolated auricles from guinea pig in 50 ml. bath at 29° C. At dots, adrenaline 10^{-7} g/ml. At *Aj*, ajmaline 10^{-6} g/ml., and at *V*, the corresponding vehicle added to bath, washed down at *W*. Note the increase of amplitude, depression of rate and prevention of cardio-accelerator action of adrenaline by ajmaline

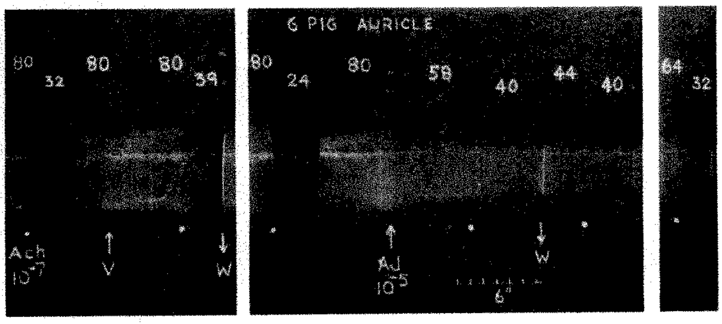

Fig. 2. Same as in Fig. 1 except that, at dots, acetylcholine 10^{-7} g/ml. is given. Note the depression of rate and amplitude by 10^{-5} g/ml. of ajmaline, and its anti-acetylcholine action

man La Roche), adrenaline tartrate (May and Baker) and noradrenaline bitartrate (Winthrop).

The results of the action of ajmaline on isolated muscles are presented in Table 1.

It has been noted that ajmaline has spasmogenic action on isolated smooth muscle preparations like guinea pig ileum and guinea pig seminal vesicles, and on isolated skeletal muscles, for example, frog rectus abdominis. On the other hand, ajmaline has depressive effects on the rate and amplitude of isolated perfused hearts of frogs and guinea pigs. In isolated auricles of both rabbits and guinea pigs, ajmaline depresses the rate but increases the amplitude (Fig. 1). High doses, however, depress both the rate and the amplitude of the auricles. The spasmogenic actions of acetylcholine on smooth and skeletal muscles are enhanced by low doses and antagonized by high doses of ajmaline. The ED_{50} of ajmaline for inhibiting contractions by acetylcholine in isolated guinea pig ileum was found to be $1 \cdot 26 \times 10^{-4}$ g/ml. The depression of rate and of amplitude of isolated auricles of guinea pigs and of isolated frog hearts caused by acetylcholine is antagonized by ajmaline (Fig. 2). The actions of adrenaline and noradrenaline in effecting an increase of the rate (cardio-acceleration) of isolated frog hearts and guinea pig auricles are antagonized by ajmaline, but the increases of amplitude caused by these catecholamines are not inhibited (Fig. 1).

The close similarity of the foregoing effects of ajmaline on the actions of acetylcholine, with those of paludrine as described by Vane[2], is shown in Table 2.

The foregoing results indicate that ajmaline reduces the cardiac rate in the same way as paludrine and other antiarrhythmic drugs, but the difference is that the reduction in rate is accompanied by an increase in amplitude when a lower concentration of ajmaline (10^{-6} g/ml.) is used. It shows, in addition, a specific anti-accelerator action (that is, inhibition of the action of adrenaline and noradrenaline on cardiac rate) which has not been described for paludrine or quinidine. This anti-accelerator property has, however, been shown to be possessed by veratrum alkaloids[9], but the latter has no antiarrhythmic property. This selective property of ajmaline in producing partial inhibition of adrenaline-action, namely, of only the increase of rate and not of amplitude after administration of adrenaline, is an additional factor in ajmaline over and above its antiacetylcholine action on isolated auricles of heart, which is a property common to both ajmaline and quinidine.

From the comparative results presented in Table 2 it is further evident that there is a very close similarity between ajmaline and paludrine so far as their cholinergic, antiacetylcholine and potentiation of cholinergic actions are concerned.

Dawes[1] postulated that drugs which inhibit the action of acetylcholine in smooth, skeletal and cardiac muscles have quinidine-like properties. This postulate was found to be satisfied by paludrine[2], which was also found to possess antiarrhythmic action. The investigation recorded here shows that ajmaline also fulfils these conditions.

Burn[3], in analysing the pharmacological actions of paludrine on isolated skeletal, cardiac and smooth muscles, suggested that drugs like paludrine act on cholinergic receptors and, therefore, can compete for such receptors.

From the point of view of this similarity (Table 2) and also because of the hypothesis of Burn[3] and Dawes[1], the conclusion becomes irresistible that ajmaline, like paludrine, acts on cholinergic receptors and can sensitize these in small concentration to enhance the action of acetylcholine, with which it can also compete at a higher dose-level and can antagonize the action of acetylcholine.

These findings on the action of ajmaline on cardiac functions offer an explanation for its useful therapeutic applicability[10] in cases of cardiac arrhythmias, for example, auricular fibrillation, and offer also a promise for its use in combination with a good antihypertensive agent like reserpine for treatment of cases of hypertension complicated by cardiac irregularities.

These experimental observations also lend support to the clinical finding[11] which shows that the extract of total alkaloids of *Rauwolfia serpentina* is preferable to a single alkaloid such as reserpine. Obviously, the beneficial pharmacological actions of ajmaline or similar alkaloids in the total alkaloid-extracts of *Rauwolfia serpentina* will counteract some of the undesirable effects, such as cardiac depression and even chances of cardiac failure, which have been met with after administration of reserpine alone[12].

We thank Dr. F. Gross, of Ciba, Ltd., and Dr. Debi Chakravarti, of Bethune College, Calcutta, for the supply of ajmaline.

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TRANSPORT OF IONS ACROSS MEMBRANES

A Model for the Transport of Sodium and Potassium Ions

THE chemical reactions between nutriments cause a decrease of these substances and are a source of metabolic end-products in the living cell. As a result, the chemical reactions give rise to continuous vectorial processes with nutriments flowing in and end-products flowing out of the cell. Owing to coupling between processes of the same tensorial order, these processes may produce stationary non-equilibrium distributions even of substances present in the system the amounts of which are not changed by the chemical reactions[1,2]. Regarding the problem of the uneven distribution of Na^+ and K^+ ions in living systems, these considerations call for an examination of the distribution of these ions in stationary-state models that include processes produced by cellular metabolism.

Acids and water are continuously produced by oxidative metabolism and supplied from the cell to the extracellular fluid[2,3]. In the present work, the ion distributions have been investigated in a stationary-state system in which an acid and water are simultaneously supplied to an aqueous solution of sodium chloride and potassium chloride.

The apparatus (Fig. 1) was of the same type as that used by Teorell[3] and by Nims and Thurber[4]. It was made of 'Plexiglas' and consisted of a small chamber (10 ml.) containing a liquid phase α, and a large chamber (1,500 ml.) containing a liquid phase β. Both phases were stirred at constant rates. Between the phases there was a porous membrane ('Membranfilter No. 9', Membranfiltergesellschaft, Göttingen). Its area was 10 cm² and thickness 60μ; the average pore diameter was 125 mμ. The membrane as such was freely permeable to all the species in the aqueous solution of sodium chloride, potassium chloride, and hydrochloric acid.

The experiments were performed at room temperature; during the stationary state the temperature in phase β was 22° ± 1·5° C.

Phase β was composed of an aqueous solution of sodium chloride and potassium chloride. The system was brought to 'life' by filling the small chamber with an aqueous solution of hydrochloric acid and supplying this fluid continuously to phase α with hydrostatic pressure. The hydrochloric acid flowing through the membrane to phase β was neutralized to pH 7 by continuous addition of an aqueous solution of sodium hydroxide and potassium hydroxide to this phase. As a guide of its neutrality phase β contained a few drops of an indicator (bromothymol blue). The dilution effect of the water flowing to phase β was cancelled out by making the concentrations of Na^+ and K^+ in the base solution just so much higher than those in phase β that, when the acid solution was neutralized, the resulting solution had the same composition as the initial solution of phase β. The hydrostatic pressure difference between the levels of the acid solution and of phase β was kept constant by continuous addition of solution to the acid bottle and removal of an adequate volume from phase β. Thus there were steady flows of hydrochloric acid and water from phase α through the membrane to phase β.

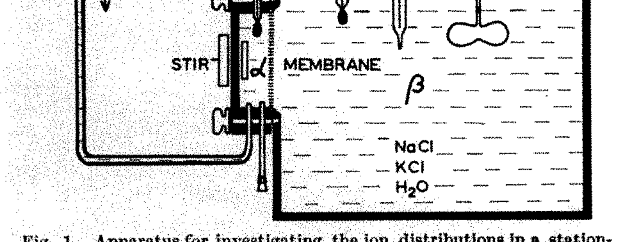

Fig. 1. Apparatus for investigating the ion distributions in a stationary-state system produced by the continuous supply of an acid and of water to the aqueous solutions of sodium chloride and potassium chloride

Against the flows of hydrochloric acid and water, Na^+ and K^+ migrated from phase β to phase α, until a time-independent non-equilibrium stationary-state distribution of all species in the system was attained. The time course towards this state was followed by measuring the potential difference between Ag/AgCl electrodes in the two chambers with a compensation potentiometer. Its decline to a constant value took 1·5–2 h. By taking successive samples from phase α, it was also found that within this time the concentrations of Na^+ and K^+ had increased and that of H^+ decreased to constant levels in this phase. The actual time for the attainment of the stationary state was taken as 3 h. The concentration of H^+ was titrated with sodium hydroxide, while those of Na^+ and K^+ were determined with a flame photometer.

The selectivity coefficient, r, for Na^+ and K^+ between the two phases was calculated as follows:

$$r = [Na^+]_\beta \cdot [K^+]_\alpha / [Na^+]_\alpha \cdot [K^+]_\beta$$

The flow rates of hydrochloric acid and water were changed in different experiments by changing the normality of the hydrochloric acid and the hydrostatic pressure difference between the levels of the acid solution and phase β. Since the normality and volume of acid solution lost in a given time were known, the rates of flow of hydrochloric acid and water through the membrane could be calculated.

Table 1 shows the stationary-state ion distributions as a function of various flow rates of hydrochloric acid and water. The experiments Nos. 3 and 10 represent extreme cases. In experiment No. 3, hydrochloric acid of high normality was supplied to phase α at low hydrostatic pressure, with the result that the distribution of other ions is determined chiefly by the diffusion effect of hydrochloric acid. This experiment resembles those of Teorell[3]. In this system the diffusion potential, arising from the low mobility of Cl^- in relation to that of H^+, leads to the accumulation of Na^+ and K^+ in phase α. However, the selectivity coefficient is low, that is, the distributions of Na^+ and K^+ are equally influenced. On the other hand, in experiment No. 10, hydrochloric acid of low normality is supplied to phase α at high hydrostatic pressure, with the result that the ion distributions are determined chiefly by the water flow. This experiment resembles those of Nims and Thurber[4]. This system increases K^+/Na^+ ratio in phase α, but the concentrations of both ions are lower in this phase than in phase β.

However, with simultaneous flows of hydrochloric acid and water, Na^+ and K^+ migrate continuously in the membrane pores with accelerated rates[5] against the reverse water flow, and since the mobility of K^+ is higher than that of Na^+, the final stationary-state concentration of K^+ may become even higher in phase α than in phase β,

Table 1. THE ION DISTRIBUTIONS IN A STATIONARY-STATE SYSTEM PRODUCED BY THE CONTINUOUS FLOWS OF AN ACID AND OF WATER TO THE AQUEOUS SOLUTION OF SODIUM CHLORIDE AND POTASSIUM CHLORIDE

Exp. No.	HCl solution (N)	Pressure (cm/H_2O)	Flow rates		Phase α (m.equiv./l.)			Ag/AgCl P.D. (mV)	r.
			HCl (m.equiv./min)	Water (ml./min)	(Na^+)	(K^+)	(H^+)		
1	1·6	4	0·049	0·030	175	7·4	140	36	1·27
2	3·1	4	0·074	0·024	206	8·8	195	48	1·28
3	6·4	4	0·140	0·022	272	12·0	360	71	1·33
4	1·6	30	0·85	0·53	17	1·7	1,250	123	3·00
5	3·1	18	0·85	0·27	80	5·2	1,550	130	1·95
6	6·2	12	0·96	0·15	120	8·4	1,990	165	2·10
7	6·1	16	1·22	0·20	99	7·3	2,400	173	2·20
8	6·2	18	1·36	0·22	93	7·2	2,400	180	2·32
9	3·2	30	1·67	0·52	21	2·4	2,200	156	3·43
10	1·9	59	2·22	1·17	8	0·8	1,700	143	3·08
11	3·2	50	2·46	0·77	10	1·2	2,500	163	3·60
12	6·2	30	3·10	0·51	41	4·9	3,900	201	3·58

In all the experiments phase β consisted of an aqueous solution of sodium chloride (150 m.equiv./l.) and of potassium chloride (5 m.equiv./l.). The Ag/AgCl electrode in phase α was negative.

while that of Na^+ remains lower in phase α than in phase β (exps. Nos. 6, 7 and 8).

Thus, this supply of hydrochloric acid and water leads to the transport of Na^+ and K^+ ions in opposite directions across the membrane; K^+ ion to phase α and Na^+ ion to phase β.

Physico-chemically this model deals with the problems of polycomponent processes. The recent progress of irreversible thermodynamics has facilitated the theoretical treatment of these systems. An attempt along these lines has been made in the following paper[6].

Regarding the possible biological usefulness of the present 'ion-pump' model, it is to be noted that the anions of an acid supplied to the system, which in the present model are Cl^- ions, are heterogeneous in living systems, including slow-moving polyanions. This is of interest, since the ability of the present model to accumulate K^+ depends on the relatively low mobility of Cl^- and not on the chemical interactions between the ions involved. It is true that natural anions can bind alkali ions, even with a certain selectivity, but as found so far, this binding seems to be too low to alone explain the distribution of these ions in living systems[7,8]. Concerning the other significant factor in the present experiments, that is, water movement, it is known that the cell membrane is relatively freely permeable to water and that the latter is apparently distributed between the cell and the extracellular fluid according to its chemical potential[8]. However, it is a fact that water, besides acids, is an end-product of oxidative metabolism. Thus there must exist places in which these substances are supplied to the system. Probably we may imagine that there are in the cell membrane different sites for the distribution of water and other uncharged substances on one hand, and for the 'ion-pumps' on the other.

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SIMO SALMINEN

Wihuri Research Institute,
Helsinki.

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Stationary Distribution of Ions across a Membrane in the System Sodium Chloride – Potassium Chloride – Hydrochloric Acid – Water

THE transport of ions across membranes has been treated theoretically by purely phenomenological methods, using the formalism of irreversible thermodynamics[1-3], or by a more 'kinetic' approach[4]. The former method gives a clear description of the transport processes, but in the general case it leads to equations containing unknown constants which depend on the properties of both the solutions and the membrane. Therefore the equations obtained are not applicable to numerical solutions of concrete problems.

However, if the membrane does not affect the properties of the solution, it is possible, in certain simple cases, to calculate approximately the ion distributions across the membrane, and to obtain relations between measurable quantities. It has already been suggested by Simo Salminen[5] that the system in which an acid and water are continuously supplied to the aqueous solution of sodium chloride and potassium chloride may be of interest as a model of biological ion transport systems. In the present work the ion distributions in this system have been studied theoretically with the aid of the concepts of irreversible thermodynamics.

Consider the isothermal system (Fig. 1) containing two homogeneous phases α and β, which are joined by a capillary k. The capillary diameter is large when compared with molecular dimensions, thus corresponding to the pores of the membrane used[5]. To the constant-volume part α a constant flow of aqueous solution of hydrochloric acid is supplied. The concentration of the hydrochloric acid is denoted by c_3^0. The compartment β contains an aqueous solution of sodium chloride and potassium chloride. The concentrations of these salts in the compartment β, c_1^β and c_2^β, respectively, are kept constant. Let us consider the system in a stationary state in which the concentrations of the components sodium chloride, potassium chloride and hydrochloric acid in compartment α are denoted by c_1^α, c_2^α and c_3^α respectively. It is assumed that the concentrations of the components sodium chloride, potassium chloride and hydrochloric acid in the capillary are continuous functions of the distance x, but are independent of the other spacial co-ordinates.

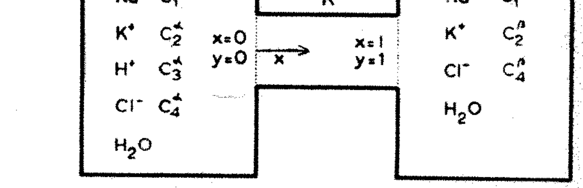

Fig. 1. Schematic representation of the system

The species Na^+, K^+, H^+, Cl^- and water are denoted by the suffixes 1, 2, . . . 5. In an isothermal system in quasi-mechanical equilibrium the flows of the species J_i may be presented in the form:

$$J_i = c_i(v_i - v_5) = \sum_{j=1}^{4} \alpha_{ij} X_j = -\sum_{j=1}^{4} \alpha_{ij}(\nabla \mu_j + z_j F \nabla \varphi) \quad (1)$$

where v_i is the velocity of the species i The conjugate force X_i is assumed to have been composed of two parts, the gradient of the 'chemical potential' μ_i and the gradient of the 'electrical potential' φ multiplied by the charge of the ion z_i and by the Faraday constant F The phenomenological coefficients α_{ij} are connected by the Onsager reciprocal relations

$$\alpha_{ij} = \alpha_{ji} \quad (2)$$

The electric current density is

$$I = F \sum_{i=1}^{4} z_i c_i v_i \quad (3)$$

The specific conductance is defined by the equation

$$I = \varkappa E \quad (4)$$

when

$$\nabla \mu_i = 0 \quad (5)$$

where E is the electric field strength

The mobilities of the ions and their transference numbers are defined by the equations

$$I t_i = z_i F J_i \quad (6)$$

$$(v_i - v_5) = \varepsilon_i u_i E \quad (7)$$

For cations $\varepsilon_i = +1$ and for anions $\varepsilon_i = -1$

From equations (1) to (7) we obtain

$$\varkappa = F \sum_{i=1}^{4} |z_i| \; c_i u_i = F^2 \sum_{i,j=1}^{4} z_i z_j \alpha_{ij} \quad (8)$$

$$u_i = c_i^{-1} F \varepsilon_i \sum_{j=1}^{4} z_j \alpha_{ij} \quad (9)$$

$$t_i = \frac{|z_i| c_i u_i}{\sum_{j=1}^{4} |z_j| c_j u_j} = \frac{z_i \sum_{j=1}^{4} z_j \alpha_{ij}}{\sum_{j,k=1}^{4} z_j z_k \alpha_{jk}} \quad (10)$$

In the present system $I = 0$, or according to (3)

$$\sum_{i=1}^{4} z_i J_i = 0 \quad (11)$$

As a consequence of (11) it is possible to use diffusion currents of the components sodium chloride, potassium chloride and hydrochloric acid instead of the diffusion currents of the corresponding ions

The chemical potentials of these compounds are

$$\mu_{\text{NaCl}} = \mu_1^4 = \mu_1 + \mu_4 \quad \mu_{\text{KCl}} = \mu_2^4 = \mu_2 + \mu_4$$
$$\mu_{\text{HCl}} = \mu_3^4 = \mu_3 + \mu_4 \quad (12)$$

For the diffusion current densities of the components the following phenomenological equations are valid

$$\begin{aligned} -J_{\text{NaCl}} &= -J_1^4 = a_{11}\nabla\mu_1^4 + a_{12}\nabla\mu_2^4 + a_{13}\nabla\mu_3^4 \\ -J_{\text{KCl}} &= -J_2^4 = a_{21}\nabla\mu_1^4 + a_{22}\nabla\mu_2^4 + a_{23}\nabla\mu_3^4 \quad (13) \\ -J_{\text{HCl}} &= -J_3^4 = a_{31}\nabla\mu_1^4 + a_{32}\nabla\mu_2^4 + a_{33}\nabla\mu_3^4 \end{aligned}$$

where the constants a_{ij} satisfy the Onsager reciprocal relations

$$a_{ij} = a_{ji} \quad (14)$$

According to equations (1), (2), (8), (10)–(13), the following relation between the phenomenological coefficients (2) and (14) is obtained

$$a_{ij} = \alpha_{ij} - t_i t_j \frac{\varkappa}{F^2} \quad (15)$$

The chemical potentials of the components μ_i^4 may be expressed by the equation

$$\mu_i^4 = \mu_i^4(T\,P, c_1\,c_2, c_3) = \mu_i^{4\circ}(T,P) + RT\ln c_i c_4 + 2RT\ln y_i^4 \quad (16)$$

where y_i^4 is the mean molar activity coefficient

Using the notation

$$\mu_{ij}^4 = \frac{\partial \mu_i^4}{\partial c_i^4} \quad (17)$$

from (13), (16) and (17), we obtain

$$\begin{aligned} -J_1^4 &= D_{11}\nabla c_1 + D_{12}\nabla c_2 + D_{13}\nabla c_3 \\ -J_2^4 &= D_{21}\nabla c_1 + D_{22}\nabla c_2 + D_{23}\nabla c_3 \quad (18) \\ -J_3^4 &= D_{31}\nabla c_1 + D_{32}\nabla c_2 + D_{33}\nabla c_3 \end{aligned}$$

where

$$D_{ij} = \sum_{l=1}^{3} a_{il}\mu_{lj}^4 = RT\sum_{l=1}^{3} \frac{a_{il}}{c_l}\left(\delta_{lj} + \frac{c_l}{c_4} + 2c_l \frac{\partial \ln y_l^4}{\partial c_j}\right) \quad (19)$$

δ_{lj} being the Kronecker symbol

If we introduce the following approximations

$$\alpha_{ij} = 0 \; (i \neq j) \quad (20)$$

$$\alpha_{ii} = \frac{c_i u_i^0}{F} = \frac{c_i \lambda_i^0}{F^2} \quad (21)$$

$$\frac{\partial \ln y_i}{\partial c_j} = 0 \quad (22)$$

where $\lambda_i^0 = F u_i^0$ and u_i^0 is the limiting ionic mobility. (8)–(10) (15), and (19)–(22) yield

$$D_{ij} = \frac{RT}{F^2} \lambda_i^0\left(\delta_{ij} - \frac{c_i}{\sum_{i=1}^{4} c_i \lambda_i^0} \; (\lambda_j^0 - \lambda_4^0)\right) \quad (23)$$

By solving the equations (18) for the concentration gradients we obtain

$$\begin{aligned} \frac{dc_1}{dx} &= -\frac{1}{D}(D_1^1 J_1^4 + D_2^1 J_2^4 + D_3^1 J_3^4) \\ \frac{dc_2}{dx} &= -\frac{1}{D}(D_1^2 J_1^4 + D_2^2 J_2^4 + D_3^2 J_3^4) \quad (24) \\ \frac{dc_3}{dx} &= -\frac{1}{D}(D_1^3 J_1^4 + D_2^3 J_2^4 + D_3^3 J_3^4) \end{aligned}$$

where the assumption that the concentrations c_i are functions of x alone has been taken into consideration In the equations (24) D is the determinant

$$D = |D_{ij}| \quad (25)$$

and D_i^j is the co-factor of the element D_{ij}

Since $c_4 = c_1 + c_2 + c_3$ we may write instead of the last equation of (24)

$$\frac{dc_4}{dx} = -\frac{1}{D}\left(\sum_{i=1}^{3} D_1^i J_1^4 + \sum_{i=1}^{3} D_2^i J_2^4 + \sum_{i=1}^{3} D_3^i J_2^4\right) \quad (26)$$

In the stationary state under consideration we have

$$v_1 = v_2 = 0 \quad (27)$$

From the equations (1) and (24)–(27) we obtain the system of differential equations

$$\begin{aligned} \frac{dc_1}{dx} &= c_1\left(A_1 + B_1\frac{c_1}{c_4} + B_2\frac{c_2}{c_4} + E\frac{1}{c_4}\right) \\ \frac{dc_2}{dx} &= c_2\left(A_2 + B_1\frac{c_1}{c_4} + B_2\frac{c_2}{c_4} + E\frac{1}{c_4}\right) \quad (28) \\ \frac{dc_4}{dx} &= c_4\left(\frac{v_5}{D_{33}} - B_1\frac{c_1}{c_4} - B_2\frac{c_2}{c_4} - \frac{c_3 v_3}{B_{33}^0}\right) \end{aligned}$$

where

$$A_i = v_5\left(\frac{2}{D_{ii}^0} - \frac{1}{D_{33}^0}\right), \quad B_i = v_5\left(\frac{1}{D_{33}^0} - \frac{1}{D_{ii}^0}\right) \quad (i = 1,2) \quad (29)$$

$$E = c_3 v_3 \frac{F^2}{2RT}\left(\frac{1}{\lambda_3^0} - \frac{1}{\lambda_4^0}\right), \quad D_{ii}^0 = \frac{2RT}{F^2}\left(\frac{1}{\lambda_i^0} + \frac{1}{\lambda_4^0}\right) \quad (i = 1,2\,3)$$

In the simplified case, when $c_3 = 0$, it follows from the equations (28) and (29) that

$$D^0_{11}\mathrm{d}\ln c_1c_4 = 2v_5\mathrm{d}x = D^0_{22}\mathrm{d}\ln c_2c_4 \tag{30}$$

Integration from $x = 0$ to $x = l$ yields

$$D^0_{11}\ln\frac{c_1^\beta c_4^\beta}{c_1^\alpha c_4^\alpha} = D^0_{22}\ln\frac{c_2^\beta c_\beta^4}{c_2^\alpha c_4^\alpha} \tag{31}$$

Nims and Thurber[6] have studied ion distributions across a membrane in a sodium chloride–potassium chloride–water system and Nims[7] has derived an equation for the distribution of Na+ and K+ ions in this case. The equation (31) is the same as the equation (35) in Nims's paper[7], if the activity coefficients are left out of consideration.

Returning to the quaternary system, the first two equations (28) yield

$$\frac{\mathrm{d}\ln\frac{c_1}{c_2}}{\mathrm{d}x} = 2\left(\frac{1}{D^0_{11}} - \frac{1}{D^0_{22}}\right)v_5 \tag{32}$$

Integrating (32) from $x = 0$ to $x = l$ we obtain

$$\ln r = \ln\frac{\frac{c_1^\beta}{c_2^\beta}}{\frac{c_1^\alpha}{c_2^\alpha}} = 2\left(\frac{1}{D^0_{11}} - \frac{1}{D^0_{22}}\right)v_5 l \tag{33}$$

From the equation (33) it will be seen that the selectivity coefficient[5], r, depends on the velocity of water v_5 and on the length of the capillary l, but not, with the approximations (20)–(22), on the concentration of hydrochloric acid.

The coefficients (29) in the equations (28) may be calculated from the known mobilities u_i^0, but the velocities v_3 and v_5 are not experimentally known quantities.

In a stationary state, the amount of hydrochloric acid supplied to the part α equals the amount escaping through the capillary k, and we may write

$$c_3v_3 = c_3^0v_5 \tag{34}$$

A new variable is defined as

$$y = \frac{x}{l} \tag{35}$$

Substitution of equations (33)–(35) and the values[8] of the ionic equivalent conductivities at 25° C λ_i^0 (cm² ohm⁻¹ equiv.⁻¹) $\lambda_1^0 = 50.10$, $\lambda_2^0 = 73.50$, $\lambda_3^0 = 349.8$, $\lambda_4^0 = 76.35$ in the equation (28) yields

$$\begin{aligned}
\frac{\mathrm{d}c_1}{\mathrm{d}y} &= -\ln r\, c_1\left(3.943 - 1.345\frac{c_1}{c_4} - 0.845\frac{c_2}{c_4} - 0.803\frac{c_3^0}{c_4}\right)\\
\frac{\mathrm{d}c_2}{\mathrm{d}y} &= -\ln r\, c_2\left(2.943 - 1.345\frac{c_1}{c_4} + 0.845\frac{c_2}{c_4} - 1.253\frac{c_3^0}{c_4}\right) \qquad (36)\\
\frac{\mathrm{d}c_4}{\mathrm{d}y} &= -\ln r\, c_4\left(1.253 + 1.345\frac{c_1}{c_4} + 0.845\frac{c_2}{c_4} - 1.253\frac{c_3^0}{c_4}\right).
\end{aligned}$$

The system of differential equations (36) is not solvable in closed form. The equations can be integrated numerically, however, when the values of $\ln r$, c_3^0, and of the c_i's on one of the boundaries are known. In the experiments[5], the boundary conditions to the right of the membrane were

$$c_1^\beta = 150 \text{ mC},\ c_2^\beta = 5 \text{ mC},\ c_4^\beta = 155 \text{ mC}$$

The concentration of hydrochloric acid c_3^0 and the rate of water flow were varied. The distribution ratio r is, in turn, a function of the water flow, according to equation (33). The numerical integration of the differential equation system (36) was made by the Runge–Kutta method, using an Elliot 803 electronic digital computor. The results for the values $r = 1.1$, 1.3, 2.0, 3.0 and $c_3^0 = 1.6$, 3.0, 6.0 mol./l. are given graphically in Fig. 2, where the stationary-state concentrations of Na+, K+ and Cl− ions in the capillary are presented as functions of the length of the capillary.

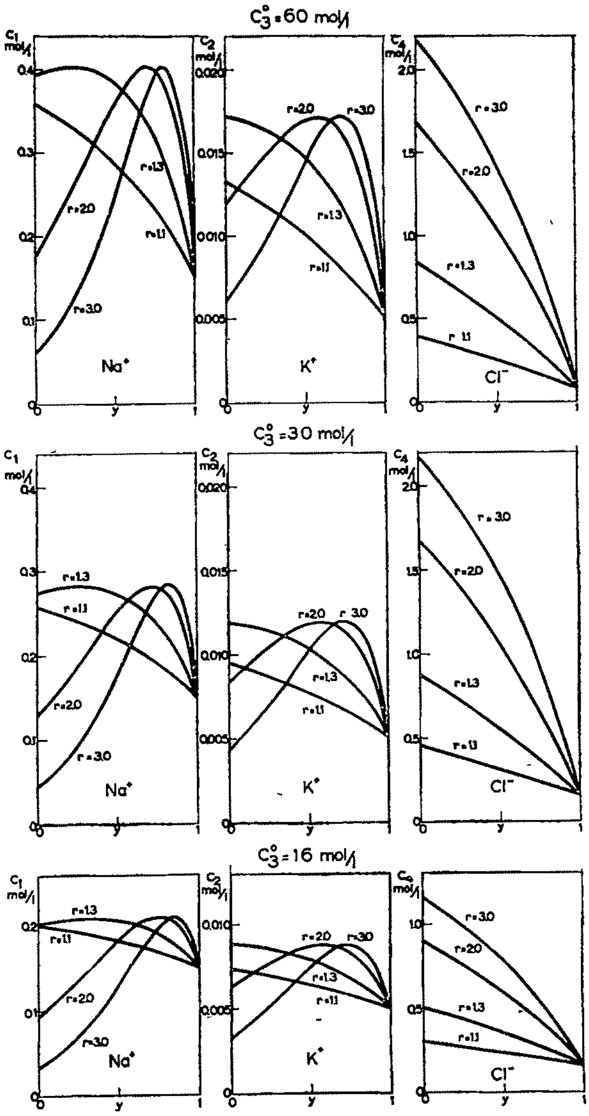

Fig. 2. Stationary state distribution of Na+, K+ and Cl− ions in the capillary

The values at the left end of the capillary represent the calculated ion concentrations in compartment α. If we take into consideration the rather rough approximations (20)–(22), the agreement between the present theoretical and previous experimental[5] results is fairly good.

The selectivity coefficient r depends, as was previously pointed out, only on the rate of water flow. The relation between the selectivity coefficient and the water flow depends, according to (33), on the length and effective cross-section of the capillary and is thus a property of the membrane and usually unknown. On the other hand, the ratios c^α/c_1^β and c_2^α/c_2^β depend on the concentration of hydrochloric acid as well.

From Fig. 2 and from the equations (36) it may be seen that the concentration maxima of Na+ and K+ inside the capillary are independent of the ratio r, that is, of the water flow. The maxima increase with increasing concentration of hydrochloric acid. They are relatively greater and lie more to the left for K+ than for Na+.

As the water flow rate is increased, the stationary concentrations of Na+ and K+ in compartment α at first increase and reach their maxima, Na+ at a lower rate of flow than K+ With a further increase of the water flow rate then concentrations again decrease

Furthermore, it may be seen from Fig 2 that at certain water flow rates and hydrochloric acid concentrations a stationary state may be reached in which the K+ concentrations in compartment α are higher and the Na+ concentrations lower than the corresponding concentrations in compartment β, as has also been verified experimentally[5] Thus the present system acts as an ion 'pump' which transports the Na+ and K+ ions in opposite directions across the membrane against their concentration gradients Consequently, this system is a rational demonstration of a type of ion transport which in biology has often been termed active transport of ions

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Aarne Ekman
Jussi Rastas

Laboratory of Physical Chemistry,
Institute of Technology,

Simo Salminen

Wihuri Research Institute,
Helsinki

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RECOVERY EFFECTS OF HOMOLOGOUS TESTES DNA INJECTED INTO TOTAL-BODY IRRADIATED (600 R) INFANTILE RATS AND MICE

By Miss N. SAVKOVIĆ

'Boris Kidrič' Institute of Nuclear Sciences, Belgrade

THE problem of discovering remedies for post-irradiation treatment of irradiated organisms is of importance The therapeutical results are theoretically important because the solution of this problem would render more comprehensive the mechanism of lesion formation caused by irradiation From the practical point of view, the problem is important because appropriate therapeutic remedies could be used in clinical radiotherapy

It has been shown that in different animals the effects of lethal radiation can be modified by certain treatments applied after irradiation Some results show that irradiated animals can recover after being treated with the bone marrow[1,2] and homogenate spleen[3] Cole *et al*[4] found that extracts of blood-forming tissues are therapeutically efficient They assume that the humoral factor is identical with deoxyribonucleoproteins

Kanazir *et al* assume that damage and mortality of irradiated organisms are due to damage of the genetic system In view of the fact that the genes are composed of DNA molecules, the primary initial lesions induced by radiation can cause damage of the DNA and RNA structure[5]

It is also assumed that the recovery process and the survival of the radiation syndrome after irradiation depends on the metabolism structure and the biological function of nucleic acids It was on this basis that I investigated the effect of DNA on the irradiated organism These experiments have given positive results The work of D Kanazir[6–8] has shown that DNA and RNA administered after exposure to lethal X-irradiation protects adult irradiated organisms Spleen and liver DNA have proved to be most efficient Histological analyses of the spleen, bone marrow[9], digestive tract, liver[10] and the adrenal gland[11] made by D Kanazir *et al* showed that damage to lethally irradiated and DNA treated animals is less than in the controls Other authors have also shown that nucleic acids administered after lethal irradiation increase the survival percentage of rats, mice and bacteria[12–14]

The toxic effect of these natural products after short treatment is minimum

The literature, however, does not provide any data on the restorative effect of DNA on animals irradiated in the infant period

I have investigated whether homologous testes DNA in sexually mature rats and mice exerts influence on the radiation syndrome and the survival of rats and mice irradiated during the infant period

White male Wistar rats and *C57Bl* male mice 8 and 17 days old were used in the experiments The animals were exposed to an X-ray dose of 600 r A Siemens Stabilipan apparatus was used under the following conditions 200 kV , 14 m amp , aluminium 3, 150 r /min at a target distance of 46 cm The animals were irradiated in wooden boxes and were then returned to their unirradiated mothers

Immediately after irradiation the animals were treated with DNA extracted from the testes of sexually mature animals (2 5 months old) The extraction was performed by the Zamenhof method[15] Each irradiated male animal was administered the quantity of DNA extracted from one pair of testes It was injected intraperitoneally in fractions for 48 h The first fraction was injected immediately after irradiation

Fig 1 shows the results of the survival of rats irradiated at early ages

The amount applied per rat varied from 0 940 to 1,300 mg

Rats, 8 days old, irradiated and treated with DNA (after irradiation) showed a 75 per cent (±7 0) survival, while only 8 per cent (±2 3) of untreated controls survived

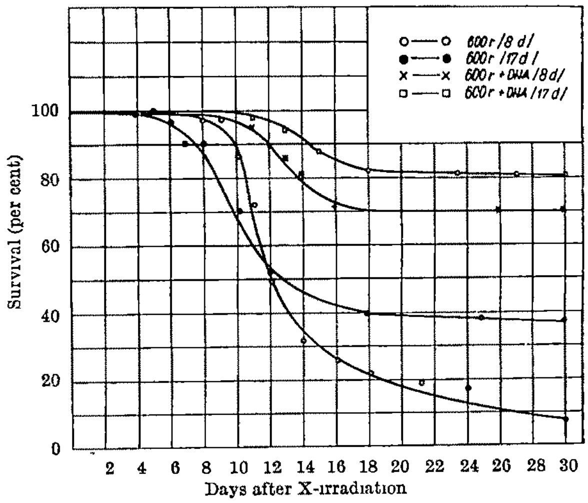

Fig 1

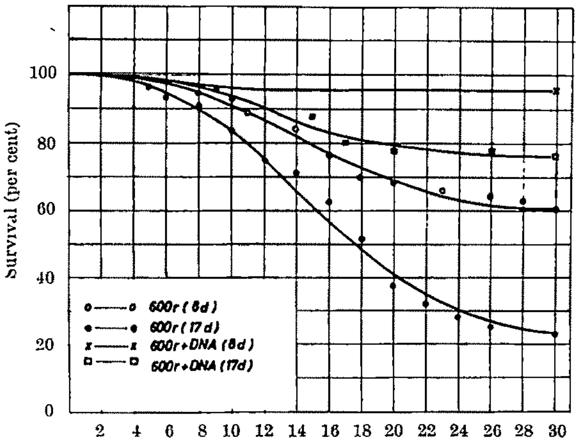

Fig 2

irradiation, the survival effect was observed in 82 treated animals and 100 controls This indicates that DNA reduces the radiation effect by a factor of 8 7 ($P < 0\ 001$)

Rats, 17 days old, irradiated and treated with DNA showed a 70 per cent (± 3 4) survival, while the controls showed 37 per cent (± 3 5), the effect was observed in 79 treated animals and 100 controls DNA reduces the effect by a factor of 2 ($P < 0\ 001$)

Fig 2 shows the restorative effect of testes DNA on the survival of mice irradiated in the infantile period

The amount of DNA was 1,500 mg

The results obtained show that DNA injected into mice irradiated at the infant stage also conditions a high degree of survival

(1) In irradiated-treated 8-day-old mice the survival was 95 (± 4 per cent) and in controls it was 60 (± 7 3) per cent, the number of animals was 40 and 100, respectively The results are statistically significant ($P < 0\ 01$)

(2) Mice which were 17 days old at the moment of irradiation and were treated with DNA after irradiation showed a 76 (± 3) per cent survival Controls of the same age showed a 23 (± 9 0) per cent survival, 58 and 80 animals, respectively, were observed These results are also statistically very significant ($P < 0\ 001$)

These results show that the administration of testes DNA increases the percentage of survival in both rats and mice in the infantile period These results are, therefore, more reliable because these two types of animals do not react in the same way Namely, 8-day-old rats are more sensitive to radiation and their percentage of survival is lower, whereas 17-day-old rats are more resistant and their percentage of survival is higher The behaviour of mice, however, is quite different 17-day-old mice are more sensitive than 8-day-old ones We also observed this phenomenon in our experiments with mice to which we gave chemical protectors[16]

Quastler also found that 1-week-old mice are more resistant to radiation than adult animals According to Ingbaru the sensitivity increases in the course of the first weeks of life, reaching the maximum one month after birth, and slightly decreasing in the following weeks[17]

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CHEMOSTATIC REGULATION OF APPETITE IN SHEEP

By J. M. HOLDER

Agricultural Research Station, Wollongbar, New South Wales

THE presence of hypothalamic centres associated with regulation of voluntary intake of food has been clearly demonstrated in many species[1] There is still considerable conjecture as to the mechanisms by which the so-called 'appetite' (lateral hypothalamic) and 'satiety' (ventro-medial hypothalamic) centres are, in the short term, stimulated or depressed Various suggestions have included the influence of specific dynamic action of food eaten[2], of osmotic effects of the diet[3], of sensory impulses from the digestive tract[4], and the concentration of certain metabolites in the blood passing through the brain centres[5,6]

Although evidence available[5,7,8] suggests an important role for glucose utilization by hypothalamic centres in monogastric appetite control, there is little to suggest that alterations in the level of this metabolite will be of importance in ruminants Blood glucose-levels in normal cattle and sheep are remarkably constant with little fluctuation associated with feeding[9] Manning *et al* [10] produced significant increases in the blood sugar of adult ewes by injection of 200–1,000 ml 50 per cent glucose solution in two equally divided doses, 60 min apart However, in the 60 min following the second injection, the appetite of their ewes was not affected when compared with controls They suggest that blood volatile fatty acids would be more likely to influence hypothalamic centres in the sheep because levels of blood acetate, particularly, rise following feeding and then gradually fall until the next meal[11] In support of this hypothesis, Dowden and Jacobson[12], with continuous infusion over 8 h of acetic acid, sodium acetate or propionic acid, produced highly significant depression in voluntary intake of lucerne hay by monozygous twin heifers during the infusion period Appetite apparently returned shortly after the infusion ceased

As part of an investigation of factors affecting the voluntary intake of food by ruminants, the role of circulating volatile fatty acids and of glucose in appetite regulation in the sheep has been assessed Merino ewes and wethers were trained to eat in 2 h the food offered to them each day Intravenous infusion of test solutions usually commenced at least 2 h before the time the sheep would normally have been fed To eliminate variation between animals in daily food intake, each sheep served as its own control, in receiving a continuous infusion of normal saline on the day prior to or succeeding the test infusion

Table 1 EFFECT OF INTRAVENOUS INFUSIONS OF GLUCOSE OR SODIUM ACETATE ON APPETITE OF SHEEP

Series	Sheep	Weight (kg)	Infusion solution	Duration infusion (min)	Feed Offered (g)	Feed Consumed (g)	Maximum plasma-level of metabolite achieved Glucose (mg/100 ml)	Maximum plasma-level of metabolite achieved Volatile fatty acids (m mol/l)
1	1♀	40 4	N saline	210	600 lucerne + 200 oats at 155 min	All by 200 min	89	0 81
			40% acetate	210		All by 180 min	60	2 22
	2♀	31 3	N saline	210	600 lucerne at 155 min	576 by 270 min	65	0 88
			40% acetate	210		583 by 270 min	64	2 44
2	3☿	43 4	N saline	210	800 lucerne at 143 min	550 by 263 min	62	—
			25% glucose	210	,	790 by 263 min	85	—
3	4☿	35 4	N saline	210	800 lucerne at 155 min	790 by 275 min	69	—
			40% glucose	210	As above at 150 min	All by 270 min	126	—
4	4☿	39 0	N saline	480	1,500 lucerne at 0 min	1,280 by 480 min	—	1 62
			40% acetate	480	,	810 by 480 min	—	1 63
			N saline	480	,	940 by 480 min	—	Not recorded
5	5☿	39 0	40% acetate	480	1,500 lucerne at 0 min	640 by 480 min	—	1 89
			N saline	480	,	630 by 480 min	—	1 45
			40% acetate	480	,,	960 by 480 min	—	Not recorded

All infusions were given at the rate of 1/3 ml per min by a slow injection apparatus (C F Palmer Ltd , London) The effect on appetite of infusions of 25 or 40 per cent glucose or 40 per cent sodium acetate solutions over 210-min periods is given in Table 1

On no occasion was there any apparent effect on voluntary intake caused by infusion of the glucose solutions Blood glucose-levels did not reach those achieved by Manning *et al* , but the continuous infusion of 40 per cent glucose produced venous levels approximately twice the normal values during most of the feeding period Sodium acetate infusions over 210 min did not produce a fall in intake despite blood volatile fatty acid levels almost three times the normal range This result is quite the opposite to that obtained by Dowden and Jacobson with their heifers, despite the relatively far greater quantity of acetate infused into the sheep

There was a possibility that the experimental sheep in being trained to expect their feed at a fixed time each day may have had the action of eating sufficiently 'imprinted' to eat despite the raised levels of metabolite To examine this possibility sheep were offered lucerne chaff *ad libitum* Weighing of residues and refeeding occurred daily at 0900 h An infusion of 40 per cent sodium acetate commenced at feeding and continuing for 8 h had no apparent effect on voluntary intake over this period, in one series (No 5, Table 1) In a second series (No 4) there was a fall in intake over the acetate infusion period, compared with the saline infusion the previous day However, a check saline infusion on the day following the acetate, produced a similar fall in intake, suggesting that the appetite depression was provoked by factors other than the acetate solution

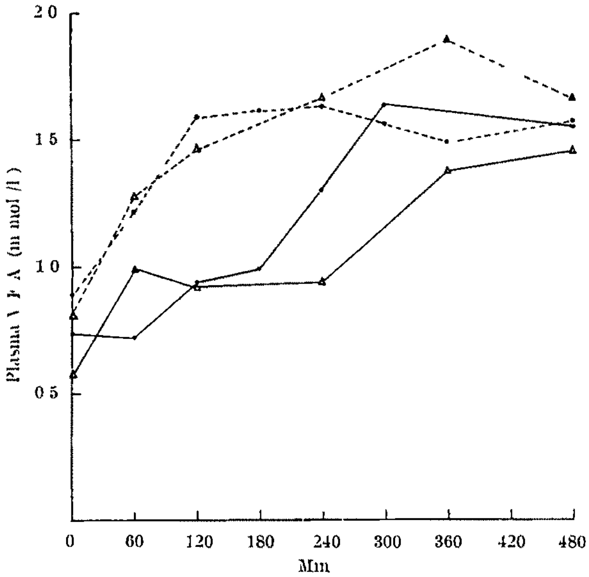

Fig 1 Plasma volatile fatty acid levels in sheep during infusion of acetate or N saline solution — ● —, Sheep 4, saline, – – ● – – sheep 4, 40 per cent acetate —△—, sheep 5, saline, – – △ – – sheep 5, 40 per cent acetate

Two points should be noted here The high blood volatile fatty acid levels achieved in series 1, following acetate infusion, relative to those in series 4 and 5 are presumably due to the 'explosive' nature of volatile fatty acid production in sheep accustomed to eating quite large quantities of feed over a short period Secondly, maximum blood volatile fatty acid levels achieved by acetate infusion in series 4 and 5 are not greatly different from those produced during saline infusion However, it was demonstrated (Fig 1) that acetate infusion achieved high levels within 1 h of the infusion commencing Similar levels were not achieved until 4–6 h after saline infusion commenced These levels were presumably the effect of feed eaten by that time

The reason for the disparity between present results with acetate and those of previous workers[12] is not clear A species difference does not seem likely It may be significant that an appreciable depression of food intake was also recorded by these workers with the only other acid metabolite, propionic acid, infused in comparable quantities This metabolite is not normally present above trace-levels in the peripheral circulation, as it is converted to glucose in the liver after absorption from the rumen and transfer via the portal vein[13] Yet a larger infusion of glucose had no effect on appetite This suggests that an unphysiological level of anion in the peripheral system may have inhibited appetite in the cattle, although no data on blood levels achieved were presented

The results indicate that neither blood glucose level nor blood acetate level is a major factor in regulating appetite in the sheep

This work was conducted while I held a Commonwealth Post Graduate Scholarship in the School of Rural Science University of New England, Armidale

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ACTION CURRENT AS A POSSIBLE SOURCE FOR THE NEGATIVE AFTER-POTENTIAL OF STRIATED MUSCLE

By Prof. HOWARD JENERICK

Department of Physiology, Emroy University, Atlanta 22, Georgia

INSUFFICIENT evidence is at present available concerning the genesis of the negative after-potential which follows the spike response in striated muscle While it is fairly certain from measurements of the time-course, that the after-potential represents a passive electrotonic decay[1], the processes which determine its initial magnitude are poorly understood Recently, analytical methods were developed for computing the trans-membrane ionic current which accompanies the propagated spike potential in the frog sartorius muscle fibre[2] It was felt that such results might aid in elucidating the after-potential, and the following is a report of my findings.

The propagated response is associated with a massive inflow of current which lasts less than 0 5 msec and is followed by a smaller, outward current of several msec duration In the work recorded here, I measured the net uptake and loss of trans-membrane ionic charge caused by the inflow and outflow of the action current (Fig 1) Since charge is equal to the product of current × time ($q = \int I dt$), these measurements were made by planimetric integration of the area under the curve of the trans-membrane ionic current plotted against time The areas indicated in Fig 1 correspond to a net uptake, q_i, of 6 4 p moles/cm^2 and a net loss, q_0, of 4 5 p moles/cm^2, leaving an unbalanced intracellular charge ($\delta q = q_i - q_0$) of 1 9 p moles/cm^2 Note that the outward current has declined virtually to zero near the end of the spike response (at 4–4 5 msec in Fig 1) Hence, the rapid outflow of charge ended approximately at the beginning of the negative after-potential V_n, before a complete exchange occurred

Sample calculations showed that if the charge, represented by δq, is distributed on the membrane capacitance C_m, a potential is generated which is of proper sign and approximate magnitude to account for the initial value of the negative after-potential, namely, $V_n \sim \delta g/C_m$ (Although our methods are not sensitive enough to detect any ionic current during the decay of the after-potential, such current must occur to drain off the internal charge)

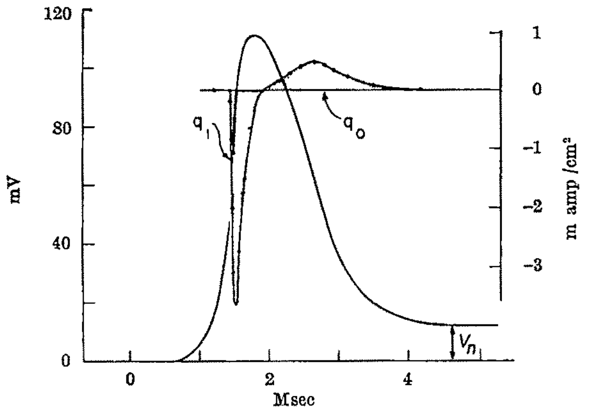

Fig 1 A propagated trans-membrane spike-potential traced from the original record and the computed ionic current are aligned in time for comparison The inward current (plotted downward) reaches its peak value before the maximum spike height is reached and reverses shortly thereafter Note that the outward current subsides markedly during the terminal phase of the spike potential and approaches the base-line before the initial value of the after-potential is reached (V_n) Current magnitudes in m amp/cm^2 of membrane are based on an assumed value of 5 μF/cm^2 for the membrane capacitance The areas under the inward and outward current plots correspond to a charge uptake q_i, of 6 4 p moles/cm^2 and a loss, q_0, of 4 5 p moles/cm^2

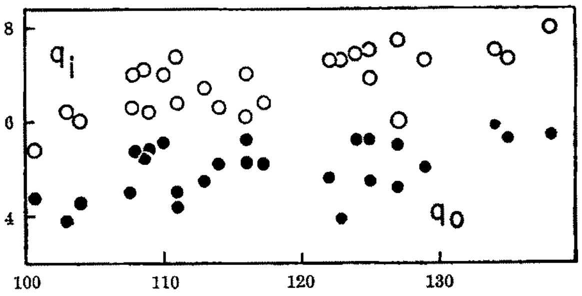

Fig 2 Effect of varying Na$^+$ and K$^+$ concentrations on the transient uptake (○) and loss (●) of intracellular charge (p.moles/cm^2 of membrane) during the response as a function of the spike-potential

A systematic study of the magnitudes of q_i, q_0, and particularly of δq, was made to determine whether or not the foregoing relationship between charge accumulation and the initial value of the after-potential was an accidental one In the initial series of experiments, I used Ringer's fluid with the following variations sodium chloride, 35–110 mM, potassium chloride, 1–5 mM, pH 7 2–7 4 No consistent increase or decrease, within the limits of error in q_i, q_0 or δq as related to the spike height (V_s), was observed (Fig 2) However, my methods for the determination of charge may have suffered from a 5–10 per cent error introduced by the graphical analysis, and if any changes did occur, they were possibly masked by these errors There was some dependence of q_i and q_0 on the spike potential magnitude, based on computations of 26 separate responses (Fig 2) Note the vertical separation of the data which indicates the magnitude of δq This gradually decreases with the spike potential, as does the after-potential However, these changes are too small, perhaps, to be convincing When sodium chloride in a standard Ringer fluid was replaced by sodium nitrate, the spike potential magnitude and q_i were unaffected, whereas the net charge loss, q_0, decreased by 20–30 per cent during the downstroke of the spike Since the initial charge gain, q_i, was unchanged, $\delta q = (q_i - q_0)$ was necessarily raised Simultaneously, the negative after-potential was increased by ~ 25 per cent in the nitrate–Ringer This gave encouraging evidence for the postulated role of δq in the genesis of the after-potential

The relationship of charge and the after-potential was further tested by using the pH of the Ringer fluid as an experimental variable, since it has been observed in our laboratory that acidic fluids (pH 5–6) markedly increased and basic fluids (pH 9–10) decreased the initial value of the negative after-potential in frog sartorius fibres While the charge uptake, q_i, remained unaffected by solution pH, the charge loss, q_0, was significantly diminished in acidic fluids and enhanced in basic fluids Under these conditions the net accumulation (gain minus loss) at the beginning of the after-potential varied between 4 1 and 'zero' pmoles/cm^2, while the associated negative after-potential varied from 50 to $\frac{1}{2}$ mV respectively (Fig 3) (Fig 3 includes the data shown in Fig 2) The solid line in Fig 3 represents the predicted relationship $V_n = \delta q/C$, between δq, the initial value of the negative after-potential V_n, and the membrane capacitance C, chosen at 5 μF/cm². The dashed lines are least-squares regression lines of δq on V_n and vice versa. Although the hypothesis requires that δq be the independent variable, δq values are subject to a greater measurement error and have a greater variance than V_n. This in turn causes a clockwise rotation of the regression line of V_n on δq and tends to present a misleading picture of the relationship. In the circumstance, both regression lines were calculated and indicate a correlation coefficient, $r = 0.86$. All data for δq in Fig. 3 lie to the right of the predicted locus for capacitor charging. They suggest that a constant 'excess' charge ($= \delta q - V_n/C$) of 0.7–0.8 p moles/cm² is temporarily accumulated (regression of δq on V_n), or that the excess is a constant percentage ($\sim$ 40 per cent) of the total charge accumulation (regression of V_n on δq). While we cannot choose between these possibilities on the basis of the experimental evidence, it seems permissible to conjecture, in any event, that the 'excess' charge is unavailable for capacitor charging by virtue of (1) a transient interaction with ionizable materials in the membrane or cytoplasm, and/or (2) a participation in excitation-contraction coupling. In the latter connexion, the increase of δq and the excess charge by nitrate–Ringer fluid is highly suggestive and warrants further investigation.

As described in the previous communication, transmembrane current magnitudes are obtained from an analysis of the phase-plane record of the spike-potential by means of the cable equation. This results in determinations of the ratio, I/C (ionic current/membrane capacitance) at any selected point on the recorded response. In this report, these ratios were converted into units of current/cm² by assumption of a definite value (5 μF/cm²) for the membrane capacitance. It must be stressed that the capacitance enters Fig. 3 only as a scale factor for the abscissa and that its actual value cannot change the observed relationship between the predicted locus and δq, that is, since $V_n = \delta q/C$ and $\delta q = \int I dt$, therefore, $V_n = \int (I/C) dt$. The right-hand member of this equality is obtained directly by the graphical integration of the original calculations in (I/C) and is therefore independent of the actual value of the membrane capacitance (provided C does not change during the response). In addition, the analysis provides separately for the capacitative current during the response. Hence, δq is independent of the 'normal' movement of charge on the capacitance and represents an additional source serving to affect the capacitor potential.

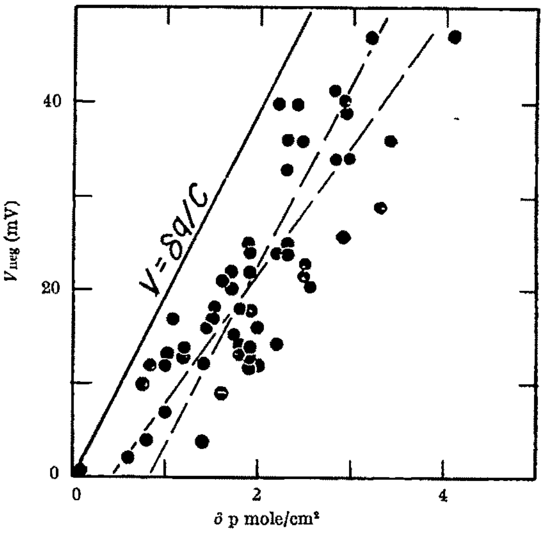

Fig. 3. The initial value of the negative after-potential (V_n) from 52 separate responses plotted as a function of the net accumulation of cationic charge, δq, during the spike potential. The solid line represents a predicted relation between the accumulated charge and the voltage on the membrane capacitance. The dashed lines are calculated least-squares regression lines.

The method described here does not reveal the mechanism by which the outflow of membrane ionic current is terminated before a complete exchange of cationic charge is effected. Since the amount of initial charge uptake, q_i, is fairly constant under a variety of conditions, including pH, it appears that the main determinant of the magnitude of the net charge accumulation, δq, is the degree to which the charge outflow q_0 can equalize q_i.

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DEVELOPMENT OF CHLOROPLASTS AND THE SYNTHESIS OF PROTEINS IN LEAVES

By Dr. M. J. C. RHODES and Prof. E. W. YEMM

Department of Botany, University of Bristol

DURING normal vegetative growth of higher plants a rapid synthesis of proteins usually occurs in the young leaves. As the mesophyll cells expand and differentiate, chloroplasts are formed from proplastids and there is now much evidence that a major part of the leaf protein is structurally held in the chloroplasts, amounting in some species to 70–80 per cent of the total protein[1]. In an extension of earlier studies of the biosynthesis of proteins in barley seedlings[2–4], a more thorough investigation has recently been made of the development and metabolism of the first leaf. When the seedlings assimilate nitrogen, supplied as nitrate or ammonium salts under controlled conditions, most of it is transformed to protein in the shoot system. A preliminary investigation with isotopic nitrogen-15 indicated that the plastid fraction, separated by differential centrifugation, rapidly became labelled with nitrogen-15 and had an isotopic abundance more than twice that of the microsome fraction[5]. Particular attention has therefore been directed to the development of chloroplasts in the expanding leaf. The results so far obtained indicate that the plastids are active centres in which protein synthesis is closely associated with high levels of ribonucleic acids (RNA) in the young leaves. Moreover, the pronounced effects of light on the formation of proteins suggest that there is a close relation between protein metabolism and photosynthetic activities of the chloroplasts.

(1) *The changes of proteins and nucleic acids in developing leaves.* When barley seedlings are grown under controlled conditions in the light, the first leaf emerges from the coleoptile after 3 days. The leaf blade increases rapidly in size, its fresh weight and dry weight reaching fairly steady values after about eight days. Etiolated leaves, grown in continuous darkness, expand rather more slowly,

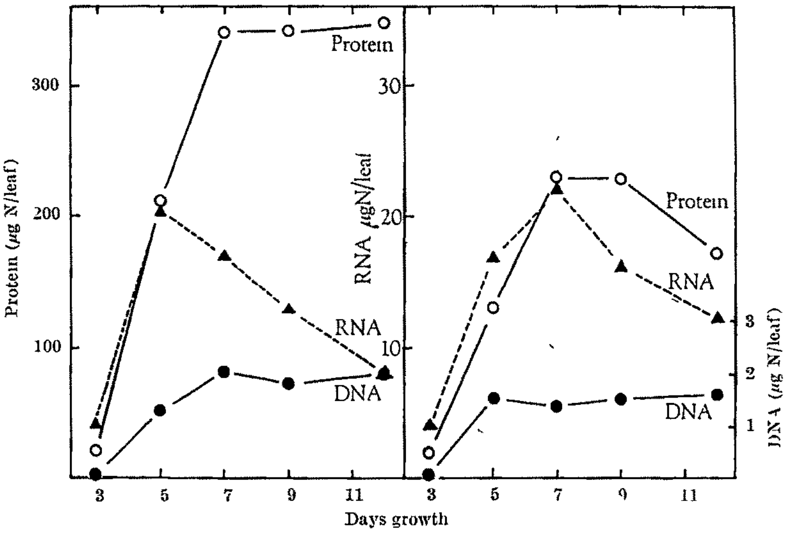

Fig. 1. Changes of proteins, RNA and DNA during the development of the first leaf of barley seedlings grown in the light (left) or in the dark (right). Samples of about 50 leaves were taken at each stage, and extracted exhaustively with ethanol, ice-cold 5 per cent perchloric acid and ether. The dried powder was subdivided for estimation of RNA (▲), DNA (●) and proteins (○).

but reach a similar maximum size after about nine days. Analytical data have been obtained to show the changes during development of some of the chief constituents of the leaves, including proteins and nucleic acids. For this purpose, nucleic acids were separated by a modification of the procedure of Schmidt and Thannhauser[6]. RNA was estimated after chromatographic purification of the nucleotides on an ion-exchange resin. Some of the results of an experiment, in which normal and etiolated leaves were compared, are illustrated in Fig. 1.

A rapid increase of RNA occurs during the first phase of leaf development. After reaching a maximum when the leaf is about half grown, the RNA content then falls to a level less than half that of the peak. The sequence and magnitude of these changes are very similar both in green and in etiolated leaves. Deoxyribonucleic acid (DNA) is present in much smaller amounts than RNA, its increase during leaf development follows a more regular course which roughly parallels that of leaf growth. The most rapid formation of proteins occurs during the early stages of development when RNA is maintained at a relatively high level. But substantially greater quantities of protein are formed in the light than in the dark, a difference which is further emphasized by the loss of protein from the etiolated leaves as they mature. One of our primary objectives in these experiments has been to relate the synthesis of proteins and other constituents to leaf development and, in particular, to changes in the structure and activity of the chloroplasts.

(2) *The composition and structure of plastids.* The fine structure and development of chloroplasts, including those of barley leaves, have been extensively studied by electron microscopy; several accounts have been given of the chief stages in the formation of the highly organized structure of the pigmented grana and colourless stroma[7-9]. On the other hand, much less is known of the composition of plastids and the way in which the main structural components, such as proteins, lipids and nucleic acids, change during differentiation. An attempt was made, therefore, to correlate changes in structure and composition by examining plastids separated from leaves at different stages of development. In this way it was also possible to estimate to what extent changes in the composition of the leaves were attributable to the differentiation of plastids.

Analyses of cell fractions, separated by differential centrifugation from young leaves, have shown consistently that a substantial part of the total RNA is sedimented with the plastids by relatively low centrifugal forces. Purification of the plastids, either by washing at the centrifuge or by sedimenting on a sucrose gradient, indicates that 20–30 per cent of the total RNA in the cell fractions of homogenates prepared from 4-day-old leaves is held in the plastids. High values of RNA in the plastid fraction have also been reported for young leaves of other species and for some algae[10,11]. However many tests with barley leaves have shown that the partition of RNA between the different cell fractions is strongly influenced by the conditions under which the separation is carried out. If preparations are made at pH 7·0, or above or are treated with deoxycholate a greater proportion of the RNA is recovered in the high-speed fractions and under extreme conditions most of it remains in the supernatant solution. It is probable that these changes result from the dispersion and leaching of nucleic acids from the plastids and other cell fractions; similar processes are now known to cause extensive losses of protein from chloroplasts during their separation in aqueous systems[12]. For comparative purposes, it is necessary to standardize the conditions of grinding and centrifugation as fully as possible. Table 1 summarizes an experiment in which the distribution of protein and nucleic acid was examined in cell fractions separated from normal and from etiolated leaves at different stages in their development.

Table 1. PROTEIN AND RNA IN CELL FRACTIONS OF NORMAL AND ETIOLATED LEAVES

(A) Protein (μgN/leaf)

Fractions	Light 6-day	Light 13-day	Dark 7-day	Dark 13-day
1 Plastids	56·5	49·0	31·2	16·7
2 Mitochondria and grana	17·3	15·4	15·0	11·4
3 Microsomes	13·4	5·7	16·5	9·4
Total (1–3)	87·2	70·1	62·7	37·5
Total/leaf	260	234	197	144
% Recovery	34	30	32	26

(B) RNA (μgN/leaf)

Fractions	Light 6-day	Light 13-day	Dark 7 day	Dark 13-day
1 Plastids	2·4	0·8	2·7	1·3
2 Mitochondria and grana	1·4	0·4	1·9	0·9
3 Microsomes	3·9	1·0	5·9	2·8
Total (1–3)	7·7	2·2	10·5	5·0
Total/leaf	21·4	7·0	21·0	13·0
% Recovery	36	32	50	38

Samples of 50 or more leaves were ground in cold 0·4 M sucrose buffered at pH 6·5 (5 ml./g tissue). Cell debris was removed by 'Terylene' gauze and centrifugation at 365g for 5 min. Fractions were then separated by centrifugation at 0° C as follows: (1) 5,000g for 10 min.; (2) 40,000g for 15 min.; (3) 75,000g for 90 min. Examination by light and electron microscopy indicated that the chief components of the fractions were (1) intact plastids (2–5μ); (2) mitochondria and grana (0·5–1μ); (3) microsomes (0·1–0·2μ).

Separate analyses of total protein and RNA in the leaves were made and the recovery in the fractions is shown.

It is evident from the results that the protein content in the plastid fraction of normal leaves is much greater than that of etiolated leaves; other cell fractions show relatively little difference between leaves grown in the light or in the dark. Having regard to the probable loss of proteins by leaching from separated plastids, it seems likely that the development of chloroplasts largely accounts for the greater formation of proteins in the light. On the other hand, the changes of RNA in the leaves appear to be fairly evenly distributed between the different cell fractions. The relatively high levels of RNA in the fractions from etiolated leaves reflect the comparatively slow loss of total RNA during the later stages of development in the dark. In general, the data indicate that the high levels of RNA in the young expanding leaves extend also to the plastids, in which a rapid formation of structural proteins is closely associated with the development and differentiation of chloroplasts in the light.

Electron microscopy has been used to investigate cellular differentiation in the leaves with the main object of correlating the changes in their composition with the development of plastids. For this purpose leaf tissues were fixed either in permanganate or osmic acid, embedded in 'Araldite' and sectioned in a Huxley–Cambridge microtome for examination in a Philips *EM* 200 electron microscope. The development of plastids in the light or in the dark diverges at an early stage in the expansion of the young leaf. Conspicuous primary lamellæ are rapidly formed in the light and they appear to arise either from small vesicles or from irregular extensions of the inner component of the plastid membrane. In 4-day seedlings after 24 h exposure to light the differentiation of grana can be seen; the older cells near the tip of the leaf contain many plastids with a few grana consisting of 3–6 disks closely associated with the primary lamellæ, while young cells at the base of the leaf have smaller plastids in which single grana disks are apparently present alongside the lamellæ. An illustration of cell and plastid structure at this early stage of development is given in Fig. 2. In comparable leaves grown in the dark, the lamellar structure of the plastids is much less conspicuous; commonly numerous small vesicles are seen which show some arrangement into layers, but as the plastid develops, a dense reticulate or vesicular structure, the prolamellar body, is formed in the central part. The fairly frequent occurrence of constricted and dumb-bell shaped plastids indicates that their division continues at this stage in both normal and etiolated leaves.

During the period of rapid leaf growth (days 4–7) in the light, the plastids increase in size and complexity, so that when the leaf blade is fully expanded all the mesophyll cells contain well-differentiated chloroplasts. This involves about a five- to ten-fold increase in volume, compared with typical plastids at day 4, and a major part of the structure is taken up by grana commonly consisting of 8–10 disks with irregular interconnecting lamellæ, similar to those already described[8]. A dominant feature of the development of the chloroplasts appears to be an extension and organization of specific lipoprotein membranes and this coincides with the increase of chlorophyll and proteins induced by light.

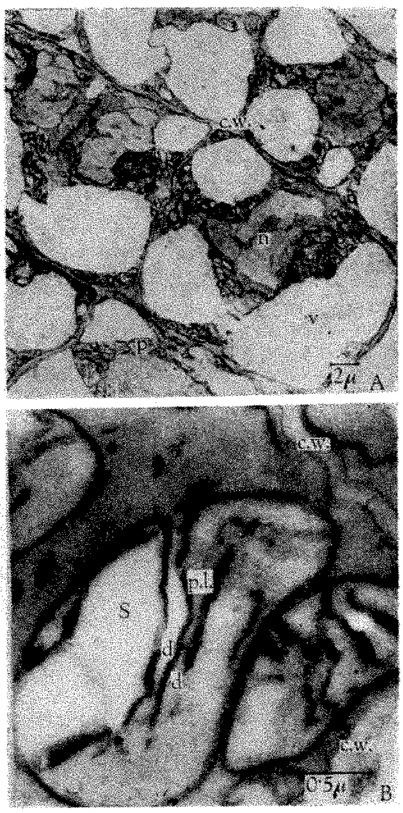

Fig. 2. Electron-micrographs of young cells from leaf of 4-day seedling after 24 h in the light (fixation in 2 per cent permanganate for 30 min at 0–4° C). *A*, vacuolating cells containing numerous plastids: plastids (*p*), cell-wall (*c.w.*), nucleus (*n*), vacuole (*v*); *B*, typical plastid at this stage showing large starch vacuole (*S*), primary lamellæ (*p.l.*) and rudimentary disk formation alongside (*d*)

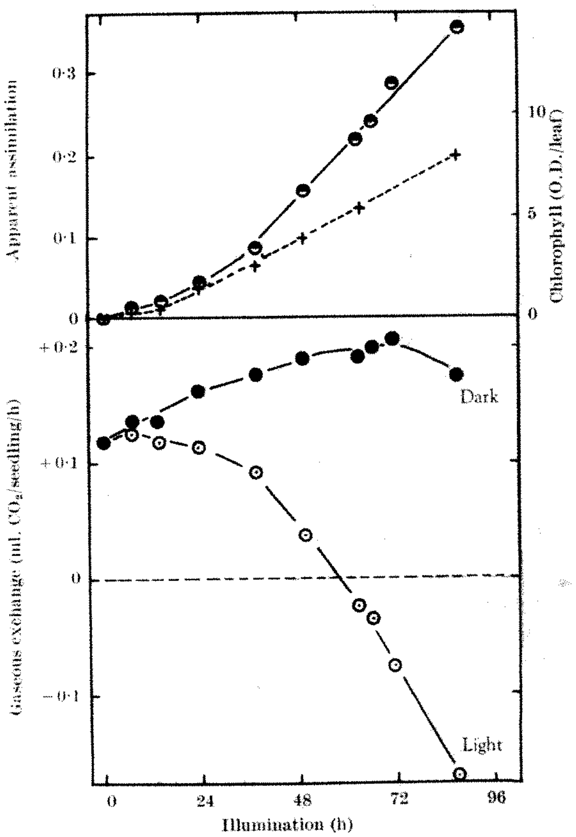

Fig. 3. The photosynthetic activity of young leaves. Measurements of carbon dioxide exchanges, shown in the lower part of the figure, were made on groups 40–50 seedlings in the dark (●) or in the light (⊙) (about 1,000 ft. candles) at 22·5° ± 1° C. Photosynthetic assimilation (◓) (estimated by difference between consecutive light and dark values) is shown here, together with chlorophyll content (+) measured spectrophotometrically at 665 mμ after extraction in ethanol

(3) *Photosynthetic activity of young leaves.* The formation of chlorophyll, probably at first by a photochemical conversion of protochlorophyll[13], occurs in the leaves within a few hours of exposure to light; the possibility is indicated that photosynthesis may play an important part in the metabolism of young leaves at an early stage in their development. However, it is well known that in some seedlings normal photosynthesis may lag behind the formation of chlorophyll[14]. In our experiments we have therefore obtained some further information on the photosynthetic activity of leaves grown under these conditions. The seedlings were germinated in glass chambers through which a steady stream of air could be passed and its carbon dioxide content monitored by means of an infra-red gas analyser. Measurements were made over short periods (30–60 min) alternating in the dark and in the light, and thus provided a sensitive method for detecting the effects of illumination on the carbon dioxide exchanges of the seedlings. The results of one of the experiments, in which estimates of the chlorophyll content of the seedlings were also made, are shown in Fig. 3.

An appreciable effect of light on carbon dioxide production was found consistently after illumination for 4–6 h of 3–4-day-old seedlings. The effect increased fairly steadily so that a compensation point between respiration and assimilation was reached after about 60 h. The rise of apparent assimilation in light followed a course similar to that of chlorophyll content, but there was evidence of an increase in the efficiency of the pigment system, since the ratio of assimilation rate to chlorophyll content rose from a value of 0·25 after 12 h to 0·36 after 80 h.

It is uncertain to what extent these results can be attributed simply to the photosynthetic assimilation of carbon dioxide. There is now evidence that respiratory and photosynthetic processes may interact at intermediate levels, such as the oxidation-reduction, phosphorylation or carbon substrate systems of the cell[15]. Some experiments, in which labelled carbon dioxide was used as a tracer, suggest that the chief products of assimilation during greening of etiolated leaves are amino-acids, such as aspartic and glutamic acids, together with malate; formation of sugar phosphates and full operation of the photosynthetic cycle seem to require longer exposure to light[16]. Despite these uncertainties, the data for developing barley leaves indicate that photosynthetic activity is an important feature of leaf metabolism during the phase of rapid differentiation of the chloroplasts. The rudimentary grana, rapidly formed in the plastids in the light, seem to be active at least in primary photochemical reactions and within about 48 h the coupling with normal carbon dioxide assimilation is effective.

(4) *Chloroplasts and the synthesis of proteins.* It is generally considered that light has an indirect effect on the synthesis of proteins in plants as a result of the formation of carbohydrates by carbon dioxide assimilation. There are, however, several lines of evidence that indicate a more direct coupling between photosynthesis and the metabolism of amino-acids and proteins in leaves. The early investigations of photosynthesis with $^{14}CO_2$ demonstrated that amino-acids such as alanine and aspartic acid are among the first compounds to become labelled[17], and this has been confirmed more recently with cell-free preparations of chloroplasts[18]. When ammonia is supplied to algal cells during the assimilation of $^{14}CO_2$, a major part of the radioactivity is recovered in amino-acids[19], rather than in sugars and sugar phosphates, while kinetic investigations strongly suggest that a relatively independent pool of amino-acids is maintained in the chloroplasts which could account for more than 60 per cent of the synthesis of proteins in the cells[20]. The action of ammonium salts as reversible uncouplers of photophosphorylation in the Hill reaction[21] and as inhibitors of carbon dioxide assimilation[22] may be important in relation to the photosynthesis of amino-acids. In leaves there is some evidence that intermediates of photosynthesis, rather than carbohydrates, are diverted to the synthesis of proteins especially in the young chloroplasts[23], and light promotes the incorporation of amino-acids labelled with carbon-14 into proteins[24]. The presence of amino-acid activating enzymes in chloroplasts has been indicated together with the possibility of a coupling with photophosphorylation by means of adenosine triphosphate[25]. Other data which suggest that proteins may be synthesized in illuminated chloroplasts have been briefly reported and reviewed[26]. Recently, strong evidence has been obtained that the synthesis of fatty acids also occurs in chloroplasts and is dependent on photophosphorylation[27-29]. It is therefore probable that the synthesis of both lipids and proteins, two of the chief structural components of chloroplasts, is closely related to photosynthetic activity in developing leaves.

On the other hand, there is evidence that light may influence the size and protein content of the plastids of some etiolated leaves by mechanisms other than those of normal photosynthesis. In leaves of bean (*Phaseolus vulgaris*), in contrast to barley, light has an important morphogenetic action on leaf expansion; relatively brief exposure to red light causes the lamina to develop as well as changes in the plastids[30]. But it is evident that prolonged exposure to light is necessary for the differentiations of grana and normal development of chloroplasts in these leaves[31].

The relationship between nucleic acids and protein synthesis in chloroplasts is of particular interest with regard to the control of their development and the inductive action of light. Distinctive ribosomes have been isolated from chloroplasts of spinach[32] and of *Euglena*[33]. The RNA of *Euglena* plastids has a base composition different from that of the microsomes; moreover, the formation of this distinctive RNA is induced by light and precedes the increase of proteins associated with the differentiation of chloroplasts[34]. In young barley leaves the relatively high level of RNA during protein synthesis does not appear to be greatly influenced by light, but the possibilities of changes in base composition have not yet been fully explored. With regard to the metabolism of nucleic acids, additional evidence for the presence of DNA in the chloroplasts of some algae and higher plants has recently been reported[35,36] and may have an important bearing on the mechanisms underlying the genetic autonomy of plastids and the control of their differentiation.

The hypothesis of a relatively independent mechanism of protein synthesis, closely coupled with the photochemical activities in developing chloroplasts, is consistent with many of the observations we have so far made with young barley leaves. It is probable that the mechanism is particularly active during the development of grana and other lamellar structures of the plastids and that a phase of protein synthesis precedes that in which carbohydrates are the chief stable products of photosynthetic assimilation of carbon dioxide. A more critical test of this hypothesis is now being made by means of labelled metabolites.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Deficiency of Metals in Population II Stars

In a recent communication under this title Bashkin[1] has attempted to explain the deficiency of heavy elements in Population II halo stars by arguing that a separation between neutral atoms and ions is possible through electromagnetic means and that, since the ionization potentials of the metals are in general low, they will be confined to the disk while the neutral atoms can diffuse into the halo. This proposal can be severely criticized on several grounds.

(1) Abundance analyses which were not available at the time of Bashkin's communication have now been completed for several extremely metal-poor stars[2,3]. These analyses are of considerably higher accuracy than previous work because the stars are relatively bright and spectra of high dispersion were utilized. In Table 1 we list abundances of various elements, representative of different ionization potentials, in metal-poor stars. After each element we list the first and second ionization potentials, since the second ionization is probably important for some elements. Finally, we list the logarithm of the ratio of the element to hydrogen minus the same quantity for the Sun, and the source of the data. The abundances are probably accurate to a factor of 2 and are surely better than to a factor of 3. We have listed only a few elements, each of which is representative of a certain first or second ionization potential. Abundances of numerous other elements are listed in the references, most of the other observable elements have first ionization potentials between 6 and 8 volts. they generally show the same deficiency as does iron except for some heavy elements in *HD* 122563 which behave like barium.

Table 1 IONIZATION POTENTIALS AND MEASURED ABUNDANCES RELATIVE TO THE SUN

Element	First ionization potential (eV)	Second ionization potential (eV)	Log $\frac{N_t}{N_H}$ − Log $\frac{N_t}{N_H}$ ($\odot$)	Source
O	13.6	35.0	−1.7	*TY* Vir and *SX* Her (ref. 3)
			< −1.7	3 metal-poor giants (ref. 2)
C	11.2	24.3	−2.0	*HD* 140283 (ref. 4)
Fe	7.8	16.2	−3.0	*HD* 122563 (ref. 2)
			−2.7	*HD* 165195 and *HD* 221170 (ref. 2)
			−2.0	*HD* 140283 (ref. 5)
			−1.7	*TY* Vir and *SX* Her (ref. 3)
Mn	7.4	15.6	−3.3	3 metal-poor giants (ref. 2)
Ca	6.1	11.8	−2.75	3 metal-poor giants (ref. 2)
Ba	5.2	10.0	−4.75	*HD* 122563 (ref. 2)
			−2.90	*HD* 165195 and *HD* 221170 (ref. 2)
Na	5.1	47.1	−2.95	3 metal-poor giants (ref. 2)

Bashkin predicts that elements with large ionization potentials should behave as does hydrogen. We note from Table 1 that oxygen, which has almost exactly the same ionization potential as hydrogen, shows the same deficiency as iron in the two variable stars *TY* Virginis and *SX* Herculis[3]. In the three metal-poor red giants[2] oxygen was not discussed in the original paper because the lines could not be found and hence only an upper limit could be set on the abundance. This upper limit, however, is 1/50 of the normal ratio of oxygen to hydrogen. Carbon has the second largest ionization potential in Table 1 and was found by Baschek to have the same deficiency as iron in the metal-poor sub-dwarf *HD* 140283 (refs. 4, 5). Sodium has the lowest ionization potential and shows the same deficiency as does iron. Calcium and barium have such low second ionization potentials that they are mostly doubly ionized in interstellar space, but the ratio of calcium to iron is normal in metal-poor stars as is barium except in *HD* 122563 in which barium and other elements from strontium to europium are deficient by a factor of 50 more than is iron.

Next we point out that manganese, the ionization potential of which is within 0.4 eV of that of iron, is deficient with respect to iron by a factor of 3. This strange ratio of manganese to iron is found in numerous metal-poor stars[6]. To illustrate these arguments we use all the data for the two metal-poor red giants, for which we have abundances of the largest number of elements, in Fig. 1.

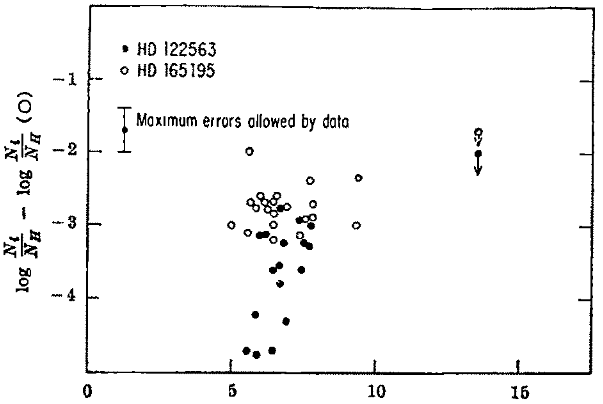

Fig. 1

Finally we direct attention to the *B*-type star in the galactic halo, *BD* + 33°2642, studied by Traving[7]. He found this star to be deficient in carbon, nitrogen, oxygen, magnesium and silicon by factors of 5–10 with no correlation with the first ionization potentials which range from 7.6 eV for magnesium to 14.5 eV for nitrogen.

(2) We wish to point out that all metal-poor stars were not formed in the halo. Of the three extremely metal-poor red giants discussed here, two (*HD* 122563 and *HD* 165195) have very small velocity components perpendicular to the galactic plane[2]. The sub-dwarf already mentioned, *HD* 140283, is also moving in a highly elliptical orbit, but its motion perpendicular to the plane is small.

The points discussed here concern the observations. Further objections can be raised on theoretical grounds against the proposed hypothesis.

(3) It is implied in Bashkin's first paragraph that ions will be confined to the galactic disk where there is a magnetic field, and neutral atoms will not. However, the evidence from the halo radio emission indicates that the magnetic fields extend into the halo region and there is no reason for believing that the magnetic field configurations of disk and halo are closed and separate. If a single ion could be given a velocity sufficient for it to be ejected into the halo, there is every possibility that the magnetic field connexion between disk and halo would allow this to occur.

(4) For any separation of the type proposed in Bashkin's first paragraph it is necessary for an atom to be given an outward velocity of several hundred kilometres a second. No mechanism for doing this is proposed other than a comment that particles in the high-velocity tail of a Maxwell–Boltzmann distribution may have high enough velocities. However, if the interstellar material is

composed of cool clouds with temperatures ~ 100° K and ionized clouds with temperatures ~ 6,000° K, the evaporation of particles with these energies is negligible.

(5) Bashkin seems to assume that the interstellar medium can be considered as made up of single particles moving in magnetic and gravitational fields. However, it must be stressed that it can only be considered as a continuous medium containing a variety of energy sources which is acting under gravitational and magnetic forces. That the viscous forces are strong is shown by the fact that only a very small ionized component of the gas is sufficient to couple the magnetic field to the neutral component[8,9]. Thus the picture of single atoms migrating into the halo is unrealistic.

Bashkin also remarks that the abundance of helium in Population II stars is incompatible with the nucleosynthesis theory. However, it has been shown elsewhere[10] that this is not so.

We conclude that Bashkin's proposal is unacceptable, and that the usual explanation of the abundance differences among stars as being due to the processes of nucleosynthesis and stellar evolution is to be preferred.

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GEORGE WALLERSTEIN

Berkeley Astronomical Department,
University of California.

E. MARGARET BURBIDGE

University of California,
San Diego.

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THE objections raised by Wallerstein and Burbidge to my proposals are themselves subject to question.

(1) The accuracy quoted by Wallerstein and Burbidge for the abundance measurements exceeds or ignores the claims of the original authors. Thus Wallerstein *et al.*[1] mention (maximum) errors of a factor of 4 in the (Fe/H) ratio for *HD* 165195 and *HD* 221170, and a factor of 2 for *HD* 122563. For the other elements cited by Wallerstein and Burbidge, an additional error of a factor of 3 is given by Wallerstein *et al.*[1] in the (element/iron) ratio. These errors omit the effect, possibly large and in the direction to reduce the under-abundances, of the contribution of Raleigh scattering to the opacity[1,2]. Deutsch[2] in particular has found that the apparent under-abundance of the metals in Mira stars is reduced from a factor of one hundred to one of three when Raleigh scattering is considered. Baschek[3] indeed finds carbon to be deficient in *HD* 140283, but his result is a factor of ten higher than that of Aller and Greenstein[4] and he notes that a change of 100° K in the assumed effective temperature causes the deduced carbon abundance to vary by a factor of 1.7. Baschek's result[5] on the abundance of nickel in *HD* 140283 differs by a factor of five from the value obtained by Aller and Greenstein[4]. Wallerstein *et al.*[1] state that the data on elements from strontium to europium are so scanty that any theoretical treatment must be cautious. The alleged[6] under-abundance of manganese relative to iron is to be viewed with reserve because of discrepancies between authors. For example, for *HD* 140283 Baschek[5] gives [Mn/Fe] as +0.51, while Aller and Greenstein[4] give +0.01. One must remark that a small systematic error in Wallerstein's results[6] on [Mn/Fe] could account for his under-abundance of manganese, and further work on this point would be helpful. The foregoing uncertainties in the data cannot be neglected in attempts to interpret stellar abundances.

The relevance of Traving's *B* star[7] is not understood. Since that star is almost certainly far younger than the galaxy, its chemical composition is not simply related to the initial condition of the galaxy. It is entirely reasonable that young, isolated, halo stars should be deficient in all elements more massive than helium simply because of gravitational attraction between those elements and the disk. What is surprising about *BD* +332642 is that the helium content is normal[7]. This evidence that uncondensed matter in the halo is helium-normal may be further justification for the idea presented in my original communication that ionization and the galactic magnetic field significantly affect stellar compositions in the halo region.

The statement that Burbidge[8] has satisfactorily explained the (He/H) ratio in Population II stars seems to me to be in serious error. He emphasizes the discrepancy between the observed (He/H) ratio (0.17) and that calculated (0.019) on the assumption that all the galactic helium was built out of hydrogen in stellar interiors and lists four different, speculative hypotheses to account for this divergence. One of his suggestions leads to the conclusion that stars of small mass should have abnormally low (He/H) ratios. This is apparently contradicted by Traving's work[7], on Barnard's star, which he finds to have a mass of 0.2 M_0 but a (He/H) ratio only half normal.

(2) Of the stars mentioned by Wallerstein and Burbidge in the preceding communication, only *HD* 165195 is definitely not a halo star, but no evidence is advanced that this star was formed near the galactic plane.

(3) Certainly the original galactic field extended into the halo region. Equally certainly, that field had a gradient and a time-dependence which could have contributed the disk-ward force I suggested. Lynds and Sandage[9] have shown how, under some conditions, a galactic magnetic field can very effectively channel the motion of galactic matter. It would be interesting, if observationally possible, to look for element differentiation in *M* 82. According to my model, the filaments should be rich in metal and poor in helium. The coupling between ions and neutral particles by virtue of collisions and induced-charge effects is an important point. My original communication noted that charge exchange could occur and that the magnetic field would be effective only for such time as a given particle was actually ionized.

(4) I reiterate that the normal (He/H) ratio in Population II stars and the virtual identity of the (metal/H) ratios for young and old stars in the galactic disk present an unsolved problem in the theory which attributes all stellar abundance differences to stellar nucleogenetic processes.

I thank Miss Barbara Middlehurst for useful discussions.

STANLEY BASHKIN

Department of Physics,
University of Arizona,
Tucson.

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GEOPHYSICS

Lunar Tides in the Equatorial Electrojet and in the Ionosphere over the Magnetic Equator

An abnormally large value of the solar daily range of the horizontal magnetic intensity, H, found at stations within a few degrees from the magnetic equator has been ascribed due to eastward electric currents flowing in the lower ionosphere during the daylight hours and known as equatorial electrojet[1] A longitudinal effect in the strength of the electrojet with a maximum in the American zone has been shown by Rastogi[2]

Bartels and Johnston[3] showed the existence of abnormally large lunar tidal effects in the range of H at Huancayo I (ref 4) showed that the enhancement in the tidal effects in the range of H is confined, within a few degrees. around the magnetic equator very similar to the equatorial electrojet I have also demonstrated a longitudinal effect in the lunar tides in range of H similar to that in the equatorial electrojet[5]

The existence of enhanced lunar tidal effects in the critical frequency f_0F_2 of the F_2 region of the ionosphere confined within a few degrees around the magnetic equator similar to the latitudinal variation of electrojet strength has been shown[6,7] The amplitude of lunar tide in f_0F_2 over the magnetic equator was largest in American intermediate in African and least in Indian zones, which is very analogous to the longitudinal variation of the equatorial electrojet

To study further the relations between the lunar tidal effects in f_0F_2 and in the electrojet analyses of midday (11–13 h mean) values of f_0F_2 at Huancayo over the years 1938–46 plus 1951–60 have been analysed by me and have been compared with the similar analyses of range in H by Bartels and Johnston[3]

In Fig 1 are drawn the harmonic dials showing the seasonal variations in the coefficients of lunar semimonthly variations in f_0F_2 computed by me and in the range of H derived from Bartels and Johnston's[3] paper The phase of lunar tide is denoted in terms of lunar age when the maximum positive deviation is obtained The data covering the period 1938–46 and 1951–60 are used for f_0F_2 and 1922–39 for range of H Each point is thus derived from about 20 lunations Probable error circles are drawn to show the statistical significance of each determination It is seen that the errors are much smaller than the amplitude and each of the determinations may be taken as significant

There is a remarkable similarity in the seasonal variations of the lunar tidal effects in the f_0F_2 and the range of H The change of the amplitude of lunar oscillation in both f_0F_2 or in range of H from their greatest values in January to their smallest values in June or July is quite marked The total change of L_2 amplitudes between the two seasons are 0 56 Mc/s and 19γ for f_0F_2 and the range of H respectively The sum of probable errors in the smallest and largest values of L_2 amplitudes is 0 06 Mc/s and 5γ The difference of L_2 amplitudes is thus about 5–10 times the sum of the probable errors

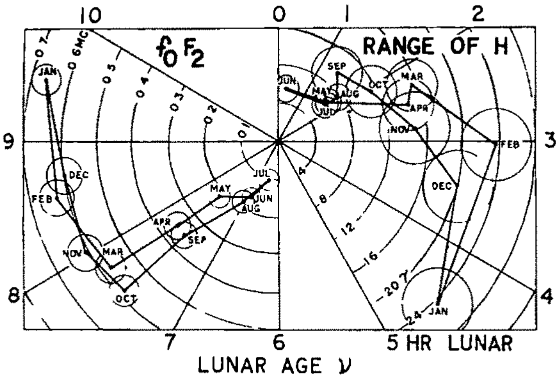

Fig 1 Harmonic dials showing the coefficients of lunar semi-monthly oscillations in f_0F_2 and in the solar daily range of H during different months at Huancayo

Further, there is a systematic shift of the phase of L_2 oscillation with season for both f_0F_2 and range of H, the crest of the wave occurring earlier in June–July than in January The difference of phase between the L_2 oscillations in f_0F_2 and range of H for any of the particular months, is very nearly 6 h, indicating an antiphase relationship between the two oscillations

The seasonal and latitudinal variations in the lunar tidal oscillations in f_0F_2 close to the magnetic equator demonstrate a very close association between the higher regions of the ionosphere and the equatorial electrojet situated in the lower portions of the ionosphere The exact mechanism of such an association is still to be worked out A fuller paper describing the lunar variations in H and f_0F_2 at Huancayo will be published later

R G Rastogi

Physical Research Laboratory,
Ahmedabad,
India

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SEISMOLOGY

Seismo-magnetic Effect and the Possibility of Forecasting Earthquakes

The existence of a correlation between magnetic disturbances and seismic activity has been suspected for nearly a century, but so many of the early observations were due solely to the mechanical vibration of magnetometers by seismic waves that when Lapina[1] reviewed the subject in 1953 she concluded that no effect had been satisfactorily demonstrated However, sufficient evidence had been accumulating in Japanese literature to justify a renewed examination of the problem

The approach of Japanese scientists was summarized by Kato and Takagi[2], who compared magnetic surveys before and after earthquakes and plotted differences which they associated with the seismic activity They presumed that the magnetic effects were due to thermal changes accompanying the earthquakes since a local rise in the Curie point isotherm (depth at which the temperature is equal to the Curie point of the dominant magnetic mineral) demagnetizes a certain volume of rock and causes a change of field at the surface However, the required movement of the isotherm is much more rapid than thermal diffusion will allow

The thermal explanation is untenable, but its acceptance has delayed recognition of the fact that seismomagnetic effects must be due to the piezomagnetic properties of rocks the magnetizations of which change reversibly with the stress applied to them Kalashnikov[3] appears to have been first to consider seriously the piezomagnetic properties of rocks He initiated laboratory experiments, the most significant of which are those of Kapitsa[4], who found that the susceptibilities of typical basaltic type rocks were reduced in the direction of an axial compression by 0 8 × 10^{-4} to 1 3 × 10^{-4} per 1 kg/cm^2 of stress, and increased by rather less than half this amount in the transverse direction Using these results, Kalashnikov[3] calculated possible magnitudes of seismo-magnetic effects, but he made very unfavourable assumptions about the geometry of the stressed rocks in the region of an earthquake and his conclusion was discouraging The maximum change he expected, of the order 0 5γ (0 5 × 10^{-5} œrsted), is scarcely observable

More recently the controversy over the influence of piezomagnetic effects on palæomagnetism has led to renewed investigation of piezomagnetic effects in rocks The theory of the subject recently reviewed by me[5], but still not entirely adequate, shows that susceptibility χ and thermoremanence I_R, measured in the direction of an axial compression σ kg/cm², are reduced by the stress approximately as

$$\frac{1}{\chi}\frac{d\chi}{d\sigma} = -1\ 1 \times 10^{-4} \text{ cm}^2/\text{kg}$$

$$\frac{1}{I_R}\frac{dI_R}{d\sigma} = -3 \times 10^{-4} \text{ cm}^2/\text{kg}$$

In the transverse direction magnetization is increased by nearly half these amounts

These figures allow a direct calculation of the surface magnetic field anomaly due to any distribution of stress in magnetic rocks The piezomagnetic moment of any element of rock due to shear stress across a fault which makes an angle φ to the direction of magnetization is an incremental moment ΔI, the magnitude of which is represented by the foregoing equations and the orientation of which is $(2\varphi \pm \frac{\pi}{2})$, the alternative signs representing right-lateral and left-lateral faults

The pattern of shear stress release due to transcurrent movement at a shallow earthquake, epicentred at (0 0) in (x, y) co-ordinates, expressed as a fraction of the stress σ_0 at the origin, can be conveniently represented by a simple analytical expression of the form

$$\frac{\sigma}{\sigma_0} = \frac{1 + y^2 - x^2}{(1 + y^2 + x^2)^2}$$

the stress being assumed constant along any vertical line

By numerical integration of the dipole law of force the magnetic field at the surface, due to a distribution of dipoles corresponding in strength to the intensity of the stress, can be calculated for any field component and for any orientations of magnetization and fault movement In Fig 1 the anomaly in vertical field due to movement along a fault directed magnetic east–west is plotted, assuming that the horizontal component of magnetization in the rock is directed to the magnetic north Its similarity to the vertical field anomaly of the Riku-U earthquake, plotted by Kato and Takagi[2] and reproduced in Fig 2, is obvious, although the Riku-U measurements were made more than 60 years ago and were too limited to give more than an indication.

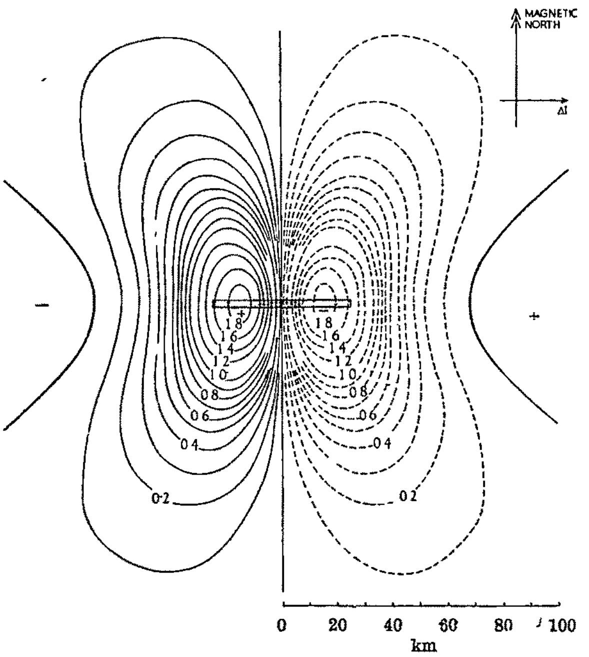

Fig 1 Intensity contours of the change in vertical component of magnetic field arising piezomagnetically from movement along an east west fault Figures on contour lines give the ratio of change in field to the vectorial change in magnetization at the point of maximum stress intensity

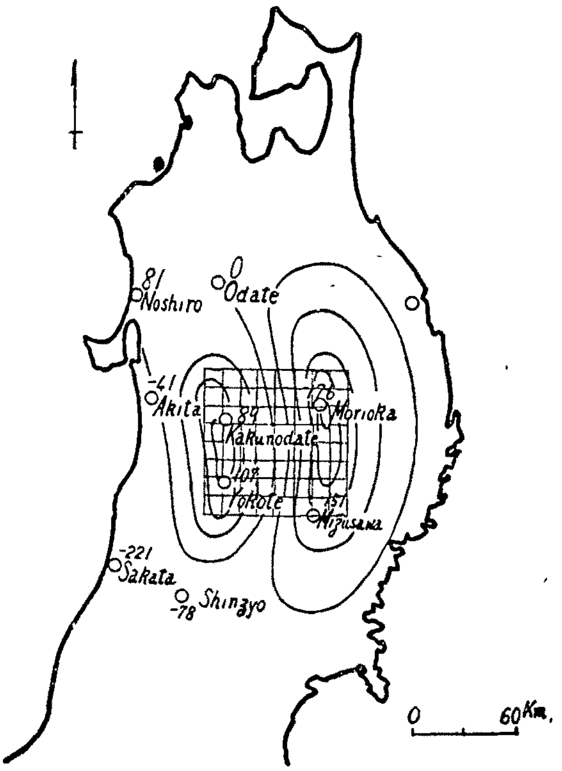

Fig 2 Contours of change in vertical field derived from magnetic surveys before and after an earthquake at Riku-U, Japan Figure taken from the paper by Kato and Takagi (ref 2)

The magnitude of the magnetic changes is determined by the intensity of magnetization of the rock and intensity of the stress released, both of which may be very variable Considerations of earthquake energy release indicate that 100 kg/cm² is a reasonable value to assume for the maximum shear stress release, so that maximum changes of 3 per cent in remanence and 1 per cent in induced magnetization are to be expected Magnetic anomalies of 10γ (10^{-4} œrsted) to 100γ (10^{-3} œrsted) are then expected in regions where the remanence of underlying igneous rock is 10^{-3} E M U to 10^{-2} E M U A surface layer having a kilometre or so of non-magnetic sediments scarcely affects this result if the magnetic rock extends to a depth of about 25 km

Such anomalies are readily observable Further, an earthquake must be preceded by a build-up of stress, normally over a period of months, and the magnetic effect must therefore give a pre-indication at least in many cases[6] The possibility of forecasting earthquakes on this basis deserves urgent consideration because magnetic instruments of the required accuracy are cheap, simple and reliable A weekly survey of the total magnetic field, using a proton precession magnetometer with a second one as a base instrument for comparison may well be sufficient to indicate the location of an impending earthquake with considerable accuracy

More extensive calculations of seismo-magnetic anomalies are in preparation for publication

This communication is submitted by permission of the Director-General of the Meteorological Office.

F. D. Stacey

Meteorological Office Research Unit,
Huntingdon Road,
Cambridge.

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GEOLOGY

Geological Effects on Surface Waters in Natal

During the course of a hydrobiological survey of the Umgeni River, Natal, it was found that the dissolved inorganic material in various tributaries of the main river showed characteristic differences in composition. It was considered that these differences arose from the fact that the tributaries drained catchment areas formed from rocks of different geological formations. This was therefore investigated further.

Five pairs of streams in Natal were selected, care being taken to ensure that the water of each stream was unpolluted, that the catchment of each stream was geologically homogeneous and that the two streams of each pair were geographically far apart. One pair of streams drained Beaufort rocks, one Ecca rocks, one Dwyka rocks, one Table Mountain Sandstone and one outcrops of basement granite, so that the principal geological formations of Natal were all included in the investigation. Samples were taken from each of the ten streams during the dry and the rainy seasons and biological checks were made during the dry season to confirm the absence of pollution. Each of the twenty water samples thus obtained was analysed for the major inorganic solutes and the results were expressed in such a way as to eliminate the effects of mere dilution or concentration. This was accomplished by finding the total of the concentrations, in p.p.m., of calcium, magnesium, sodium, potassium, carbonate, sulphate, chloride, and silica (the concentration of carbonate used here being a conventional value obtained by multiplying by 0·6 the total alkalinity expresed as p.p.m. of calcium carbonate) and expressing each concentration as a percentage of that total.

It was found that the analyses of the streams of each pair then showed close similarities and differed characteristically from those of the streams of other pairs. Thus it was concluded that the differences between the pairs were real and due to differences in geology.

Table 1 shows the mean results for each geological formation as well as figures considered to apply to Tertiary and more recent formations (deduced from analyses from other sources).

Table 1

Geological formation	Mean T.D.S. (p.p.m.)	Calculated percentages Ca	Mg	Na	K	CO_3	SO_4	Cl	SiO_2
Beaufort	38	8·7	4·2	8·0	1·9	41·0	6·0	6·9	23·3
Ecca	52	7·3	4·7	10·1	1·1	44·1	7·6	6·2	18·9
Dwyka	314	8·3	7·3	19·4	0·7	34·3	2·8	23·0	4·2
Table Mountain Sandstone	84	4·3	4·7	19·9	2·2	20·7	8·1	23·8	16·3
Granite	134	6·8	5·3	21·2	1·0	29·5	5·4	21·0	9·8
Tertiary and younger	175	18·0	2·2	14·1	1·5	31·0	6·1	22·5	4·6

Examination of these results (which is aided by any form of graphical representation) shows that the surface waters from Beaufort and Ecca rocks are very similar. This would be expected in view of the lithological similarities between these rocks. The surface waters from Table Mountain Sandstone and granite rocks differ from these in having higher proportions of sodium and chloride, although no ready explanation of this has yet been found. The waters from Dwyka rocks show these features also, together with a lower proportion of silica and a very much higher concentration of total dissolved solids. It is thought that these characteristics arose from the presence in Dwyka formations of tillite made up of relatively unweathered rock fragments which are only now being exposed to the effects of weathering and leaching. Some confirmation of this view arises from the fact that the figures assumed for waters from younger rocks (Tertiary to Recent), which are also relatively unweathered, are rather similar to those obtained for Dwyka waters.

These results are proving very useful in establishing a background for the interpretation of water analyses made in the course of later river surveys.

P. Hayden Kemp

Natal Regional Unit,
National Institute for Water Research,
Durban, South Africa.

A Mid-Labrador Sea Ridge

J. T. Wilson[1] has recently speculated on the presence of a median ridge extending through the Labrador Sea and up Baffin Bay. He suggested that its presence had not been noted because of insufficient sounding data, and that its presence could be verified by additional sounding data.

Although the Labrador Sea is not the best-sounded area of the world oceans, there are sufficient observations to determine that no obvious ridge exists. There are, however, several isolated peaks along the centre of the sea which suggest the presence of buried topography of some magnitude[2].

During 1960 and 1961 joint cruises were made in the Labrador Sea by C.N.A.V. *Sackville*, under the jurisdiction of the Atlantic Oceanographic Group of the Fisheries Research Board of Canada, and R.V. *Vema* of the Lamont Geological Observatory of Columbia University. The research programmes during these cruises were varied and included oceanographical, biological, geological, and geophysical investigations.

Table 1

Station	Latitude *A*	Longitude *A*	Latitude *B*	Longitude *B*	Velocity (km/sec)	Thickness (km.) *A*	*B*
159–160	56–38 N.	52–01 W.	57–01 N.	51–31 W.	1·49	3·43	3·44
					1·98	0·96	0·97
					3·20	1·93	1·33
					5·47	0·84	2·19
					7·67		
161–162	58–31 N.	49–19 W.	59–05 N.	48–37 W.	1·49	3·41	3·23
					1·95	1·51	1·74
					5·54	1·68	2·32
					7·58		

As a part of the 1960 programme, four seismic refraction profiles were observed in the deep part of the Labrador Sea (> 3,000 m) on board the two vessels. The observational methods have been described by Officer *et al.*[3] and the data were reduced following the methods of Officer and Wuenschel[4], Ewing *et al.*[5], and Sutton and Bentley[6]. The results of these observations are given in Table 1 and indicate that the structure beneath the bottom of the Labrador Sea is not that of the typical ocean basin but more closely resembles that found by Ewing and Ewing[7] along the Mid-Atlantic Ridge. There was no indication of a ridge-like feature in the echo-sounding records from the two vessels, however, the major topographic feature being the extension of Mid-Ocean Canyon No. 1 (ref. 8), afterwards traced to 61° N. during the 1961 cruise.

In 1961 a new technique, seismic profiling[9], became available and was put to use during the joint cruise in the Labrador Sea. With this technique, a reflexion method using small explosive charges fired at 2-min intervals, it became possible to see the structure of the rock surface beneath the sediments. Profiler sections were made along two tracks across the Labrador Sea, one from north-east of Newfoundland to Julienhaab, Greenland, the other west from Julienhaab towards Hudson Strait at about 61° N. (Fig. 1).

The results along the two lines are quite similar. Near the continental margins of Greenland and North America, the rock surface is buried deep beneath the sedimentary cover and in these areas it is not possible to detect the rock surface beneath it. As the geographical centre of the Sea is approached, the rock surface (5·5 km/sec) appears on the profiler records and in each case the maximum elevation of this surface is very near to the geographical centre of the Labrador Sea. Along the northern line it is still buried beneath about a kilometre of sediments while to the south it appears above the surface in several places.

Possible extension of this ridge north and south is difficult to verify. It may swing around the tip of Greenland to join the Mid-Atlantic Ridge. There are a few isolated peaks indicated on the bathymetric chart[2] which might represent the highest points of buried topography. On the other hand, it may just die out and disappear. To the north, a shallow (400 m) sill separates the Labrador Sea from Baffin Bay. Lg phases from earthquakes have been observed to propagate across this sill[10]. If continental structure is required for propagation of lg phases[11], then the ridge as such must terminate against this sill and its continuation farther north is questionable. If, on the other hand, lg phases will propagate through a buried ridge structure, then the ridge may continue into Baffin Bay.

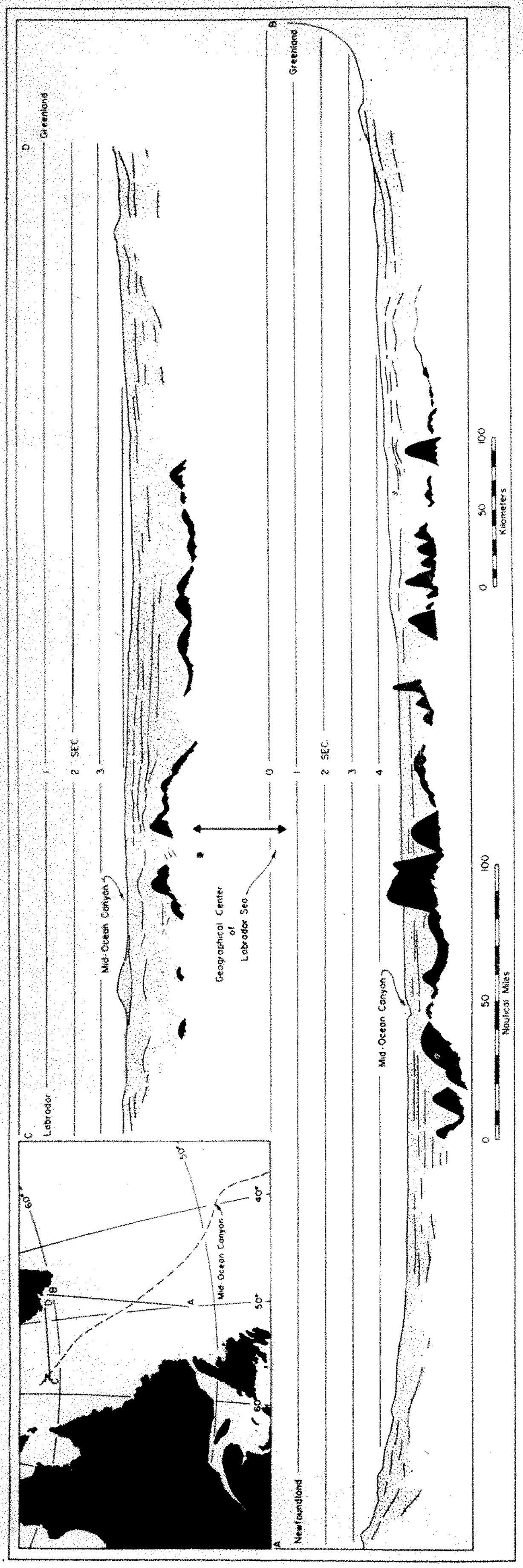

Fig. 1

The symmetry of the Labrador Sea ridge, midway between North America and Greenland, suggests a common mode of origin with the mid-ocean ridge system. There are, however, some significant differences. No distinctive magnetic anomaly, such as is frequently found over the rift in the middle of the mid-ocean ridge[11], was observed and only a few earthquakes have been recorded from this area[12]. These facts, plus the depth of burial of the ridge by sediments and its lower elevation, suggest that the Labrador Sea ridge developed at an early geological time and that its development ceased before it reached the mid-ocean ridge stage.

We thank the naval personnel and scientific parties of *Sackville* and *Vema* for their co-operation. This work was supported by the U.S. Office of Naval Research and the U.S. National Science Foundation and by the Fisheries Research Board of Canada, Royal Canadian Navy, and Dominion Observatory of Canada.

CHARLES L. DRAKE
Lamont Geological Observatory,
Columbia University, N.Y.

NEIL J. CAMPBELL
Atlantic Oceanographic Group,
Fisheries Research Board of Canada,
Halifax.

GEORGE SANDER
Dominion Observatory, Ottawa.

JOHN E. NAFE
Lamont Geological Observatory,
Columbia University, N.Y.

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New Zealand Mid-Tertiary Stratigraphical Correlation

THE apparent absence of certain stratigraphically important planktonic Foraminifera from the Australian and New Zealand Tertiary rocks has made it difficult to correlate these sequences with the more tropical Tertiary sequences[1]. A recent examination of rocks from the North Auckland region of New Zealand has yielded what appears to be a warmer water fauna.

One of the most important short-ranging species in the well-documented Trinidad Oligocene–Miocene section is *Globorotalia kugleri* Bolli[2]. In New Zealand, Hornibrook[3] recorded possible specimens of this species from the Kopuku section, south of Auckland City, from rocks of Otaian age. A few Otaian samples from North Auckland have yielded definite specimens of *G. kugleri*. Samples slightly older than this have yielded specimens of *Globigerina ciperoensis ciperoensis*.

The initial appearance in time of *Globigerinoides trilobus* (Reuss) has until recently been placed at the base of the Awamoan stage[4]; but there is slight evidence that it may start a little earlier than this, possibly in the top of the Otaian[3]. This level would be approximately equivalent to the top of the *G. kugleri* zone in Trinidad (Fig. 1).

New Zealand stages (ref. 5)	Trinidad zones (ref. 2)	New Zealand stages
Awamoan Hutchinsonian Otaian Waitakian	*C. stainforthi* *C. dissimilis*	Awamoan Hutchinsonian
	G. kugleri	Otaian
	G. ciperoensis ciperoensis *G. opima opima* *G. ampliapertura*	Waitakian Duntroonian Whaingaroan

Fig. 1 Correlation of the New Zealand stages with the planktonic foraminiferal zones of Trinidad

Eames, Banner, Blow and Clarke[5] have correlated the Waitakian–Awamoan rocks in New Zealand with the *stainforthi* zone of Trinidad. The recent discovery of definite *G. kugleri* in Otaian rocks shows that this is completely wrong. There is therefore very little evidence for their hypothesis, which shows a large disconformity below the Waitakian Stage in New Zealand.

With further work, now in progress in New Zealand on the Tertiary planktonic Foraminifera, it is hoped to establish a more accurate correlation of the Tertiary with rocks elsewhere.

D. GRAHAM JENKINS

New Zealand Geological Survey,
Lower Hutt, New Zealand.

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PHYSICS

Charge Transfer associated with Temperature Gradients in Ice

THE deviation at temperatures above −7° C from the theoretical value for the charge transfer produced by temperature gradients in ice is shown to be a surface phenomenon.

Recent calculations[1] predict that when a steady temperature difference ΔT is maintained across a uniform specimen of pure ice a potential difference of magnitude $V = 2\Delta T$ mV is developed between its ends. This equation was tested by applying temperature gradients to cylinders of pure ice and was verified for temperatures below −7° C. Above this temperature, however, the measured potentials were greater than those predicted.

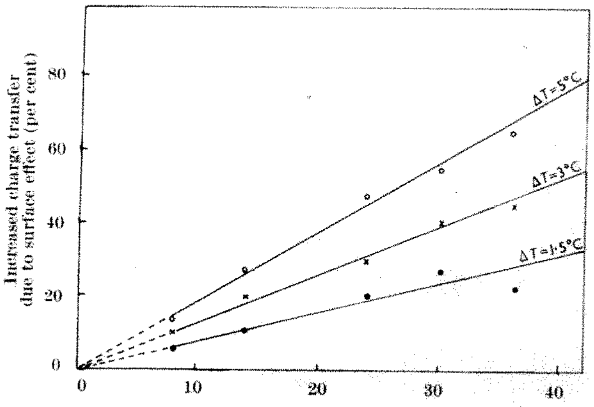

Fig. 1. Effect of the surface-volume ratio on the potentials developed across ice by steady temperature gradients (temperature of colder end of ice cylinder = −7° C)

In order to discover whether the accentuation of the potentials developed across the ice as the temperature of its warmer end approached 0° C was a surface-dependent phenomenon or not, the experiments were repeated for cylindrical specimens of ice of different surface-volume ratio. Curves obtained of V against ΔT for different values of this ratio are shown in Fig. 1. It is seen that as the ratio increases so does the divergence from the theoretical value. Although the accuracy of these experiments was not very great it is evident from the figure that if the straight lines are extrapolated back to zero surface-volume ratio they tend to pass through the origin, that is, showing that the divergence at high temperatures is solely a surface phenomenon, corresponding to an additional transport of charge along a surface layer having a larger variation of conductivity with temperature than the bulk ice. It is not possible to calculate the thickness of such a layer, since both its conductivity and its concentration of mobile ions are unknown.

Weyl[2] has suggested that the molecules on the surface of ice are disarranged and that this distortion extends a finite thickness into the ice. The thickness and adhesive properties of this 'liquid layer' vary with temperature in a similar manner to the divergence of the temperature-gradient potentials from the foregoing equation, but at present there is no direct evidence connecting these two surface properties of ice.

J. LATHAM

Department of Physics,
Manchester College of Science and Technology,
Manchester, 1.

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Epitaxial Growth of Silicon by Vacuum Sublimation

SINGLE crystal layers of silicon up to several μ thick have been deposited on to a heated single crystal silicon substrate from a sublimating source. We believe we can grow good epitaxial crystals at a lower growing temperature than previous experiments[1] by reducing the growth rate in a good vacuum free from contamination.

Silicon slabs of about 18 mm × 1·5 mm × 0·5 mm and 18 mm × 3 mm × 0·5 mm were used as the source and the substrate, respectively, with which preferably {111} planes were used. They were cut, polished, etched and cleaned before they were fixed to tungsten rods with tantalum clips so as to face the widest surfaces about

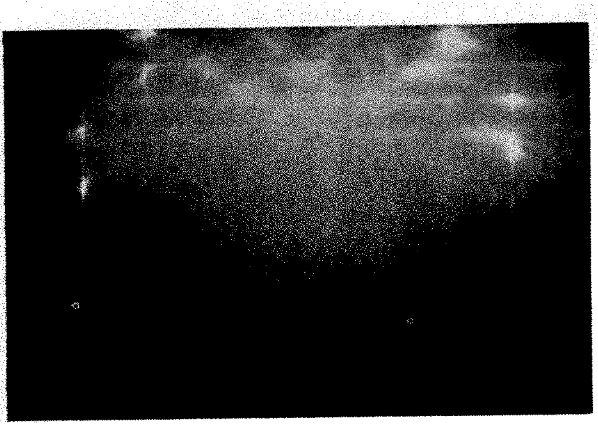

Fig. 1. Electron diffraction photograph of silicon epitaxially grown at 830° C

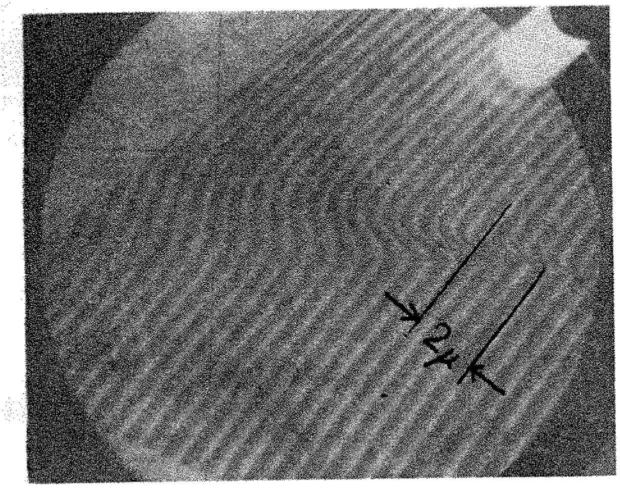

Fig. 2. Interference pattern measuring the thickness of the epitaxial deposit

10 mm apart from each other and sealed in a quartz tube which was evacuated with a Nippon Electric Co. Vac-Ion pump to as low as 10^{-8}–10^{-9} mm mercury. After a good vacuum was attained, both the source and the substrate were directly heated at 1,250° C for about 1 h by flowing a.c. current to the silicon slabs to bake out the adsorbed gas on the surfaces and to etch the substrate in order to get a clean surface[2], which is essential to obtain good epitaxial crystals.

While the temperature of the source was kept at 1,200° C for about 100 h, at which the sublimation rate is about 1×10^{-7} g/cm^2 (ref. 3), that of the substrate was maintained at various temperatures to observe the growing conditions. It was easier to grow shiny and flat epitaxial crystals at higher temperatures.

By keeping the substrate at 830° C for 50 h, we obtained a deposit about 2μ thick and the grown film was examined by electron diffraction at the electron energy of 100 keV. Fig. 1 shows the diffraction pattern revealing Kikuchi lines, which is evidence of the good quality of the crystal. The thickness of the deposited film is measured at the edges of the substrate, which are masked with the tantalum clips with an interferometer. The interference pattern in 546 mμ of a mercury vapour lamp is shown in Fig. 2.

P,n junctions with a maximum reverse breakdown voltage of 200 V have been obtained. The deposited layers of either *n*- or *p*-type were obtained, but the resistivity was not determined because the diffusion velocities of impurities[4] were comparable with the growth rate of the crystal, and the conventional bevelling and staining techniques were useless.

The advantage of epitaxial growth by sublimation is found in the result of the slow growth rate, that is, growth at lower temperatures, which may lead to the epitaxial growth of silicon on other crystals such as germanium or gallium arsenide. So far the growth on germanium is unsuccessful, probably due to the incompletely clean surface of germanium.

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YASUO NANNICHI

Fundamental Research Laboratory,
Nippon Electric Co., Ltd.,
Kawasaki, Japan.

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Colour Centres in Sodium Cyanamide

IRRADIATION of pellets of sodium cyanamide at room temperature with ultra-violet from a high-pressure mercury lamp, or with 2-MeV electrons, results in the formation of a number of absorption bands. The results are shown in Figs. 1 and 2. Looking at Fig. 1, the bands occur at 4250 Å (band *A*) and 5000 Å (band *B*). In addition there is a broad region of absorption around 6500 Å (band *C*) and the possibility of a weak band at 3200 Å (band *D*). These bands bleach on heating, and with *A* and *B* light.

At higher temperatures (110° C) bands *A* and *B* are more intense after irradiation, while at 200° C thermal decomposition can also occur, which itself gives rise to absorption bands[1]. Of these the bands at 3400 Å and 5000 Å have been attributed to the presence of sodium produced by thermal decomposition.

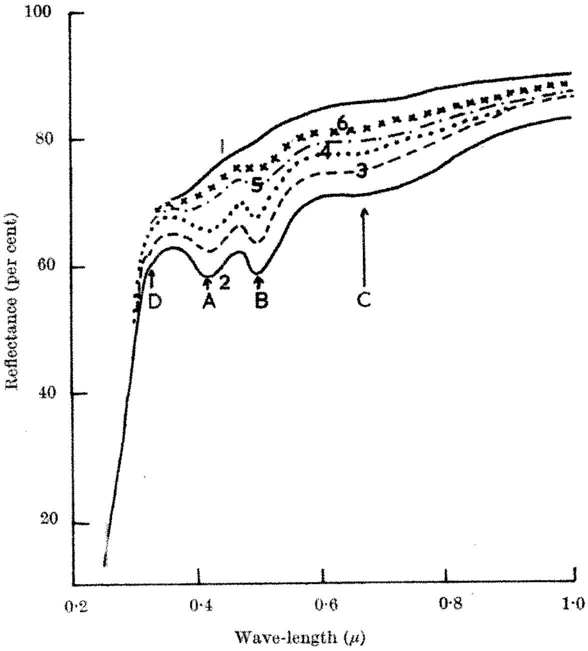

Fig. 1. Effect of ultra-violet irradiation at room temperature in a vacuum on the reflexion spectrum of sodium cyanamide (standard comparison $MgCO_3$). 1, Before irradiation; 2, after 2·5 h irradiation; 3, after 0·5 h anneal at 20° C; 4, after 1·5 h at 20° C; 5, after a further 0·5 h plus illumination with light of wave-length 5000 Å; 6, after a further 0·25 h plus illumination with light of wave-length 4250 Å

The spectra shown in Figs 1 and 2 are similar though, in the case of irradiation with 2-MeV electrons, band *D* is stronger while band *C* is scarcely resolved The intensities of *A* and *B* increase with dose, and tend to saturate, eventually forming a single band with a minimum at 4500 Å The results are similar to the build-up of *F* centres in sodium chloride[2] where the process of defect production is a two-stage one They are also similar to those obtained with sodium azide[3]

Qualitative measurements showed that both ultra-violet and electron-irradiated samples gave rise to an electron spin resonance signal with a *g* value near 2 0 , no such signal was observed in the case of unirradiated samples

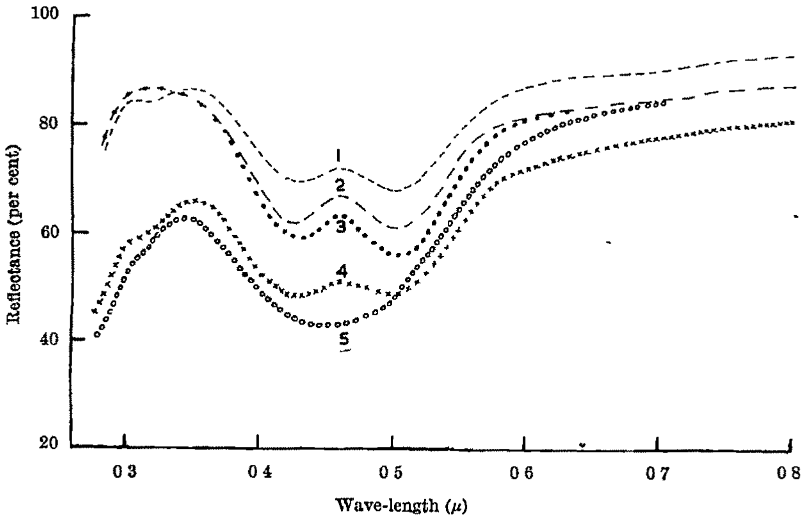

Fig 2. Reflexion spectra of sodium cyanamide pellets irradiated with 2-MeV electrons at room temperature 1, 3 megarad dose, 2, 5 megarad, 3, 15 megarad, 4, 30 megarad, 5, 60 megarad Irradiation was intermittent to avoid overheating, and dose-rate about 1 megarad/min

The results suggest that bands *A* and *B* are due to colour centres of the *F* centre type Band *C* appears more pronounced at higher temperatures of irradiation and coincides with a thermally induced band The properties of the band suggest either impurity or aggregate centres as the type most likely to be responsible The *D* band is best observed at low temperatures (about −100° C). since at high temperatures it may be masked by a thermally induced band at 3400 Å It occurs in the wave-length region normally associated with electron-deficient centres, and is therefore attributed to an unspecified *V*-type centre

It is not at present possible to give detailed information on the nature of the colour centres responsible for the *A* and *B* bands It is likely, however, that they originate from photochemical decomposition in a manner analogous to sodium azide[3], though in this case the efficiency of coloration is much less The experiments indicate that bands *A* and *B* are due to two forms of the same centre, and it is suggested that this is an R_3 centre which can have two orientations (sodium cyanamide is orthorhombic)[4] The R_3 centre would be formed from a divacancy in the manner

$$\square^{\square} + 3e \rightarrow \square^{\square} \quad (R_3 \text{ or } F'^{+}_{2}) \text{ centre}$$

M J SOLE
A D YOFFE

Physics and Chemistry of Solids,
Cavendish Laboratory,
University of Cambridge

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CRYSTALLOGRAPHY

Nucleation of Gypsum Crystals

HETEROGENEOUS nucleation is an important process, and the nucleation of ice crystals in particular has been extensively investigated. The nucleation of crystals of gypsum has a theoretical and industrial interest but has received little attention This communication presents some observations on the capacity of various substances to nucleate gypsum from solution

The hydration of calcium sulphate hemihydrate appears to be initiated by the heterogeneous nucleation of gypsum crystals at favourable sites in mixtures of calcium sulphate hemihydrate and water[1–3] It should be possible, then, to assess the nucleating efficiency of a substance from its effect upon the rate of hydration

We have used the following procedure to determine the effect of various substances on the rate of hydration The material to be tested was added as a powder to 60 0 ml water, and then 100 0 g of finely divided calcium sulphate hemihydrate was added slowly to the mixture After stirring for 30 sec the paste was transferred to a Dewar flask The mixtures were made at 20 5° C The progress of hydration was followed from the increase in temperature and the justification for this procedure has already been given[4] The effect on the rate of the reaction was assessed from the induction period and the position of the curve of $\log_{10}\alpha$ plotted against time (α is the ratio of the increase in temperature divided by the final increase in temperature) The induction period was defined as the time taken for the rate of increase in temperature to reach 0 1° C/min

Despite the fact that hydration is initiated by the heterogeneous nucleation of crystals of gypsum, of the insoluble or sparingly soluble solids tested other than gypsum itself only synthetic γFeO OH has been found to have a significant effect on the rate of hydration The materials tested so far include ground glasses, silica, hæmatite, kaolinite, talc, and many other minerals and solids that for various reasons were suspected of being able to nucleate gypsum

The γFeO OH used in the work was prepared by oxidizing ferrous ammonium sulphate with potassium iodate and sodium thiosulphate[5] X-ray diffraction yielded the pattern of γFeO OH and showed that the material was poorly crystalline Atomic absorption spectroscopy showed that the preparations were free from such quantities of sodium and potassium that could produce an acceleration of the rate by a mechanism other than that of increased nucleation

An example of the effect of γFeO OH is shown in Fig 1, where $\log_{10}\alpha$ is plotted against time for an unaccelerated reaction and for hydration in the presence of 0 05 g γFeO OH The induction period of the unaccelerated reaction was 16 min and that of the accelerated reaction was 12 min Precipitated gypsum (0 5 g) produced almost the same results as the 0 5 g of γFeO OH Several preparations were tried and all were found to be effective

Observations under the microscope were also made on aqueous suspensions of γFeO OH and calcium sulphate hemihydrate, and crystals of gypsum could be seen growing from the particles of ferric oxide monohydrate

If epitaxy is occurring it would be expected that the spacings of the substrate and growing lattice would be similar within certain limits[6] Both gypsum and γFeO OH have layers of hydrogen bonds in the 010 planes and perfect cleavages parallel to the 010 planes Seeing that the oxide has to be ground to render it suitable for experi-

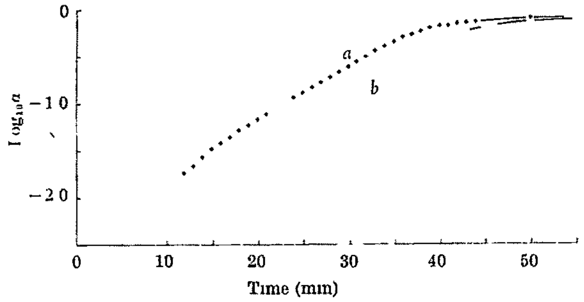

Fig 1 $\text{Log}_{10}\alpha$ plotted against time for 100 0 g calcium sulphate hemihydrate and 60 0 ml. water (*a*) 0 50 g γFeO OH added, (*b*) no additive The induction period for (*a*) was 12 min and for (*b*) was 16 min

ment, it is highly probable that there will be plenty of exposed (010) faces However, calculation shows that the agreement between the spacings of the two lattices is not such that the action of γFeO OH is obviously due to epitaxy on the 010 plane, which would seem to be difficult to reconcile with the experimental facts for another reason A mechanism of this type would result in the *b* direction of the growing gypsum crystals being perpendicular to the surface of the substrate, and growth in the *b* direction is slow If, indeed, orientated overgrowth of gypsum on faces of the microcrystals of γFeO OH is not occurring in mixtures of calcium sulphate hemihydrate and water, topographical features such as steps on the surfaces of the grains of γFeO OH may be providing favourable sites for the development of gypsum nuclei

We thank Mr C Lancucki for the X-ray examination of the samples of γFeO OH

M J Ridge
H Surkevicius

Commonwealth Scientific and Industrial Research
Organization
Division of Building Research
Highett, Victoria, Australia

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X-ray Intensity Statistics of a Pair of Related Crystals

We have been investigating the problem of the probability distributions connected with the X-ray intensities of two crystals Two ideal situations may be considered (*a*) when the two crystals are fully related, that is, when the atoms in one form a part of the atoms in the other, (*b*) when they are unrelated, that is, when the atoms in the two structures are in entirely different positions It is of course assumed that the two unit cells are exactly the same or very nearly so When a protein crystal and its heavy atom derivative form an isomorphous pair, they are related in the foregoing sense, and the present investigation has resulted in the formulæ which could be used to test the isomorphism of such a pair of crystals The term 'relatedness' is used in preference to 'isomorphism' for the more general concept, following the suggestion of some senior crystallographers

We consider a pair of crystals, one containing P, and the other N, atoms ($P < N$, $N = P + Q$) Probability distribution functions can be derived for several variables connected with F_N and F_P, the structure amplitudes of the two crystals Specifically, three such have been considered, namely, the normalized difference

$$w = (|F_N| - |F_P|)/\sigma_N$$

the normalized product

$$Z = |F_N||F_P|/\sigma_N\sigma_P$$

and the normalized quotient

$$V = \frac{(|F_P|/\sigma_P)}{(|F_N|/\sigma_N)} = \frac{|F_P|}{|F_N|} \frac{\sigma_N}{\sigma_P}$$

where σ_P and σ_N are the root mean square intensities of the two crystals, that is, $\sigma_P^2 = <|F_P|^2>$ and $\sigma_N^2 = <|F_N|^2>$

The functions $P(u)$, $P(Z)$ and $P(v)$ all depend on whether the crystal is centrosymmetric or non-centrosymmetric Two situations have been considered in either case (*a*) when the crystals are related, and (*b*) unrelated, and detailed expressions have been derived in every case These are not given here, but only some of their properties will be considered It may be mentioned that the formulæ were derived on the assumption that the number of atoms in the crystals is large, so that the asymptotic distribution may be utilized for each crystal

All the expressions contain a parameter σ_1^2, defined by $\sigma_1^2 = \sigma_P^2/\sigma_N^2$, which is the ratio of the mean intensities due to the P atoms and the N atoms It is also the mean fractional contribution to the intensity of the N-atom crystal from the P atoms in the related case

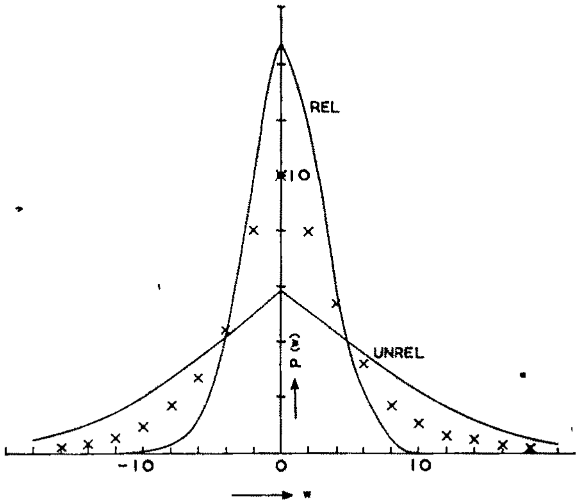

Fig 1 Theoretical probability distribution function $P(w)$ for the related and unrelated case in a centrosymmetric crystal, corresponding to $\sigma_1^2 = 0\ 9$ The crosses are observed data for the pair of crystals, caffeine and theophylline

Of the three variables w, Z and v, the distribution functions of the first and the third, namely $P(w)$ and $P(v)$, widely differ between the related and the unrelated cases These functions can therefore be used to test for relatedness As an example, the function $P(w)$ is shown in Fig 1 for the two cases, corresponding to a value of $\sigma_1^2 = 0\ 9$ It will be noticed that the curve is much sharper in the related case than in the unrelated case The data for $P(w)$, for the pair of crystals, caffeine[1] ($C_8H_{10}N_4O_2H_2O$) and theophylline[2] ($C_7H_8N_4O_2\ H_2O$) which differ only by a group CH_2 and which have very nearly the same unit cell dimensions and therefore expected to be isomorphous, are indicated by crosses It will be seen that these results fall in between the curves for the related and unrelated cases, showing that the isomorphism is not exact

In fact, it is possible to define a quantity "degree of relatedness" (or 'isomorphism'), γ, for such cases by the formula

$$\gamma = \frac{<w>_{\text{obs}} - <w>_{\text{unrel}}}{<w>_{\text{rel}} - <w>_{\text{unrel}}} \times 100 \text{ per cent}$$

which will make γ = 0 for the unrelated case and 100 per cent for the related case For the pair of crystals already mentioned, γ proved to be **38** per cent, showing that the isomorphism was far from exact A similar test with protein crystals will be very valuable for testing for isomorphism

The function $P(w)$ can also be used to calculate the R-value which is to be expected when a part of the structure (for example, the heavy atoms alone) is used for calculating the F_c's This follows from the relation

$$R = \frac{\Sigma||F_N| - |F_P||}{\Sigma|F_N|} = \frac{\int_{-\infty}^{+\infty} |w|P(w)\mathrm{d}w}{\int_{-\infty}^{+\infty} yP(y)\mathrm{d}y},$$

where $P(y)$ is the distribution function for a single crystal (centrosymmetric, or non-centrosymmetric, as the case may be), and is known A table of R as a function of $\sigma_1{}^2$ has been calculated for both the related and unrelated cases, and this has been found to be useful in checking whether the positions of the heavy atoms determined are correct or not

By calculating from $P(w)$ the integral $P_+ = \int_0^\infty P(w)\mathrm{d}w$, that is, the fraction of reflexions for which w is positive, it is possible to put the intensity data of two isomorphous crystals on the same relative scale The details are omitted

Thus, these statistical methods are found to have a number of applications to the study of isomorphous crystals However, it may be mentioned that the foregoing results correspond only to the type of isomorphism in which a small number of atoms are *added* to a crystal The extension of these studies to the case when the isomorphism is of the replacement type is possible and is under investigation

G N RAMACHANDRAN
R SRINIVASAN

Department of Physics,
University of Madras,
India

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Crystallographic Data of Two Seleno-urea Derivatives

As part of an investigation of the crystal structure of several derivatives of seleno-urea and a metal (palladium) the unit cell and space groups of α-benzoyl β-phenyl seleno-urea and α-acetyl β-phenyl seleno-urea have been determined

(*a*) *α-benzoyl β-phenyl seleno-urea* Rotation and Weissenberg photographs, taken with copper $K\alpha$ radiation, showed that the unit cell is monoclinic with the dimensions $a = 19\ 97 \pm 0\ 03$, $b = 5\ 11 \pm 0\ 03$, $c = 13\ 15 \pm 0\ 03$ Å, $\beta = 104\ 0 \pm 0\ 3°$.

The space group is $P2/c$ The observed density was 1 6 g/cm^3 in agreement with the calculated value 1 54 g/cm^3 corresponding to four molecules of α-benzoyl β-phenyl seleno-urea per unit cell The absorption coefficient gave $\mu = 41\ 8$ cm^{-1}

(*b*) *α-acetyl β-phenyl seleno-urea* Crystals are monoclinic with the dimensions $a = 10\ 26 \pm 0\ 03$, $b = 22\ 43 \pm 0\ 03$, $c = 9\ 36 \pm 0\ 03$ Å, $\beta = 113\ 0 \pm 0\ 5°$, deduced from rotation and Weissenberg photographs Copper $K\alpha$-radiation was used The systematic absences are $0k0$ for k odd and $h0l$ for l odd, so that the space group is determined as $P2_1/c$ The density measured by flotation is 1 7 g/cm^3 The calculated value for eight molecules per unit cell gives 1 69 g/cm^3 The absorption coefficient for copper $K\alpha$-radiation is 3 6 cm^{-1}

M PEREZ RODRIGUEZ
M CUBERO
A LOPEZ CASTRO

Division de Ciencias del C S I C,
Department of Physics,
University of Seville, Spain

CHEMISTRY

Detergent (Sodium Lauryl Sulphate)-splitting Enzyme from Bacteria

THE persistence of synthetic detergent compounds in treated sewage effluents and rural water supplies has become an increasing problem[1] Several investigations have been carried out, using methylene blue colorimetric and manometric techniques, of the bio-degradation of these materials by the mixed bacterial flora in sewage and river water[2] Payne and Feisal[3] succeeded in isolating from benthonic soils two strains of bacteria, of which one could utilize lauryl sulphate and the other both lauryl sulphate and alkyl benzenesulphonate as sole carbon sources Investigation of detergent metabolism at the enzyme-level has not yet been reported

Alkyl benzene sulphonates constitute the major class of detergent in use for household purposes and, therefore, play the greatest part in water pollution problems However, examination of the enzymatic degradation of these compounds is complicated by the enormous number of isomers to be considered On the other hand, alkyl sulphates are easily obtained in high purity without cross-contamination of isomers, inorganic sulphate, or other inorganic substances By examining the mode of action of alkyl sulphate-destroying enzymes as a model, clues might be obtained to facilitate investigation of enzyme action on alkyl benzene sulphonates Because it is well known that detergents denature proteins, one wonders how a detergent-destroying enzyme could escape denaturation The investigation recorded here was initiated with these problems in mind

With synthetic minimal media containing sodium dodecyl sulphate (SDS) as both carbon and sulphur source, a gram-negative bacillus was isolated from raw sewage water of the Baltimore Back River Sewage Treatment Plant (As proposed by Schwatz and Pewey, the term 'dodecyl' will be reserved for the true C_{12} radical, and the term 'lauryl' for the mixed aliphatic radicals (mainly C_{12} and C_{14}) derived from coconut oil (*Surface Active Agent*, Interscience Publishers, Inc, New York, 1949)) This strain could also utilize octyl, decyl, tetradecyl sulphate instead of dodecyl sulphate (This series of alkyl sulphate was kindly supplied by Dr R C Johnson of E I DuPont DeNemours and Co)

Detergent utilization was assayed by non-polar complex formation of detergent with methylene blue via chloroform extraction[4] As a product of enzyme activity, sulphate was measured with barium chloranilate[5]

Bacteria were grown at 37° C in the synthetic medium with sodium dodecyl sulphate as sole carbon and sulphur source The cells were collected by centrifugation, washed with 0 14 M sodium chloride solution and disrupted in a French pressure cell[6] All the detergent-splitting activity remained in the ultra-centrifugal supernatant (110,000g for 90 min) and was not lost after dialysis. The enzyme preparation from cells adapted to SDS released sulphate from decyl- and tetradecyl-sulphate as well as SDS. That the enzyme (or enzymes) is formed adaptively is suggested by the observation that the same strain grown with citrate as carbon source did not yield preparations with these detergent-splitting activities The same conclusion was reached in manometric assays using non-growing cells

With SDS as substrate, the crude enzyme preparation consumed only 0 6 μM oxygen during the release of 11 6 μM sulphate This suggests that oxygen does not participate in the release of sulphate and that the enzyme action is the hydrolysis of the sulphate ester linkage, the other product of this type of reaction would be dodecyl alcohol

When the concentration of SDS exceeded 6 7 × 10^{-3} M, enzyme activity was lost However, addition of bovine serum albumin permitted active hydrolysis of SDS at

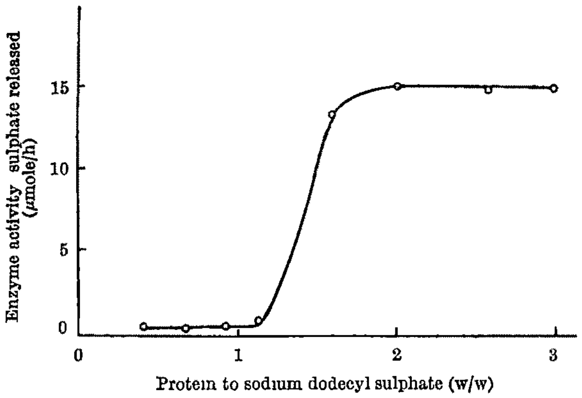

Fig 1 Micromoles of sulphate released in 1 h incubation at 37° C from equal amounts of enzyme in varying ratios of total protein to sodium dodecyl sulphate (SDS) SDS concentration 0 01 M in 0 05 M *tris*-buffer (*p*H 8) containing 0 14 M sodium chloride to a total volume of 1 5 ml

the higher levels of SDS The ratio of total protein to detergent was rather critical, and excess of SDS destroyed the enzyme activity (Fig 1)

Concomitant with the release of sulphate from SDS, protein precipitated from the reaction mixture It is not yet known whether this precipitation resulted from the release of denatured protein from a protein-SDS complex or whether the product of the enzyme reaction, dodecyl alcohol, effected it The latter is a possibility since the alcohol, when added to the system, results in precipitation of protein It is also not known whether the true substrate of the enzyme is protein–SDS complex or free SDS ion equilibrated with such a complex

In this connexion, Putnam and Neurath[7] have demonstrated the formation of protein–SDS complex with mobilities intermediate between those characteristic of the protein and SDS It is also established that SDS forms micelles at concentrations greater than $6–8 \times 10^{-3}$ M, which are in equilibration with the free ions, the latter predominate at low concentration[8] Recently, Bigelow and Sonenberg[9] have demonstrated two different shifts in the ultra-violet spectrum of protein–SDS complex depending on the concentration of detergent The authors suggest that the spectral changes are due to disruption of hydrophobic bonds involving tyrosyl or tryptophanyl residues

The mode of action of SDS concentration below 6×10^{-3} M is under investigation Our bacteria are utilizing the detergent below this level, at which considerable bacteriostatic action is exerted on non-SDS utilizing organisms

Acknowledgement is made to Dr Leonard H Frank for advice and suggestions and to Dr R C Johnson for supplying the alkyl sulphates I thank Dr C W Krusé, and Misses Beverly Jackson and Carole Beach for their help

YU-CHIH HSU

Environmental Microbiology,
Department of Sanitary Engineering,
School of Hygiene and Public Health,
The Johns Hopkins University,
Baltimore 5,
Maryland

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Solubility in Water of C_1–C_9 Hydrocarbons

THE solubilities in water of C_1–C_8 paraffin and branched-chain paraffin, 4 *cyclo*paraffin, and 5 aromatic hydrocarbons have been determined using a gas-liquid partition chromatographic technique

Because of their relatively high solubility in water, the solubilities of benzene, toluene, xylenes, ethylbenzene, and isopropylbenzene have been determined by several workers[1–7] The solubilities of methane and ethane were determined by Winkler[8] in 1901 and substantiated by the work of Claussen and Polglase[9] in 1952 Claussen and Polglase also determined the solubilities of propane and normal butane However, the only solubility data for C_5 and higher paraffin hydrocarbons were those determined by Fuhner in 1924 He cites values for normal pentane, hexane, heptane, and octane So far as is known, solubilities have not been reported for branched chain paraffin or *cyclo*paraffin hydrocarbons

With the development of the technique of directly injecting water samples into a gas chromatograph[10,11], it is possible to determine the solubilities of hydrocarbons by a different technique from those previously reported Saturated solutions of various hydrocarbons in distilled water were prepared by either shaking vigorously on a reciprocal shaker or stirring several days with a magnetic stirrer Phillips Petroleum Co 99+ per cent purity hydrocarbons were used 20–50 ml of individual liquid hydrocarbons were added to distilled water One atmosphere of the gaseous hydrocarbons was maintained over water during equilibration No attempt was made to maintain the temperature closer than the 25° ± 1 5° C maintained by the air-conditioning system in the laboratory Samples of the hydrocarbon-saturated water were obtained from beneath the hydrocarbon phase A·0 05 ml or 0 10 ml sample of the hydrocarbon-saturated water was directly injected into the gas chromatograph fitted with a suitable fractionator containing 'Ascarite' or 'Drierite' to absorb water The dissolved hydrocarbon released from the water passed into the chromatograph column The concentration of hydrocarbon was determined by measuring the area under the chromatograph peak with a planimeter The hydrogen flame ionization detector was calibrated using known amounts of pure hydrocarbons

Table 1 summarizes the solubilities of the saturated and aromatic hydrocarbons having from 1–9 carbon atoms in water at room temperature The measured solubilities for the aromatic hydrocarbons closely check those reported by previous workers The solubilities of *o*-xylene, ethylbenzene, and isopropylbenzene are somewhat lower than

Table 1 SOLUBILITY IN WATER AT ROOM TEMPERATURE OF SATURATED AND AROMATIC HYDROCARBONS

Hydrocarbon		Solubility (g hydrocarbon in 10^6 g water) This work	Previously reported
Methane	8*	24 4 ± 1 0†	21 7 (6)‡, 22 8 (7)
Ethane	4	60 4 ± 1 3	56 6 (6), 58 3 (7)
Propane	5	62 4 ± 2 1	67 0 (7)
n-Butane	8	61 4 ± 2 6	72 7 (7)
Isobutane	6	48 9 ± 2 1	
n-Pentane	12	38 5 ± 2 0	360 (5)
Isopentane	9	47 8 ± 1 6	
n-Hexane	12	9 5 ± 1 3	140 (5)
2-Methylpentane	11	13 8 ± 0 9	
2,2-Dimethylbutane	10	18 4 ± 1 3	
n-Heptane	8	2 93 ± 0 20	50 (5)
2,4-Dimethylpentane	6	3 62 ± 0 10	
n-Octane	6	0 66 ± 0 06	14 (5)
2,2,4-Trimethylpentane	6	2 44 ± 0 12	
*Cyclo*pentane	9	156 ± 9	
*Cyclo*hexane	6	55 0 ± 2 3	
Methyl*cyclo*pentane	10	42 6 ± 1 6	
Methyl*cyclo*hexane	8	14 0 ± 1 2	
Benzene	10	1,780 ±45	1,740 (1), 1,790 (3), 1,775 (4), 1,740 (6), 1,730 (7)
Toluene	7	538 ±17	530 (1), 627 (3), 470 (5)
o-Xylene	6	175 ± 8	204 (1)
Ethylbenzene	6	159 ± 8	168 (2), 208 (3), 140 (5)
Isopropylbenzene	6	53 ± 5	73 (2)

* No of determinations
† Standard deviation of mean
‡ Refs

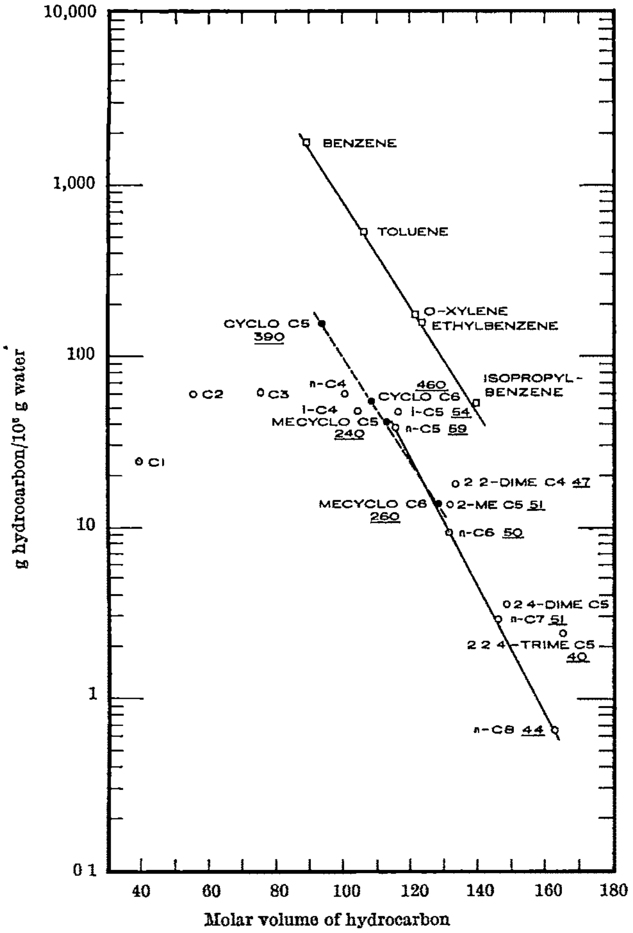

Fig 1 Solubility in water of aromatic □, *cyclo*paraffin ●, and paraffin ○ hydrocarbons as a function of their molar volumes

previously reported The solubilities of methane, ethane, propane, and normal butane are close to the values reported by Winkler, and Claussen and Polglase However, the solubilities found for normal pentane, hexane, heptane, and octane are from 10 to 20 times lower than reported by Fuhner

Lindenberg[12] proposed a relationship between the log of the solubilities of hydrocarbons in water and the molar volume of the hydrocarbons Using solubility data from the literature, he calculated constants for various saturated and aromatic hydrocarbons as well as for chlorinated hydrocarbons If the log of the solubilities of the hydrocarbons in water is plotted against the molar volumes of the hydrocarbons, a straight line is obtained Fig 1 shows such a plot for the saturated and aromatic hydrocarbons The molar volume vs log of the solubility relationship appears to hold for the saturated liquid and aromatic hydrocarbons However, as contrasted to Lindenberg's proposal, all hydrocarbons do not fall on a single line Lindenberg used Fuhner's solubilities for normal C_5, C_6, C_7 and C_8, and, as shown in Table 1, they are much too high The results reported here plot as separate straight lines The aromatic hydrocarbons have considerably higher solubilities for a given molar volume than do the saturated hydrocarbons

The solubilities of the branched-chain paraffin hydrocarbons are higher in all instances than for the normal paraffin hydrocarbons The increased solubilities apparently are not due to a structural feature of the molecules but to the higher vapour pressure (fugacity) of the branched-chain hydrocarbons If the measured solubility of each normal or branched-chain paraffin hydrocarbon with a boiling point higher than 25° C is divided by its vapour pressure in mm mercury at 25° C and multiplied by 760 mm mercury, a predicted solubility at room temperature and 1 atmosphere is obtained The calculated values are shown underlined by the names of the hydrocarbons on Fig 1 The values range from 40 to 59 p p m The lower values appear to be associated with the heavier hydrocarbons The solubilities of ethane, propane, iso- and normal butane range from 49 to 62 p p m Methane with a solubility of only 24 p p m at 1 atmosphere pressure is anomalous With this exception, it appears that to a first approximation the weight of a normal or branched-chain paraffin hydrocarbon which dissolves in water is proportional to the vapour pressure of the hydrocarbon The measurement of hydrocarbon solubilities by the gas chromatographic technique reported here is limited at a concentration of about 0 1 p p m If the solubility data of C_2–C_8 normal and branched-chain hydrocarbons can be extrapolated, the solubility of C_9 and heavier hydrocarbons can be predicted from their vapour pressures

The calculated solubilities at 25° C and 1 atmosphere pressure for the *cyclo*paraffin hydrocarbons are underlined by each *cyclo*paraffin hydrocarbon in Fig 1 The water solubilities of the *cyclo*paraffin hydrocarbons are considerably higher for a given vapour pressure than the normal and branched-chain paraffin hydrocarbons However, the decrease in molar volume for a given carbon number compared with the straight and branched-chain hydrocarbons is such that the *cyclo*paraffin solubilities fall approximately on the line with normal paraffin hydrocarbons However, the dashed line through the 4 *cyclo*paraffin hydrocarbons has a slope parallel with the aromatic hydrocarbons and different from the normal paraffin hydrocarbons

The molar volumes of the aromatic hydrocarbons are less than for *cyclo*paraffin hydrocarbons with the same number of carbon atoms, but the very marked increase in solubility caused by the polar character of the benzene ring more than offsets the decrease in molar volume so that the aromatic hydrocarbons fall on a separate and higher line

The experimental technique and further details of this work will be reported elsewhere

C McAuliffe

California Research Corporation,
La Habra, California

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Attenuated Total Reflectance Spectra of Glycyl-L-alanine in Aqueous Solutions

In a recent report[1] the spectra of various dipeptides in the isoionic, basic, and acidic states in water were examined by transmission techniques with 'Irtran-2' cells, and the use of such spectra for structural and identification purposes was discussed A drawback of the method used is that no information can be obtained in areas where water absorbs very strongly This is particularly true around 6µ (~ 1,660 cm^{-1}), where amino-acids and peptides should show characteristic bands The principle of attenuated total reflectance[2] has enabled the examination of even comparatively weak absorption bands and has permitted spectra to be obtained on difficult samples

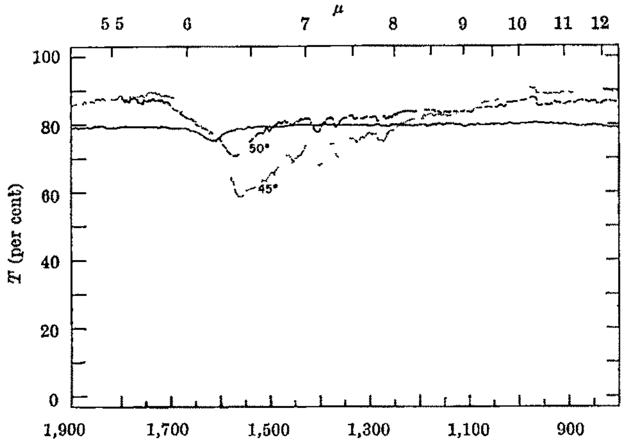

Fig 1 ——, Degree of compensation with two attenuated total reflectance cells containing silver chloride hemicylinders, – – –, the effect of a 50° angle of incidence, , the effect of a 45° angle of incidence

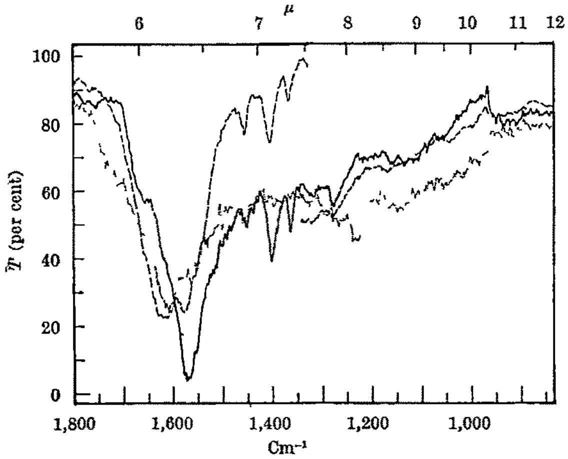

Fig 2 5 per cent (w/v) glycyl-L-alanine, in the isoionic form (——), in the basic form (– – –), and in the acidic form ( )

Spectra of sucrose in water and of whole blood have been shown by manufacturers of infra-red equipment

The purpose of this communication is to show that the use of two attenuated total reflectance accessories allows useful differential spectra to be obtained, particularly in the 6μ region, since the water is not totally absorbing by attenuated total reflectance techniques (With a silver chloride hemicylinder and water in the sample beam versus air in the reference beam, when the baseline is at 90 per cent *T*, the water band has a 50 per cent *T*) The instrument used was a Perkin–Elmer model 421 infra-red spectrophotometer, which was linear in cm^{-1} presentation Both attenuated total reflectance cells were used with silver chloride hemicylinders, the compensating reference cell containing only water

Fig 1 (solid line) demonstrates the degree of compensation and the effect of the angle of incidence (dashed and dotted lines) on band intensity and shape 50° was chosen as the angle of incidence for the runs on glycyl-L-alanine since this angle yields better band shapes at reasonable intensities than does 45° Probably 47° would be still better, but the apparatus has a detent at 50°, which permits locking the optics at this angle

Spectra were recorded for 5 per cent (w/v) aqueous solutions of the dipeptide glycyl-L-alanine in the isoionic, basic, and acidic forms Fig 2 shows these spectra Five times expansion was used on the ordinate scale Significant differences can be seen in the 1,750–1,000 cm^{-1} region Whereas the isoionic and basic forms show what appears to be COO$^-$ absorption (from comparison with transmission spectra[2]) at 1,401 and 1,402 cm^{-1} respectively[3], this band is expectedly missing in the acidic form Another band at 1,364 cm^{-1} in the isoionic form and at 1,368 cm^{-1} in the basic form is missing in the acidic form also, this is also probably a COO$^-$ band[7]

In the region near 1,600 cm^{-1} there are distinct differences between the isoionic, basic, and acidic forms The isoionic form has a band at 1,571 cm^{-1}, which is probably due either to stretching vibrations (CO and CN) arising from the resonating peptide linkage[4] or from NH stretch[5,6] The shoulder at 1,656 cm^{-1} may be associated with the NH_3^+ grouping, but may be associated also with the carbonyl group of the peptide link, which absorbs normally at 1,655 cm^{-1} in the solid-state[7] In the basic form there are bands at 1,579 and 1,620 cm^{-1}, which are probably NH stretching absorptions[7] In the acidic form the band at 1,605 cm^{-1} is probably due to stretching vibrations from the resonating peptide linkage[4] The shoulder at 1,658 cm^{-1} may be associated with the CO of the un-ionized carboxyl group[7] The shoulder at 1,712 cm^{-1} may be the amide-I CO stretch

As with transmission methods, characteristic spectra are obtained, which can be used for identification purposes The advantage of the attenuated total reflectance method lies in the information obtainable in the area where water absorbs strongly by transmission methods

I thank Dr Robert W Hannah, of the Perkin–Elmer Corporation, for recording the spectra and for his advice

FRANK S PARKER*

Department of Biochemistry,
State University of New York,
Downstate Medical Center,
Brooklyn, New York

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* Present address Department of Biochemistry, New York Medical College, New York 29

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Annealing by Compression following Chemical Radiation Damage in Potassium Bromate

RECENTLY it has been shown[1,2] that fragments generated in solid substances by molecular rupture initiated by radiation thermal neutron capture can recombine under the influence of compression These results suggested that other kinds of chemical radiation damage might also be susceptible to annealing by compression At present the only quantitative investigation on annealing of chemical radiation damage in ionic compounds appears to be the thermal annealing investigations on nitrates by Maddock and Mohanty[3–5] In a recent communication Mohanty and Upadhyay[6] have reported that compression of radiation damaged lead nitrate before thermal annealing accelerates the annealing process

Annealing by compression and isothermal annealing investigations have been carried out on radiolysed potassium bromate Boyd *et al* [7] have reported that radiolysis of bromate ion gave bromite, hypobromite, bromide and oxygen gas They also reported, that the oxidizing species in radiolysed alkali metal bromates are susceptible to thermal annealing, but no attempt was made to determine the annealing kinetics The experiments were conducted on a sample of potassium bromate crystals sieved to between 80–100 mesh The crystals were irradiated to a dose of about 50 Mrads with 2-MeV electrons The annealing by compression was carried out using the Tuttle-type of pressure bomb

Table 1 gives the results of annealing by compression at 20° and 135° The samples were compressed in 2 min The experiments at 135° were carried out allowing the sample to reach the temperature before the pressure was applied The equilibrium temperature was obtained after 10 min The 'oxidizing power' representing the oxidizing fragments produced by radiolysis (bromite, hypobromite, etc) was determined by an arsenite–iodide titration The total bromide content representing the total non-bromate bromine produced by radiolysis was determined by a micro-potentiometrical titration with silver nitrate after the oxidizing fragments were reduced by arsenite[7]

Table 1

Treatment	Temperature (°)	'Oxidizing power' (m equiv mole^{-1})	Direct bromide content (m mole Br^{-} mole^{-1})	Total bromide content (m mole Br^{-1} mole^{-1})
None	20	17 1	7 2	12 2
Compressed, 1,000 atm	20	15 9	7 4	12 1
Compressed, 2,000 atm	20	14 3	7 7	12 0
Heated, 10 min	135	16 4	7 3	12 1
Heated and compressed, 10 min, 1,000 atm	135	10 7	8 0	11 3
Heated and compressed, 10 min, 2,000 atm	135	7 1	8 6	10 7

The results show that the chemical radiation damage can be annealed by compression The oxidizing fragments forming hypobromite and bromite on solution are annealed to bromate and bromide forming fragments by compression of the material The isothermal annealing experiments show that these reactions occur by heating the material, too The possibility that the observed effects by thermal annealing arose from aerial oxidation was excluded by showing that virtually the same annealing occurred whether the crystals were heated in air or *in vacuo* Combinations of compression and thermal annealing show that the compression produces two different effects a rapid annealing directly induced by the application of pressure, and the second an acceleration of the thermal annealing processes

I thank Dr J D C MacConnell, Department of Mineralogy, Cambridge, for arranging the compressions

T. ANDERSEN

Department of Chemistry,
University of Aarhus,
Denmark

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Viscosity - Molecular Weight Relationship for Polyvinyl Chloride

THE expression $[\eta]=KM^a$, where $[\eta]$ is the intrinsic viscosity, M, the molecular weight, and K and a are constants for a given polymer-solvent system, is commonly used to relate $[\eta]$ and the molecular weight of the polymer Values of K and a for polyvinyl chloride-solvent systems often show considerable variations for the same solvent Table 1 gives values obtained for *cyclo*hexanone as solvent with the temperature, type of molecular weight and whether unfractionated polymers or fractions were used in the determination of K and a

Table 1 VALUES OF K AND a FOR POLYVINYL CHLORIDE–*Cyclo*HEXANONE

Author(s)	K	a	Comments	Ref
Hengstenberg	$1\ 95=10^{-4}$	0 79	25°, $\overline{M}_n$, unfractionated	1
Breitenbach *et al*	$1\ 16=10^{-4}$	0 85	20°, $\overline{M}_n$, unfractionated	2
Mencık	$2\ 04=10^{-3}$	0 56	25°, $\overline{M}_n$, fractions	3
Danusso *et al*	$2\ 4\ =10^{-4}$	0 77	25°, $\overline{M}_n$, unfractionated	4
Ciampa and Schwindt	$1\ 1\ =10^{-5}$	1 0	25°, $\overline{M}_w$, unfractionated	5
Bier and Kramer	$1\ 13=10^{-3}$	0 63	25°, $\overline{M}_n$, fractions	6

Endo[7] fractionated five different commercial samples and obtained values of a varying from 0 72 to 1 08 from values of $[\eta]$ and $\overline{M}_n$ in *cyclo*hexanone at 30°

We have fractionated a commercial polyvinyl chloride prepared by emulsion polymerization (Breon 121) The polymer was first extracted with hot ethanol to remove emulsifying agent and then fractionated by stepwise addition of 80/20 by volume tetrahydrofuran/water mixture to a 1 per cent solution in tetrahydrofuran Five primary fractions were each refractionated to give a total of fifteen sub-fractions of which six were used in further work Number average molecular weights $\overline{M}_n$ of these were determined osmotically in *cyclo*hexanone at 25 ± 0 01° using Pinner–Stabin osmometers[8] statically The membranes used were of cellulose, treated with 5 per cent aqueous caustic soda before washing in water and conditioning to *cyclo*hexanone This treatment was found to prevent diffusion of low molecular weight material which occurred when untreated membranes were used with solutions of the unfractionated polymer Plots of π/c, where π is the osmotic pressure and c the concentration, against c were slightly curved and plots of $(\pi/c)^{\frac{1}{2}}$ against c were extrapolated to zero concentration to obtain values of $\overline{M}_n$ Weight average molecular weights $\overline{M}_w$ in *cyclo*hexanone at 25° ± 0 01° were determined using a Brice-Phoenix light scattering photometer The refractive index increment was 0 0867 Intrinsic viscosities in *cyclo*hexanone at 25° ± 0 01° were obtained by use of suspended level capillary viscometers Flow times were such as to prevent shear effects and kinetic energy corrections were made where applicable The *cyclo*hexanone was purified and fractionally distilled just before use All solutions were heated for 1 h at 80° and then cooled before being used This procedure, also used by Mencık[3], is necessary to break down rapidly aggregates which, if of high molecular weight material, break down very slowly at 25°

Fig 1 shows logarithmic plots of $[\eta]$ against $\overline{M}_n$ and $\overline{M}_w$ Values of K and a, obtained from these by the method of least squares, are

$$\overline{M}_n \quad K=2\ 08\times10^{-3}\ ,\ a=0\ 56\pm0\ 02$$
$$\overline{M}_w \quad K=1\ 74\times10^{-3}\ ,\ a=0\ 55\pm0\ 02$$

The values of a are in good agreement and those of K and a for $\overline{M}_n$ are in excellent agreement with those of Mencık[3] Values of $\overline{M}_w/\overline{M}_n$ for the fractions are all about 2. The fact that the plots are essentially parallel suggests that the fractions not only have similar values of $\overline{M}_w/\overline{M}_n$ but also have similar molecular weight distribution[9]

Reasons for the values of K and a given by other workers are not entirely clear Diffusion of low molecular weight material in osmotic measurements or the presence of aggregates not removed by prior heating might lead to higher values of the exponent a Different values of K and a could also result from the use of unfractionated polymers with different molecular weight distributions Lower values of a tend to be obtained when fractions are used

It is also possible that polyvinyl chloride prepared by emulsion polymerization differs in chain structure from that obtained by other methods Hengstenberg[1], Mencık[3] and Bier and Kramer[6] used emulsion polymers Other workers[2,4,5,7] used polymers prepared by bulk, solution and suspended polymerization although Danusso *et al* [4]

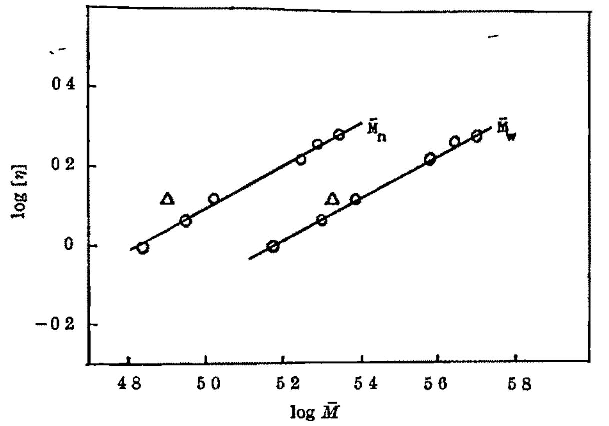

Fig 1 Plots of log [η] versus log $\bar{M}_n$ and log $\bar{M}_w$ for polyvinyl chloride–*cyclo*hexanone at 25°C O, Fractions, Δ, unfractionated polymer

included emulsion polymers Hengstenberg's value apart, values of *a* obtained using emulsion polymers are less than those obtained by use of polymers prepared by other methods It seems probable that this is a consequence of chain branching Bier and Kramer state that their value of 0 63 for *a* refers to branched material Peterlin[10] has shown that appreciable branching of polyvinyl chloride may occur and lead to formation of aggregates Emulsion polymers are generally of higher molecular weight than those obtained by other methods and may be more highly branched

Emulsion polymers of vinyl chloride may generally be more branched than suspension polymers apart from any specific influence of higher molecular weight Polyvinyl chloride is virtually insoluble in vinyl chloride but vinyl chloride is soluble in the polymer to the extent of about 30 per cent This means that during polymerization the effective monomer concentration is lower and the effective polymer concentration higher in emulsion polymer particles than in suspension polymer particles Chain branching is therefore more likely in the former and it has been suggested[11] that the incidence of branching may be three or more times greater in emulsion polymers of vinyl chloride than in polymers obtained by suspension polymerization

It would thus seem to be likely that the low values obtained for the exponent *a* reflect chain branching The values of the polymer–solvent interaction parameter χ_1 was found, in the present work, to be 0 22 This relatively low value suggests that *cyclo*hexanone is a thermodynamically good solvent for polyvinyl chloride and a higher value of the exponent *a* might be expected if the chains were linear

We thank Mr J Benton, of British Geon, Ltd, for useful advice, especially on chain branching of emulsion polymers, and British Geon, Ltd., for the supply of materials

W R MOORE
R J HUTCHINSON

Polymer Research Laboratories,
Department of Chemical Technology,
Institute of Technology,
Bradford

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BIOCHEMISTRY

Action of Bovine and Human Pancreatic Phospholipase *A* on Lipoprotein-bound Phospholipids

IN previous reports from our laboratory some characteristics of the *in vitro* and *in vivo* activity of snake venom phospholipase *A* were described The enzyme was found to split lipoprotein-bound phospholipids present in egg yolk and serum at a higher rate than in their total lipid extracts or than purified ovolecithin[1] Intravenous administration of snake venom phospholipase *A* into rabbits produced a rapid and marked decrease in plasma lecithin, an increase in plasma lysolecithin and attachment of lysolecithin to the red blood cells with ensuing red cell sphering[2] In the work recorded here we investigated whether phospholipase *A* preparations from sources other than snake venom had similar action Phospholipase *A* preparations from both human and bovine pancreas were obtained according to the method of Rimon and Schapiro[3] A standard suspension of the enzymes containing 10 mg protein/ml in 0 04 M veronal buffer *p*H 7 6 was used

The human and bovine phospholipase *A* preparations were acted on egg yolk and plasma, on total lipid extracts of egg yolk and plasma, and on purified lecithin The total lipid extracts and the purified lecithin were prepared as described previously[1] Both the egg yolk and the egg yolk lipid extracts were 5-fold diluted in 0 04 M veronal buffer *p*H 7 6, and the purified lecithin was suspended in the buffer to a concentration of 10 mg/ml The enzymatic splitting of the substrates was followed by free fatty acid release according to the method of Dole[4] The comparative splitting of plasma lipid extract by the two enzyme preparations was followed by the decrease in lecithin content Phospholipids were extracted, chromatographed on silicic acid impregnated paper, and the lecithin spot analysed for lipid phosphorus as described by Klibansky[2]

As seen in Tables 1 and 2, both human and bovine pancreatic phospholipase *A* preparations split the lipoprotein-bound phospholipids at higher rate than the phospholipids in the lipid extracts or than purified lecithin The human pancreatic phospholipase preparation showed higher activity than the bovine on all substrates tested

Table 1 COMPARATIVE ASSAY OF FREE FATTY ACIDS RELEASED IN THE COURSE OF ACTION OF HUMAN AND BOVINE PANCREATIC PHOSPHOLIPASE *A* ON VARIOUS SUBSTRATES

Pancreatic phospholipase *A*	Substrates	Free fatty acids released (µequiv /ml) 10 min	20 min	40 min	60 min
Human	Egg yolk	3 31	6 12	7 74	9 07
Human	Egg yolk lipid extract	2 12	4 62	5 50	5 50
Human	Lecithin	0 47	1 52	2 15	2 32
Bovine	Egg yolk	1 01	1 92	2 99	3 79
Bovine	Egg yolk lipid extract	0 41	0 76	0 76	0 85
Bovine	Lecithin	0	0	0	0

Incubation system 0 5 ml substrate, 0 5 ml enzyme suspension, 0 1 ml ether, 0 05 ml 5 mM calcium chloride, temperature 37° C

Table 2 COMPARATIVE SPLITTING OF LECITHIN IN PLASMA AND PLASMA LIPID EXTRACT BY HUMAN AND BOVINE PANCREATIC PHOSPHOLIPASE *A*

Pancreatic phospholipase *A*	Substrate	Lecithin phosphorus (mg %) Zero-time	After 1 h incubation	Per cent lecithin split
Human	Plasma	2 60	0	100
Human	Plasma lipid extract	2 16	1 25	42
Bovine	Plasma	3 36	1 83	54
Bovine	Plasma lipid extract	3 12	2 50	20

Incubation system 1 ml substrate, 1 ml enzyme suspension, 0 1 ml ether, 0 05 ml 5 mM calcium chloride, temperature, 37° C

Pancreatic phospholipase *A* preparations were injected into the ear vein of rabbits kept on a regular diet After various time-intervals blood was withdrawn by heart puncture for determination of plasma lecithin and lysolecithin by means of silicic acid paper chromatography[2] Blood samples from the ear vein were examined for red blood cell shape The bovine pancreatic phospholipase preparation was administered in two portions, each

Table 3 CHANGE IN PLASMA LECITHIN AND LYSOLECITHIN AND IN RED CELL SHAPE FOLLOWING INTRAVENOUS ADMINISTRATION OF BOVINE PANCREATIC PHOSPHOLIPASE *A* IN THE RABBIT

Time of blood collection	Lecithin phosphorus (mg %)	Lysolecithin phosphorus (mg %)	Red cell shape
Before injection	3 10	0 41	Bidiscoid
20 min after 1st injection	2 22	0 60	Coarsely crenated spheres
20 min after 2nd injection	1 61	0 41	Coarsely crenated spheres

Second injection 45 min after first injection

containing 50 mg protein suspended in 5 ml veronal buffer, at 45-min interval As seen in Table 3, plasma lecithin decreased during a 65-min observation period, while lysolecithin first increased and afterwards reached the pre-injection level The red blood cells became coarsely crenated spheres and retained this shape during the observation period In contradistinction to the well-tolerated bovine pancreatic phospholipase preparation, intravenous administration into rabbits of human pancreatic phospholipase preparation, in an amount containing 50 mg protein, caused rapid death Using smaller amounts, the animals survived but no red cell shape change appeared, pointing to a lower degree of plasma lecithin splitting The cause of the toxicity of the human pancreatic phospholipase *A* preparation has not been elucidated.

These results are in agreement with the previously reported results concerning the action of snake venom phospholipase *A*[1,2], both as to the higher rate of splitting of lipoprotein-bound phospholipids *in vitro* and the conversion of plasma lecithin to lysolecithin with associated red cell sphering *in vivo* A similar action *in vivo* has been described for bee venom phospholipase *A* by Habermann and Krusche[5] It is evident that lipoprotein-bound phospholipids are readily attacked, both *in vitro* and *in vivo*, by phospholipase *A* obtained from diverse sources

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ELEANOR CONDREA
CHAYA KLIBANSKY
RUTH KERET
ANDRE DE VRIES

Rogoff Medical Research Institute,
Department of Experimental Biology,
Tel Aviv University and Labour Sick Fund,
Beilinson Hospital, Petah Tikva, Israel

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Kinetics of Collagen Synthesis by Established Mammalian Cell Lines

IT was recently reported that certain established lines of mouse fibroblasts, capable of indefinite propagation in culture[1], were able to synthesize appreciable quantities of collagen[2] The collagen appeared as extracellular fibres of characteristic periodicity between cells growing in multiple layers on the surface of a Petri dish This communication examines the temporal relationship between cell growth and the expression of this differentiated function

A trypsinized suspension containing 5 × 10^4 log phase cells of established line *3T6* was inoculated into 50 mm plastic Petri dishes The cells attached to the surface and began to grow within approximately 12 h They then grew exponentially for about 3 days with a mean doubling time of 13 h (Fig 1*A*) Thereafter, the growth rate declined rapidly, and the culture soon reached a saturation density of about 9 × 10^6 cells, no further increase in cell number occurred for the 24 days of this experiment, although the supernatant liquid medium was changed three times weekly Throughout this period the majority of the cells remained viable, trypsinization producing a suspension of healthy-appearing cells with a plating efficiency of 80 per cent on X-irradiated feeder cells

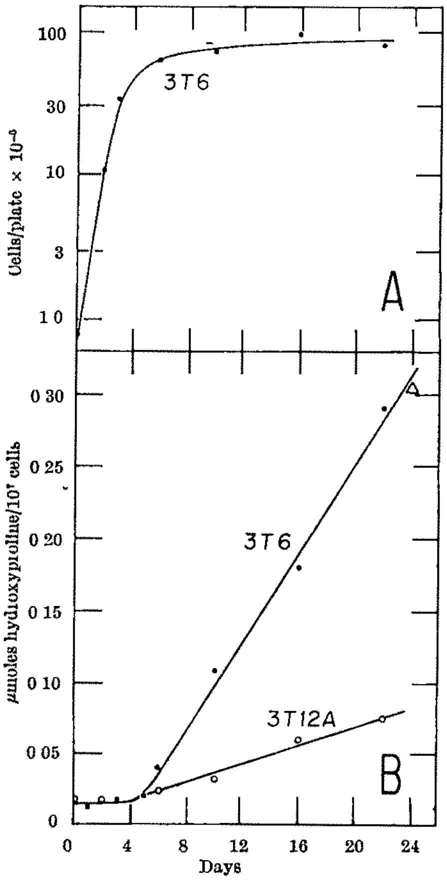

Fig 1 *A*, Growth curve of line *3T6* Day 0 is the day following inoculation *B*, Hydroxyproline formation by established lines *3T6* and *3T12A* Δ indicates determination of hydroxyproline on *3T6* cell layer after isolation of the pure amino-acid by chromatography

The synthesis of collagen by the cells was examined by hydroxyproline determinations[3] on the washed and hydrolysed cell layer Fig 1*B* (top curve) shows the quantity of hydroxyproline found in the cell layer at various times Through the exponential growth phase, low constant values were obtained, equivalent to 0 017 μmoles of hydroxyproline per 10^7 cells As the method used is not absolutely specific for hydroxyproline, a hydrolysate of 2 × 10^6 log phase cells was subjected to column chromatography and the eluate analysed for the pure amino-acid Since none could be detected (less than 0 0003 μmoles per 10^7 cells), the low constant values of days 0–4 in Fig 1*B* do not represent hydroxyproline but rather non-specific colour formation

At day 5, when the number of cells approached a maximum, hydroxyproline appeared in the cell layer and increased linearly with time throughout the remainder of the experiment The constant rate of synthesis during this time for line *3T6* was approximately 0 016 μmoles of hydroxyproline per 10^7 cells per day Chromatographic isolation of hydroxyproline from a replicate stationary phase culture on day 24 gave a value of 0 30 μmoles per 10^7 cells, in good agreement with determinations made directly on the hydrolysate (see Fig 1*B*) Another established line, *3T12A*, reached a similar saturation density and then began to produce collagen, though at a lower rate (0 005 μmoles hydroxyproline/10^7 cells/day) It is concluded that collagen does not begin to accumulate in the cell layer until the period of rapid growth is completed

This conclusion is supported by electron microscopic data from thin sections of replicate cultures at different times. Log phase cultures did not contain extracellular fibrils. Stationary phase cultures, beginning at day 5, showed abundant extracellular fibrils, which later developed the banding typical of collagen. Stationary phase cells showed the cytodifferentiation of protein-secreting cells.

These experiments did not rule out the possibility of liberation of soluble collagen into the liquid medium by log phase cells. Cultures in log and stationary phases were therefore incubated for 12 h with proline labelled with carbon-14, the cold trichloroacetic acid-insoluble protein of the cells and medium of each culture was then hydrolysed. Carrier hydroxyproline was added to the hydrolysates and re-isolated chromatographically. The log phase cells and medium together contained only 6 per cent of the hydroxyproline radioactivity of the saturation density cultures.

During the establishment of line 3*T*6, the cells were transferred regularly every 3 days at an inoculum size of 6×10^5 cells, for about 100 cell generations. Under these conditions, the cells never reached a stationary phase and therefore could not have synthesized appreciable collagen. Nevertheless, this differentiated cell function was not lost during the period when it could not be expressed. The synthesis of collagen by these cell lines offers a clear demonstration of the often stated antagonism between growth and differentiation in mammalian systems. The nature of the changes which result in the arrest of cell division and the onset of collagen synthesis is the object of continuing investigation.

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HOWARD GREEN
BURTON GOLDBERG

Department of Pathology,
New York University School of Medicine,
New York 16

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Ultra-violet Photolysis of Unsaturated Fatty Acids in Relation to the Thiobarbituric Acid Test

A CHARACTERISTIC pink coloration was observed by Kohn and Liversedge[1] after heating tissue suspensions with aqueous thiobarbituric acid (TBA). Colour formation was attributed to reaction of unknown oxidation products of unsaturated fatty acids[2]. The principal TBA-reactive substance is considered to be malonaldehyde[3,4]. However, the means of formation of malonaldehyde is unknown. Inasmuch as ultra-violet irradiation of unsaturated fatty acids had been used to produce TBA-reactive products[5], investigation of the radiation-induced oxidation was conducted in this work in an attempt to clarify the basis of the TBA test.

Five g of linolenic acid (Hormel Foundation, Austin, Minn.) were placed in the bottom of a Petri dish (80 mm diam.) and were irradiated for 18 h by placing two unfiltered Mineralight lamps (Ultra-Violet Products, Inc., South Pasadena, Calif., model *SL* 2537) on the dish. The lamps and dish were covered with aluminium foil and linen cloth. The ambient temperature below the foil was 40°–45° C.

The viscous yellow product was transferred, with four 10-ml water washings, into a 250-ml separatory funnel. Thirty ml of water were added, and the mixture was shaken vigorously for 30 sec. After 15 min, the opalescent lower phase of approximately 50 ml was collected. The remaining mixture was extracted three times with 50-ml aliquots of water. The aqueous extracts were combined and filtered, with gentle suction, through three glass fibre disks (H. Reeve Angel and Co., Ltd., No. 934-*AH*, 2.1-cm diam.) in a Gooch crucible mounted on a filter flask. The filtration was repeated several times with fresh disks until the extract was water-clear. The disks were discarded. The filtrate was diluted to 250 ml with water and washed by shaking successively with 60 ml of hexane and 60 ml of carbon tetrachloride. The washings did not give rise to a pink chromogen in the TBA test of Waravdekar and Saslaw[6] and were discarded. The aqueous phase was concentrated by lyophilization until about 10 ml remained. After thawing, aliquots were diluted with water and ultra-violet absorption spectra were recorded. The diluted aliquots were also assayed for malonaldehyde[6,7] by heating during 20 min with the TBA reagent. Results were expressed in absorbance units at 532 mμ and 225 mμ and were termed the activity (TBA or ultra-violet) in the particular test.

Fig. 1 shows the spectra obtained with extracts prepared in like manner from irradiated and non-irradiated linolenic acid. Irradiation resulted in the appearance of pronounced absorption at 225 mμ. Inasmuch as the hydroperoxides of unsaturated fatty acids absorb maximally at about 234 mμ and malonaldehyde absorbs maximally at 245 mμ[8], the predominant spectral feature resulting from the irradiation process may not be ascribed to the presence of either material. The TBA activity of the extract of irradiated and non-irradiated lipid was 87.5 and 0.5 units respectively. When calculated on the basis of malonaldehyde, these values corresponded to 124.0 and 0.7 μg respectively. The spectral characteristics of the chromogen solutions in the TBA test differed from those observed with malonaldehyde only in displaying slight absorption at 450–480 mμ.

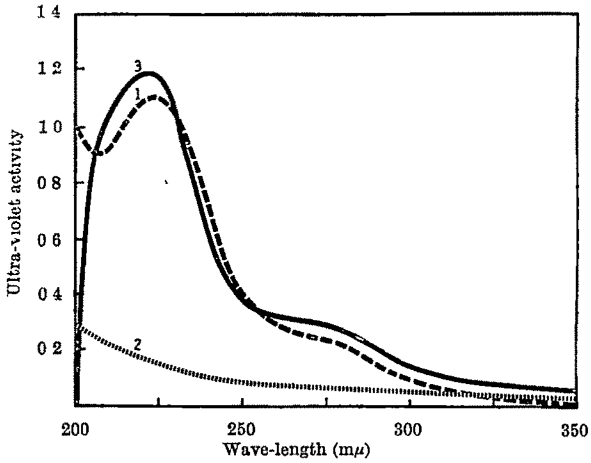

Fig. 1. Absorption spectra of diluted extracts. 1, from irradiated linolenic acid; 2, from non-irradiated linolenic acid; 3, ethanolic extract obtained from chromatogram.
Spectra were determined for a 10-mm light path with a Perkin-Elmer 202 spectrophotometer and a water blank or ethanol blank.

To investigate the nature of the TBA-reactive material, counter-current distribution studies were conducted with a glass train of 20 cells (E-C Apparatus Co., Philadelphia). A portion of the extract of irradiated linolenic acid was subjected to 19 transfers in an *n*-butanol–ethylenediamine solvent system (*p*H of aqueous phase = 8.3). Approximately 48 per cent of the TBA activity was recovered in the aqueous phase. The results (Fig. 2) revealed the presence of two TBA-reactive components. Neither component could be distinguished from malonaldehyde on the basis of the spectra obtained in the TBA test except for slight absorption in the region of 450–480 mμ. Tubes 2–4 were pooled and, in comparison with fresh aqueous phase, were found to display a pronounced absorption maximum at 225 mμ[9]. The minor component (pooled tubes 10–12) exhibited a slight absorption maximum at 220–225 mμ.

Similar results were obtained with an extract of irradiated arachidonic acid.

Extracts of irradiated methyl linolenate and irradiated methyl arachidonate were also prepared. All extracts were fractionated by using thin-layer chromatograms. Glass plates (20 cm × 20 cm) coated with a 300-μ layer of 'Camag-*D5*' silica-gel (Fluka AG, Buchs SG, Switzerland) were activated at 105° C for 3 h. The plates were spotted with 50–100 μl. of each extract. Malonaldehyde, obtained by distillation from the bisulphite addition compound[8], was spotted at references sites. A solvent mixture (*n*-butanol/dichloromethane/water, 20 : 4 : 1) was allowed to ascend along the plates during 4 h. The plates were brought nearly to dryness in a hood, sprayed with the TBA reagent, and heated at 65°–70° C for 5 min. With each extract, one yellow spot ($R_F = 0{\cdot}8$) and two pink spots ($R_F = 0{\cdot}9$ and 0·6) were obtained (Fig. 3). Malonaldehyde migrated only slightly and with considerable tailing ($R_F = 0{\cdot}25$). No proof of its presence in the extracts was evident. In further investigations with an extract of irradiated linolenic acid, parallel unsprayed regions were scraped from the chromatogram and were extracted with ethanol. The ethanolic extract corresponding to the leading spot possessed 10 times the TBA activity of the hindmost TBA-reactive region and also contained most of the ultra-violet activity of the extract. Sixty per cent of the TBA activity was recovered.

Consequently, by using different procedures of fractionation (counter-current distribution and thin-layer chromatography), two TBA-reactive components were detected. Less than 0·3 μg of malonaldehyde may be visualized on the chromatogram. Because products other than malonaldehyde account for the major portion of the TBA activity, it is considered that malonaldehyde is not present in the extracts.

It was also noted that, unlike malonaldehyde, the extracts of the irradiated lipids failed to produce colour with the Claisen reagent[10] despite the existence of an adequate level of malonaldehyde as ascertained with the TBA test.

The ultra-violet absorption spectra of all extracts were similar. With the extract of irradiated linolenic acid, the ethanolic extract of the predominant TBA-reactive site on the chromatogram exhibited a peak at about 225 mμ (Fig. 1). Absorption in the far ultra-violet region was considerably less than was observed for the unfractionated extract.

Association of the ultra-violet activity with the major portion of the TBA activity in each procedure of fractionation supports the tentative assignment of both activities to one compound. Preliminary studies have implicated an unsaturated carbonyl component.

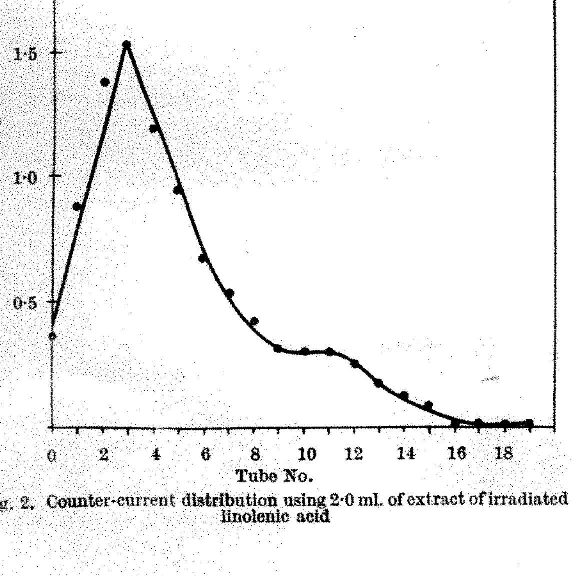

Fig. 2. Counter-current distribution using 2·0 ml. of extract of irradiated linolenic acid

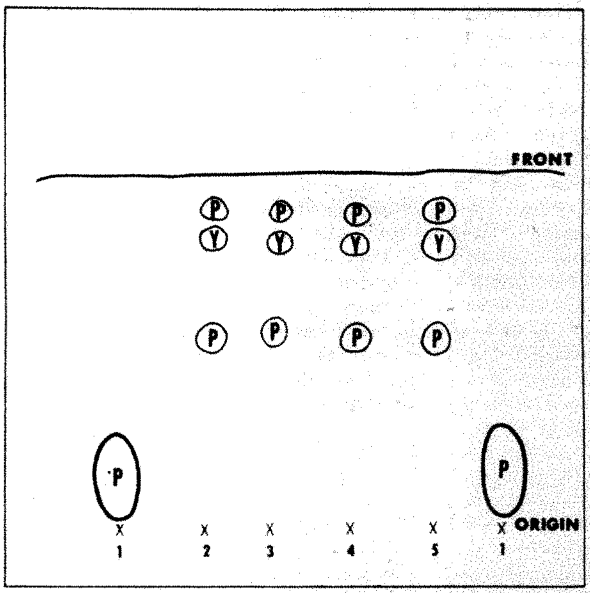

Fig. 3. Thin-layer chromatogram after visualization with TBA
P, pink ; *Y*, yellow

Extract: 1, malonaldehyde ; 2, linolenic acid ; 3, arachidonic acid ; 4, methyl arachidonate ; 5, methyl linolenate

Fränz and Cole[11] recently separated several 'TBA-positive' compounds from irradiated methyl linolenate by gas chromatography. The presence of several TBA-reactive compounds is of interest in view of the prevailing belief that malonaldehyde is responsible for the characteristic test.

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L. D. Saslaw
H. J. Anderson
V. S. Waravdekar

Biochemistry Branch,
Armed Forces Institute of Pathology and the
Veterans Administration Central Laboratory for
Anatomic Pathology and Research,
Washington 25, D.C.

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Uptake of Particulate Matter within the Thymus of Adult and New-born Mice

In 1961 Marshall and White[1] proposed the presence of a 'blood thymus barrier' to explain the failure of the thymus to react to antigenic stimuli given by ordinary routes of administration. Furthermore, because of the exclusion of the thymus from normal auto-antigenic stimuli it was suggested by these authors that the thymus might be the proliferation site of 'forbidden clones' of lymphocytes[2].

This investigation was undertaken to examine the uptake by the adult and new-born mouse thymus of materials ordinarily used to study the reticuloendothelial system, and thereby evaluate the 'blood thymic barrier'.

Adult (about 25 g) mice of *Cf* and *CFW** (Carworth Farms, New City, N.Y.) strain, and new-born mice of *B6AF*1 and *LAF*$_1$, Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine, strain were used. The following materials were administered: trypan blue (National Aniline Division, Allied Chemical Corp.), 'Thorotrast' (Testagar and Co., Inc., Detroit, Mich.), carbon 'Pelikan' ink (Gunther Wagner, Germany), and fluorescein-labelled human albumin, egg albumin and human γ-globulin. Coupling to fluorescein isothiocyanate was performed according to the method of Rinderknecht[3]. The coupled proteins were then heat denatured[4]. The dosages and route of administration of these materials are shown in Table 1.

Table 1

Substance injected	Route	Dose	Time of killing after administration (h)
		Adult mice	
Trypan blue	Subcutaneous	2% in H_2O 0·3 c.c./mouse	24–72
'Thorotrast' (24%)	Intravenous	0·2 c.c./mouse	6–8
Carbon 'Pelikan' ink	Intravenous	0·65% suspension 0·1 c.c./mouse	6–8
Fluorescein-labelled proteins (heat denatured)	Intravenous and intraperitoneal	0·6 c.c. of 0·1% solution, half-dose given intravenous; other half given intraperitoneal	6
		New-born mice	
Trypan blue	Subcutaneous	0·05 c.c. of 0·7% solution in H_2O/mouse	24
'Thorotrast' (24%)	Intravenous*	Dilution 1 : 5, 0·05 intravenous	6
Carbon 'Pelikan' ink	Intravenous*	0·05% solution 0·05 c.c./mouse	6
Fluorescein-labelled proteins (heat denatured)	Intravenous*	0·1 % solution 0·05 c.c./mouse	6

* Retro-orbital vein.

Tissue sections were prepared according to standard methods of alcohol dehydration and paraffin embedding. Sections were cut at 6μ and were stained with alcoholic eosin; at no time were they exposed to concentrations of alcohol below 80 per cent. The tissues containing the fluorescein-labelled proteins were processed in the cold according to the technique of Sainte-Marie[5], and were examined with a Leitz ultra-violet microscope.

Particulate matter within or associated with blood vessels or in fibrous septum and connective tissue was scored as negative. Only those thymuses showing particulate material widely distributed in many cells (macrophages) of the thymic parenchyma were considered positive. In each animal the liver and thymus were always examined. The spleen and lymph nodes were examined in fewer animals.

The results are shown in Table 2. The numerator indicates the number of organs showing uptake; the denominator shows the total number of organs examined. With two exceptions, the thymuses stained only in those animals with stained livers. Cells containing particulate material were present both in cortex and medulla. In adult mice trypan blue was found in the thymus in almost half the cases. 'Thorotrast' and carbon were found in the thymus infrequently. Denatured fluorescein-labelled proteins were never found in the thymus. In new-born mice trypan blue was infrequently found in the thymus. However, here the liver also showed poor uptake. In contrast, thorotrast and carbon were found frequently in the thymus (Fig. 1). Finally, fluoresceinated proteins were found infrequently in the thymus.

Most investigators have indicated that the thymus responds poorly if at all to antigen given to an animal at a site distant from the thymus[6]. However, Stoner *et al.*[7] have found that thymus can produce antibody, as demonstrated by pre-immunized intra-ocular thymus grafts functioning in irradiated mice. Marshall and White[1] have shown that antigen injected directly into the thymus of rats and guinea pigs does stimulate plasma cell production of specific antibody; they therefore proposed the concept of a 'blood thymus barrier' to explain the failure of thymic immunological activity.

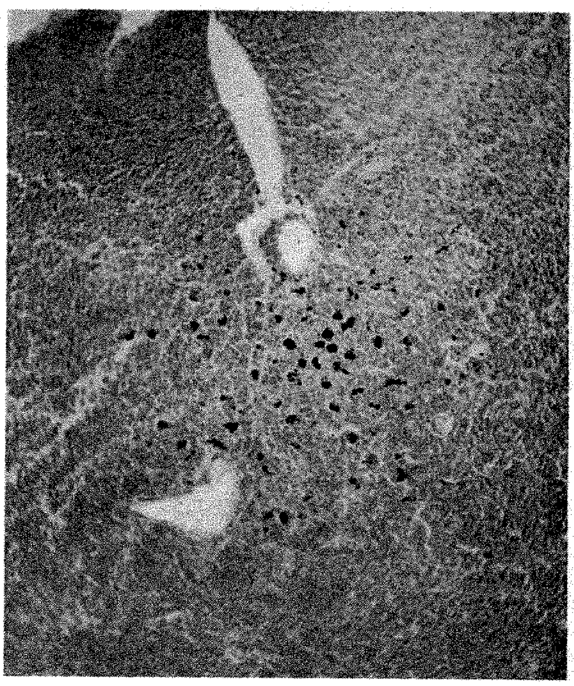

Fig. 1. Thymus of new-born mouse showing macrophages containing carbon. Localization is mainly in medulla

There has been no recent large-scale systematic investigation of the entry of materials into the thymus. However, early investigations of Kaplan and Coons[8] using pneumococcal polysaccharide indicated that this material reached the thymus in large amounts. Marshall and White[1], using a few animals, indicated that trypan blue and pneumococcal polysaccharide reached the thymus only rarely. Svet-Moldavsky *et al.*[9] have recently reported that foot-pad and subcutaneous injection of Freund's

Table 2

	No. staining/total
Adult mice	
Trypan blue	
Liver	29/33
Spleen	28/28
Thymus	15/33
Inguinal lymph node	8/18
'Thorotrast'	
Liver	17/17
Spleen	14/14
Thymus	2/17
Inguinal lymph node	4/4
Carbon	
Liver	18/18
Spleen	11/12
Thymus	2/18
Inguinal lymph node	5/8
Fluorescein-coupled protein*	
Liver	12/17
Thymus	0/17
New-born mice	
Trypan blue	
Liver	7/24
Spleen	3/23
Thymus	4/24
'Thorotrast'	
Liver	31/31
Spleen	4/4
Thymus	7/31
Carbon	
Liver	27/30
Spleen	3/3
Thymus	14/30
Fluorescein-coupled proteins†	
Liver	57/67
Thymus	4/67

* 6/17 injected with fluorescein-labelled human albumin
11/17 injected with fluorescein-labelled human γ-globulin.
†28/67 injected with fluorescein-labelled human albumin.
36/67 injected with fluorescein-labelled egg albumin.
3/67 injected with human γ-globulin.

adjuvant lead to the formation of follicular structures and plasma cells within the thymus, and have suggested that Freund's adjuvant may reach the thymus after injection at a distant site.

The present investigation indicates that trypan blue reaches the adult thymus easily, and that 'Thorotrast' and carbon are frequently found in the new-born mouse thymus. The reason that the thymus does not respond to ordinarily administered antigenic stimuli may be more complex than failure of antigen to reach thymic tissue.

The fact that the thymus of the new-born appears more permeable to particulate material than the adult thymus is of interest and deserves further investigation.

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IRA GREEN
KATHRYN BLOCH

Department of Hematology,
Laboratory Division,
Montefiore Hospital,
New York.

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Toxicity and Fluorescence Properties of the Aflatoxins

THE isolation, characterization and some of the properties of four closely related components of aflatoxin, the mixture of toxic metabolites produced by certain strains of *Aspergillus flavus*, have been described previously[1]. These components were named aflatoxins B_1, B_2, G_1 and G_2. Structural formulæ have been proposed for aflatoxins B_1 and G_1 (ref. 2) and the relationships between these compounds and B_2 and G_2 have been established[1,3], the double bond in the terminal dihydrofuran ring in the first two components having become hydrogenated in the others.

This communication is concerned with the relative intensities of fluorescence of the four major components and their acute toxicities when administered to day-old ducklings.

The peak fluorescence intensities of the aflatoxins in methanol solution were measured in arbitrary units on an Aminco-Bowman spectrofluorimeter. Freshly re-crystallized material and freshly prepared solutions (0·2 μg/ml.) were used. This concentration was in the range where a linear relationship existed between concentration and fluorescence intensity. The relative fluorescence intensities[4] KQ of the four components were obtained by comparing the intensity of the solutions (calculated to 1 μg/ml.) with that of a solution of quinine sulphate of the same concentration in 0·1 N sulphuric acid when excited at 350 mμ and measured at 450 mμ. The KQ values are recorded in Table 1. In the case of aflatoxin B_1 KQ was found to rise to 1·5 after 3 h and then fall again to 0·5 after a further 17 h.

Table 1. FLUORESCENCE PROPERTIES OF THE AFLATOXINS

Aflatoxin	$\lambda_{\text{exitation max.}}$ (mμ)	$\lambda_{\text{emission max.}}$ (mμ)	KQ*
B_1	365	425	0·5
B_2	365	425	4
G_1	365	450	2·5
G_2	365	450	6·5

$$* \; KQ = \frac{\text{fluorescence intensity of aflatoxin solution}}{\text{fluorescence intensity of standard quinine sulphate solution} \times \text{concentration of aflatoxin solution } (\mu\text{g/ml.})}$$

The acute toxicity of each compound was determined by biological assay using day-old Khaki Campbell ducklings (average weight 38 g). Various dose-levels of each compound dissolved in dimethylformamide were administered to groups of 8 fasting ducklings. Each bird received 0·1 ml. by tube into the gizzard and mortality was recorded for 6 days after administration. Histopathological examinations of the livers were carried out to determine whether death was due to aflatoxin poisoning. The four compounds were found to produce similar histopathological hepatic changes which were indistinguishable from the lesions seen in field cases of aflatoxicosis in ducklings[5]. The LD_{50} value for each compound was calculated on the basis of 50 g body-weight day-old duckling. Under these conditions the LD_{50} for B_1 (3 assays) was 18·2 μg (5 per cent fiduciary limits 14·0–23·8 μg), for B_2 (single assay) 84·8 μg (5 per cent limits 65–110 μg), for G_1 (2 assays) 39·2 μg (5 per cent limits 27·1–56·7 μg) and for G_2 (2 assays) 172·5 μg (5 per cent limits 158–188 μg). No deaths occurred in control groups dosed with 0·1 ml. dimethylformamide alone nor were any hepatic changes observed on histopathological examination.

From these results it can be seen that reduction of the isolated double bond in the terminal dihydrofuran ring of B_1 and G_1 to give B_2 and G_2, respectively, lowers the toxicity. The reduction in activity is very similar in both series, the ratio of the LD_{50} values of $B_1 : B_2$ being 1 : 4·66 and for $G_1 : G_2$ being 1 : 4·40. Expansion of the pentenone ring in the B series to give a second lactone ring in the G series also reduces the toxicity; the ratio of the LD_{50} values of $B_1 : G_1$ is 1 : 2·15 and of $B_2 : G_2$ is 1 : 2·03.

The pronounced difference between the fluorescence intensities of the four aflatoxins together with the difference in their biological activities makes it difficult to relate the toxicity of a mixture of these compounds to its fluorescence intensity.

Accordingly, if fluorescence is to be used as a precise indication of toxicity it is desirable that each constituent should be separated and individually determined. Moreover, the measurements of fluorescence should be made under carefully controlled conditions.

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R. B. A. CARNAGHAN

Central Veterinary Laboratory,
Weybridge.

R. D. HARTLEY
J. O'KELLY

Tropical Products Institute,
Gray's Inn Road, London.

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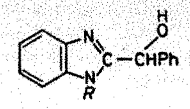
2-(α-Hydroxybenzyl)-benzimidazolium Salts and their Influence on Cultured Cells infected with Poliovirus

1-ALKYL-2-(α-HYDROXYBENZYL)-BENZIMIDAZOLES (I) give a high degree of protection against the cell degeneration which follows infection of tissue cultures with poliovirus (types 1, 2 and 3)[1]. The action of corresponding quaternary salts (II) is of considerable interest in view of: (*a*) the much greater solubility of these compounds in water; (*b*) the introduction of the positive charge; (*c*) the removal of the type of hydrogen-bonding (III) or metal-chelating (IV) propensities that might (for example, by allowing attachment to protein or nucleic acid) play some



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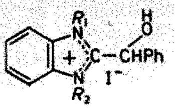

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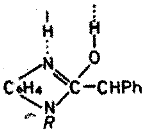

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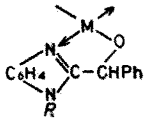

(IV)

part in the activity of the parent compound HBB (I; R=H) and of its 1-alkyl derivatives (I; R=alkyl). Infra-red spectra show that these latter compounds form pairs of intermolecular hydrogen-bonded links as indicated (III), and they also form copper chelates.

Assays were carried out by growing monolayers of *ERK* cells in a number of slowly rotating tubes at 37°. The media were decanted after three days and replaced by fresh media containing half the maximum tolerated concentration of compound and various dilutions of virus (type 1, *L Sc*, 2 *ab*; type 2, *P* 712, *Ch*, 2 *ab*; type 3, *Leon* 12 *ab*). Some controls lacked compound and virus and some lacked compound only. Control tubes containing HBB were also included. Tubes were rolled at 37° and examined twice daily, the time (to the nearest 0·25 day) being noted when half the cells in each tube remained undamaged. The delay in onset of cytopathic change gave an indication of the effectiveness of each compound[1].

The 1,3-dimethyl (II; $R_1 = R_2$ = Me) and the 1-methyl-3-ethyl (II; R_1 = Me, R_2 = Et) quaternary iodides gave no protection and the 1,3-diethyl compound gave just detectable protection against the type 1 and type 3 viruses. However, increase in chain-length produced an improvement; the 1-methyl-3-propyl (II; R_1 = Me, R_2 = ^{n}Pr) and the 1-ethyl-3-propyl (II; R_1 = Et, R_2 = ^{n}Pr) compounds both exhibited a slight, but distinct, effect similar to that shown by 100 μM HBB[1].

The compounds were found to endow cells with marked protection against the type 2 virus. The results, summarized in Fig. 1, show that each quaternary iodide, at half the maximum tolerated concentration (Table 1), had about the same effect as 50 μM HBB. However, in our experiments, as the maximum concentration of HBB tolerated by the cells was 200 μM it is seen that the quaternary iodides had a much lower selective effect than either HBB or its more potent 1-alkyl derivatives[1].

No cytopathic effects were produced in growing cells by the presence of 100 μM potassium iodide and this concentration provided just detectable protection to cells infected with type 2 poliovirus. As the simplest quaternary salts provided no protection against types 1 and 3 viruses, the iodide ion itself gave no protection against these. 1-Methyl-2-(α-hydroxybenzyl)-benzimidazolium iodide (II; R_1 = H, R_2 = Me) had both the same toxicity and offered the same protection as 1-methyl-2-(α-hydroxybenzyl)-benzimidazole (I; R = Me). Although the hydroiodide was probably converted into the 1-methyl derivative of HBB and sodium iodide in the nutrient medium, these results confirmed that the iodide ion was not responsible for the effects produced by the quaternary salts.

Table 1. 1,3-Dialkyl-2-(α-hydroxybenzyl)-benzimidazolium Iododes (II)

R_1	R_2	Maximum tolerated concentration (μM)	M.p. (° C)	Yield (%)	H-bonded OH stretching frequency (cm^{-1})
Me	Me	80	189–90	72	3,230
Me	Et	100	137–9	67	3,250
Et	Et	140	155–6	38	3,250
Me	^{n}Pr	140	170–1	66	3,230
Et	^{n}Pr	160	186–7	89	3,150

R_1	R_2	Formula	Required (per cent)				Found (per cent)			
			C	H	I	N	C	H	I	N
Me	Me	$C_{16}H_{17}IN_2O$	50·5	4·5	33·4	7·4	50·6	4·2	33·7	7·3
Me	Et	$C_{17}H_{19}IN_2O$	51·8	4·8	32·2	7·1	51·5	4·7		6·9
Et	Et	$C_{18}H_{21}IN_2O$	52·9	5·2	31·1	6·9	52·7	5·0	31·1	7·1
Me	^{n}Pr	$C_{18}H_{21}IN_2O$	52·9	5·2	31·1	6·9	52·6	5·4	30·8	6·8
Et	^{n}Pr	$C_{19}H_{23}IN_2O$	54·2	5·5	30·1	6·6	54·3	5·5	29·8	6·6

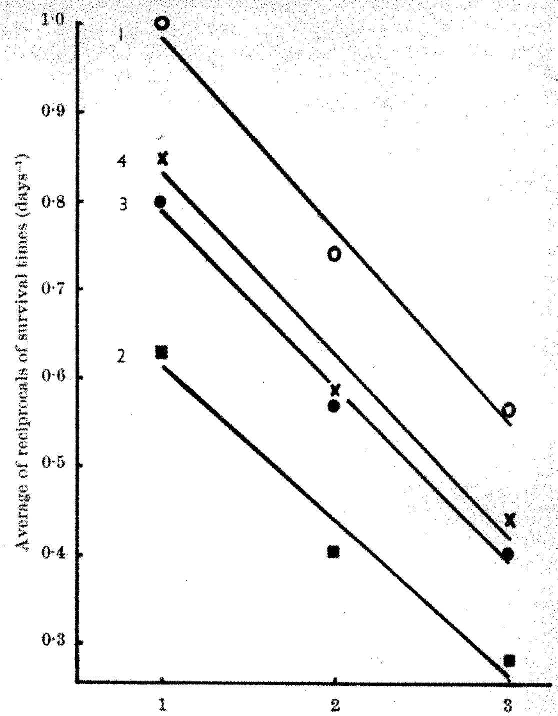

Fig. 1. Lines obtained in assay of protection given to cells against cytopathic effects of poliovirus type 2. (1) Control line (test compounds absent) (○); (2) HBB (100 μM) (strong protection) (■); (3) HBB (50 μM) and 1-methyl-3-ethyl- (50 μM), 1,3-diethyl- (70 μM), 1-methyl-3-propyl- (70 μM) and 1-ethyl-3-propyl- (80 μM) quaternary iodides (●) (same data for all these compounds); (4) 1,3-dimethyl quaternary iodide (40 μM) (×)

The general effect of quaternization is to reduce markedly the protective action of the 1-alkyl derivatives. However, notable protection remains, except with the simpler compounds, against the types 1 and 3 viruses. The OH stretching frequency near 3,200 cm^{-1} in potassium chloride disks and in chloroform (1·5 per cent) (Table 1) shows that quaternization does not reduce the ability of the α-hydroxyl groups to form intermolecular hydrogen bonds, and thus hydrogen-bonded association with other suitable molecules is still possible.

Benzimidazolium salts (II; R_1 = Me, R_2 = Me, Et, ^{n}Pr) were obtained by heating, in a sealed tube at 100° for 24 h, a solution of the 1-methyl derivative of HBB (I; R = Me) (0·002 mole) and the alkyl iodide (0·003 mole) in methanol (1 ml.). The crystalline product, obtained after adding ether, was collected and washed with ether. The 1,3-dimethylbenzimidazolium iodide (II; $R_1 = R_2$ = Me) formed white needles on crystallizing from methanol–ether, while all other benzimidazolium salts, here described, formed white prisms from ethanol–ether after treatment with charcoal.

The 1-ethyl-3-propyl derivative (II; R_1 = Et, R_2 = ^{n}Pr) was prepared in a similar way from the 1-ethyl derivative of HBB, the methanol being replaced by ethanol (1 ml.).

The 1,3-diethyl compound (II; $R_1 = R_2$ = Et) was obtained from the 1-ethyl derivative of HBB and ethyl iodide, nitromethane (1 ml.) replacing the alcohol in the reaction mixture. Otherwise the procedure was unchanged.

1-Methyl-2-(α-hydroxybenzyl)-benzimidazolium iodide (II; R_1 = H, R_2 = Me) was obtained as white prisms, m.p.

194°, by adding ethanol, saturated with hydrogen iodide, to the base (I; R=Me) in ethanol, precipitating the salt with ether and then crystallizing from ethanol–ether.

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D. G. O'SULLIVAN
A. K. WALLIS

Courtauld Institute of Biochemistry,
The Middlesex Hospital Medical School,
London, W.1.

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Isolation of an Easily Precipitable α_1-Glycoprotein of Human Serum

IN the course of investigations concerning the purification of human serum haptoglobin[1] we found in the α_1-globulin fraction obtained by preparative zone electrophoresis at pH 8·6 a glycoprotein hitherto not described. Because of its unusual ability to precipitate at relatively low concentrations of neutral salts and ethanol we propose to characterize this α_1-glycoprotein by the term 'easily precipitable'.

Preparation was made according to the method illustrated in the scheme of Fig. 1. In the analytical ultracentrifuge (Spinco model E) the glycoprotein exhibited a sedimentation constant of $S^{1\cdot5\%}_{20,w} = 3\cdot8$. The mobility in paper electrophoresis at pH 8·6 corresponds to that of an α_1-globulin. We determined its position in the immuno-electrophoresis diagram of the normal serum with a horse anti-human serum following the technique proposed by Heremans[2] and described by Osserman[3]. As can be seen in Fig. 2, its precipitation line is situated within the α_1-antitrypsin bow. The demonstration of special immuno precipitate is made possible by the addition of α_1-glycoprotein to the normal serum in the concentration of 0·2 per cent (Fig. 2). In the starch-gel electrophoresis the α_1-glycoprotein moves into the position directly behind the albumin as a 'post albumin'[4]. After treatment with neuraminidase isolated from *Vibrio cholerae* the α_1-glycoprotein migrates at a reduced mobility. In starch-gel electrophoresis, it appears to be split into several components.

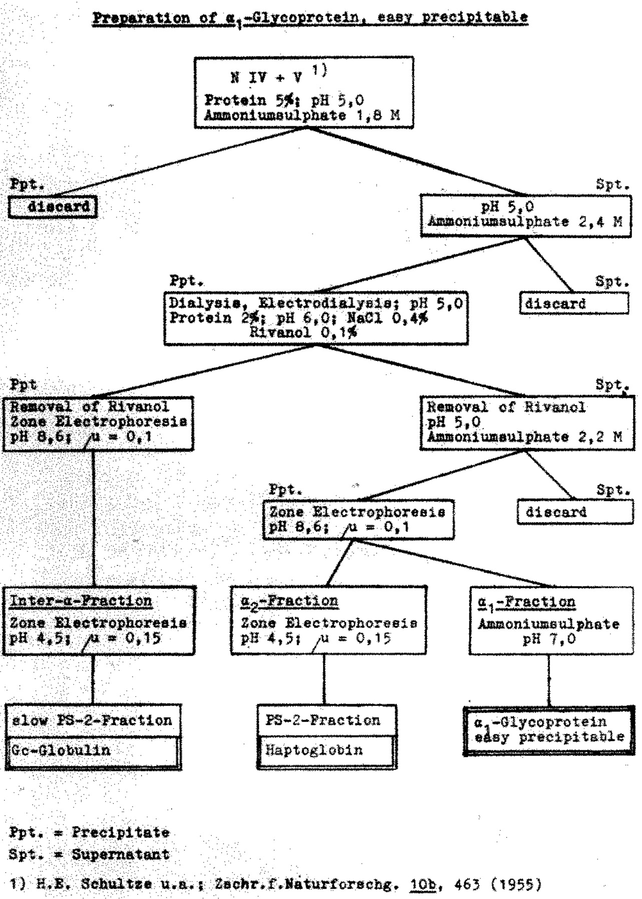

Fig. 1

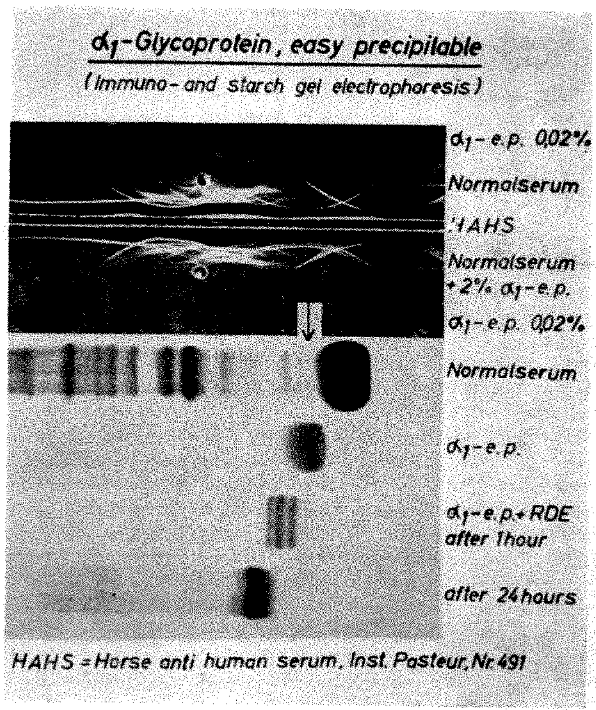
α1-Glycoprotein, easy precipitable
(Immuno- and starch gel electrophoresis)
α1-e.p. 0,02%
Normalserum
HAHS
Normalserum
+2% α1-e.p.
α1-e.p. 0,02%
Normalserum
α1-e.p.
α1-e.p.+ RDE
after 1hour
after 24 hours
HAHS = Horse anti human serum, Inst. Pasteur, Nr 491

Fig. 2

Some chemical characteristics and the precipitation behaviour of the α_1-glycoprotein 'easily precipitable' are shown in Table 1. For comparison the precipitation behaviour of two other α_1-glycoproteins, namely, the α_1-antitrypsin and the α_1-acid glycoprotein, are also presented in Table 1.

Table 1. CHARACTERISTICS OF THE α_1-GLYCOPROTEIN 'EASILY PRECIPITABLE'

		Precipitation			
			α_1-Glycoprotein e.p.	α_1-Antitrypsin	α_1-Acid-glycoprotein
Biuret value (albumin = 100)	89	Perchloric acid	0·6 M	—	—
Hexose %	4·8*	Trichloroacetic acid	0·15 M	0·15 M	—
Acetylhexosamine %	4·4	Sulphosalicylic acid	0·2 M	0·2 M	—
Acetylneuraminic acid %	3·4	'Rivanol'; pH 7·5	0·12 M	0·12 M	0·12 M
Fucose %	0·4	Ammonium sulphate pH 7·0	1·5–2·0 M	2·0–3·2 M	3·2–4·0 M
$E^{1\%}_{1\text{cm}}$ 280 mμ	9·0	Ethanol pH 5·2, μ = 0·1	18%	18%	60%

*Referred to Gal./Mann. = 1 : 1.

The biological significance of this serum protein, which is present in normal serum only in very small quantities, is still unknown.

H. E. SCHULTZE
K. HEIDE
H. HAUPT

Behringwerke AG Marburg (Lahn).

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PHYSIOLOGY

Production of Cardiac Arrest in Animals

THE basic requirement in the study of cardiac resuscitation methods is to obtain a method whereby we can arrest cardiac function in standard circumstances for a predetermined period of time. Numerous methods of inducing cardiac arrest are so far known[1-6].

With the methods of inducing cardiac arrest, the myocardium and its impulse-conducting system is mostly exposed to chemical or electrical trauma. These factors render difficult the evaluation of the methods used for cardiac resuscitation.

In the course of our experiments, we have worked out a new method of cardiac arrest concerning resuscitation, its essence being an artificial tamponade of the pericardium, the arrest of circulation, that is, the creation of clinical death. Our method was as follows: we narcotized animals with chloralose (1 g/kg body-wt.) and incubated them to secure breathing. An open method of recording blood pressure and control continuously with encephalograph (ECG) was applied. We approach the heart through a thoracotomy on the left side. Then with a short, dull-pointed needle connected with the syringe by a rubber tube, we punctured the pericardial sac fixed by tweezers and administered a solution of 150–250 ml. of isotonic sodium chlorate at a medium speed. Caution is needed in puncturing the pericardial sac, and the needle is led parallel to the axis of the heart, thus avoiding damaging any branch of the coronary artery or of the muscles of the moving heart. The needle is fixed carefully so as not to let it slip out during heart-beat. If possible, a fresh puncture is avoided, because the administered solution may flow out through the first hole during over-pressure, which may lessen intrapericardiac pressure and heart function may again spontaneously return at a decreased pressure. The pericardial sac is continuously filled up until it tightens considerably and blood pressure drops to zero.

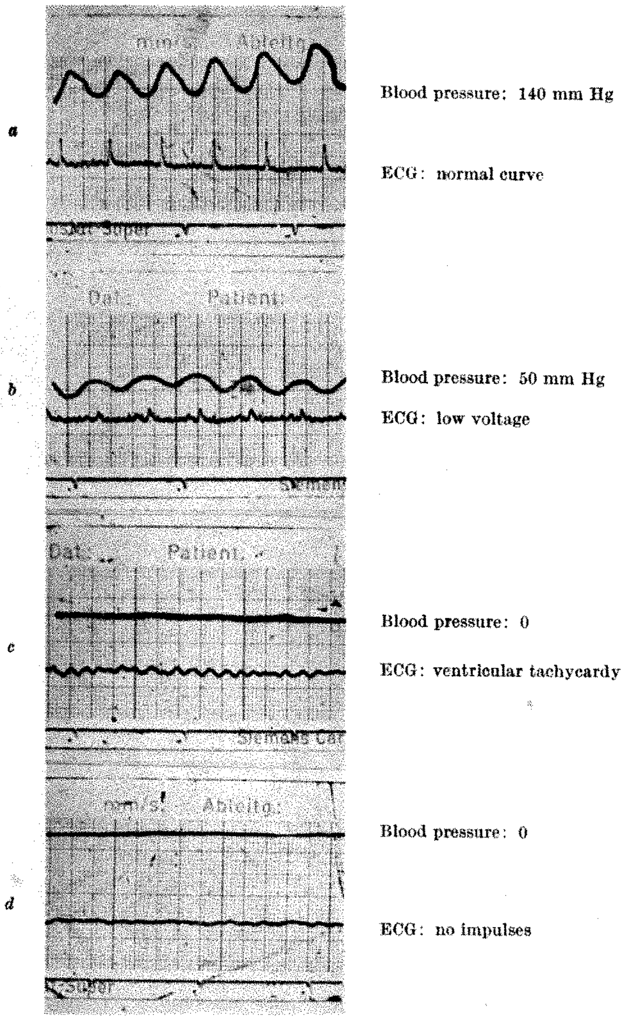

Fig. 1. *a*, Pulse curve and ECG curve of a dog under anæsthesia; *b*, during filling up the pericardial sac; *c*, after complete filling. No circulation ('weak heart'); *d*, 2 min after complete filling up

With this method we have brought about a complete cardiac arrest; at the same time electrical pathological heart action still could be traced by the ECG in a large number of cases. To demonstrate this, the pulse curve was recorded on the ECG tape with the help of a registering set applied on Marey's drum.

This method was carried out on 25 dogs. To determine the beginning of cardiac standstill—after having filled the pericardial sac—we took the tensionless state of clinical death as a beginning and measured time from this point onwards. With the first group of animals (10 dogs) we induced cardiac arrest, lasting for 2–4 min. We gave no drugs, no blood transfusion or infusion during the cardiac arrest and resuscitation. Having stopped the pericardial tamponade, cardiac function returned spontaneously in three cases and it started easily in seven cases after bimanual massage.

In the second group of animals (15 dogs), we induced cardiac arrests lasting for 5–10 min. We included the time needed for splitting the pericardium (30–40 sec), that is, the cessation of the tamponade, into the full time of cardiac arrest.

In this group of animals, resuscitation could only be achieved by bimanual massage if we combined it with intra-arterial transfusion of blood under pressure and with the administration of intravenous noradrenaline. In this group after ceasing pericardial compression we saw fibrillation in five hearts, of which, after successfully defibrillating, we could resuscitate by massage in three cases. In two cases, fibrillation could not be influenced, that is, we failed to achieve resuscitation.

J. HEID
O. SZÉKELEY

Central Institute of Traumatology,
Budapest, Hungary.

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Influence of Duration of Experiment on Rate of Uptake of Radioactive Potassium in Smooth Muscle

THE rates of uptake and loss of radioactive tracers by excitable tissues can often be described by a function involving one exponential term[1-3], but over relatively long periods deviations have been observed from such a simple course[4-7]. The rates of exchange towards the end of such periods are commonly slower than would be predicted from the initial rates and two general causes for this behaviour have been suggested: either the rates are, in fact, diminishing during the course of an experiment or the tissue is not behaving uniformly. This latter possibility has been extensively investigated[1,8-15], because biological material shows an obvious lack of uniformity, and because deviations from single exponential behaviour are observed even when the stoichiometric composition of the tissue remains constant. However, a constant composition is not proof of constant exchange rates but might be maintained if all exchanges in the system slowed down, corresponding perhaps to the slow deterioration of the *in vitro* preparation, or indeed if movement stopped altogether. One might expect deviations due to the ageing of the tissue to be more marked the longer the duration of an experiment, and the uptake of potassium-42 by the smooth muscle of the guinea pig tænia coli has now been examined for such an effect since it only shows serious deviations from single exponential behaviour after 5 h[12].

Unselected guinea pigs were stunned and bled out at the neck, and pieces of tænia coli weighing 6–12 mg were rapidly dissected and suspended with a tension of 2 g in an organ bath containing a modified Krebs solution[14] (Na^+ 137, K^+ 5·9, Ca^{++} 2·5, Mg^{++} 1, Cl^- 144, HCO_3^- 5·9 D(+)-glucose 11·5 mM equilibrated with 99 per cent oxygen/1 per cent carbon dioxide). At appropriate times, muscles were prepared for analysis by wiping them across a 'Perspex' sheet to remove adhering liquid[12], the cotton threads then being cut away and the tæniæ weighed and ashed in hard glass tubes with 0·1 ml nitric acid (sp. gr. 1·5) and 0·1 ml perchloric acid (sp. gr. 1·7) (analytical grades). Samples of the physiological solution and empty control tubes were also prepared. Potassium-42 was measured in the well-crystal of an Ecko scintillation counter *N*664*A*. The total potassium was determined later by flame photometry. All experiments were carried out at 35° C, and were repeated at least six times.

In preliminary observations, pieces of tænia were first loaded with potassium-42 and each was then transferred at 2-min intervals through a series of 20 small organ baths containing inactive Krebs solution. The activity in each organ bath was determined and two semi-logarithmic curves for the efflux were constructed[15], one from the amounts of activity left by the muscle in each organ bath and the other from the total activity in the tissue at each of the same times. This total activity was calculated by adding the residual activity in the muscle at the end of the experiment to the sum of the counts in each of the washing solutions. The rate constants calculated by these two methods should be identical for a tissue showing a single exponential exchange[15], but in the present experiments they differed by more than 50 per cent.

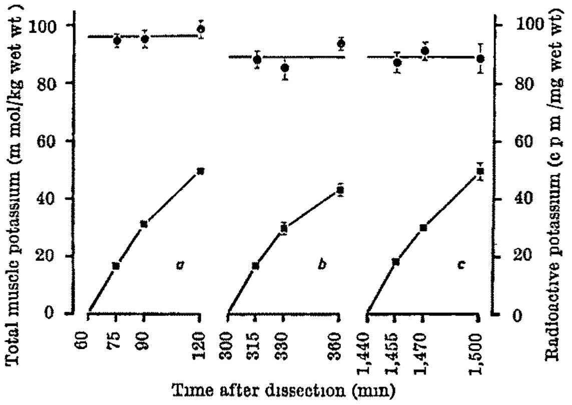

Fig 2. Total potassium (circles) and uptake of potassium-42 (squares) by the tænia coli. Muscles were transferred to radioactive solutions at different times after dissection, but no progressive change in the initial rate of uptake of potassium-42 was observed. The specific activity of solution was 10^5 c.p.m./m mole potassium

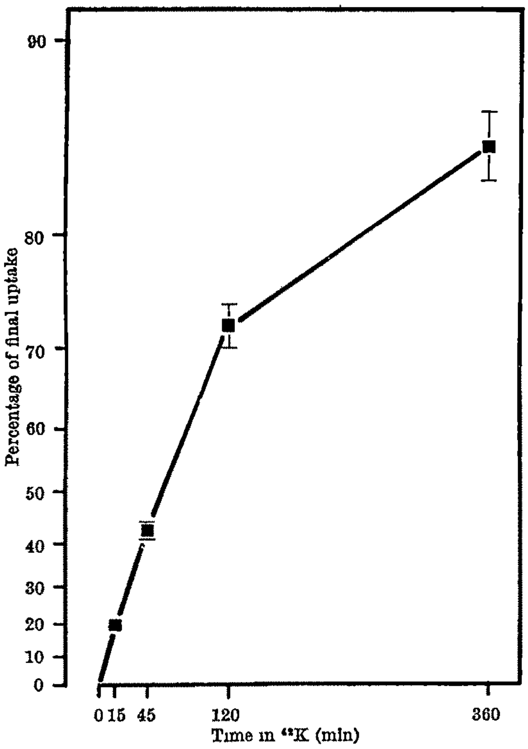

Fig 1. Uptake of potassium-42 by the tænia coli. The logarithmic ordinate scale corresponds at infinity to the uptake observed after 1,440 min. The time-scale is linear. The points show typical deviations from a straight line, even though all muscles were kept the same period *in vitro*. The *S.E.* of each mean has been individually calculated and shown where it was big enough

In further experiments a potassium-42 uptake curve was constructed for pieces of tænia which had all been kept in Krebs solution *in vitro* for 25 h before analysis. Five muscles from each guinea pig were suspended in inactive solution after dissection, and the first of these was transferred to radioactive solution 1 h later when the net potassium content had reached a steady state[12]. The remaining muscles were transferred at intervals thereafter until, 1,475 min after dissection, all were in the active organ bath together. A sixth muscle from the same animal had been placed in the radioactive bath immediately after dissection, at which time there was a restoration of the net potassium which had initially been lost from the tissue during the cold preparation period[12]. This net gain contributed to the radioactive content of the last muscle by giving it an extra opportunity to take up tracer, and it was removed with the others and prepared for analysis after 1,500 min.

If there were no ageing effects, these observations should give similar results to those found by Goodford and Hermansen: single exponential uptake over the first 120 min, followed by a progressive slowing during the next 3 h. If, on the other hand, there were a marked deterioration of the tissue *in vitro*, the shorter uptake periods (which only began towards the end of the experiments when the muscles were already ageing) should be most affected and deviations in the reverse direction might even occur. The results in fact resembled Goodford and Hermansen's[12], but suggested that exchange was virtually complete after 1,440 min, first, because the relative activity ($S.A._{\text{muscle}}/S.A._{\text{solution}}$) of 1·006 did not differ significantly ($0·9 > P > 0·8$) from 1, and secondly, because the muscle which went straightway into the active organ bath, and therefore had additional opportunities and time (1,500 min) to take up more potassium-42, in fact took up no more.

In Fig. 1 the radioactive uptake after the shorter periods in radioactive solution has been plotted with a logarithmic ordinate, adjusted so that the observed uptake after 1,440 min would be at infinity on this scale. With the axes of Fig. 1, the points should have been on a straight line if uptake followed a single exponential course, although the method of least squares was not applicable to the calculation of the best linear regression to fit the points, because after the logarithmic transformation the later observations were relatively more scattered than the earlier results This has been shown in Fig 1, where the standard error of the mean has been separately drawn for each time of observation. It may be seen clearly by inspection that no straight line could pass close to all the points, a single exponential course could describe the observations at 15, 45 and 120 min adequately, but deviations arose after 300 min just as they did in the conventional experiments of Goodford and Hermansen[12] These deviations from a single exponential were not due to variation between guinea pigs since the effect was observed in each individual animal, nor were they artefacts due to the assumption that exchange was complete after 1,440 min This was, of course, implied in the selection of the logarithmic ordinate scale, but the relative activity could not exceed 1 at equilibrium and any smaller value would only accentuate the curvature in Fig 1

It may be concluded from the previous considerations that the potassium in the tænia coli was not uniformly distributed However, the effect of duration of experiment on rate of exchange was investigated in a further series of experiments in which nine muscles were dissected from each guinea pig and placed in inactive Krebs solution Three were transferred to the radioactive organ bath after 60 min and were removed for analysis during the next hour (Fig 2*a*) Three more pieces were transferred after 300 min and similarly observed (Fig 2*b*), while the remaining three muscles were only transferred to radioactive solution after 1,440 min (Fig 2*c*) The results in Figs 2*a*, *b* and *c* are similar, and in particular do not show any progressive change in the initial rate of uptake of potassium-42 after *in vitro* pre-incubation periods as long as 24 h The later, slower exchange of potassium in the tænia coli[12] is therefore largely, or entirely, due to a lack of uniformity in the tissue, as is commonly assumed, and is not appreciably due to any deterioration of the preparation *in vitro*

H Bauer
P J Goodford*
J Huter

Pharmakologisches Institut,
Johannes Gutenberg-Universitat,
Mainz,
West Germany

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* Present address: University Department of Pharmacology, South Parks Road, Oxford

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Effect of Calcium Ions on Melanophore Dispersal

It has been suggested that calcium ions act as the coupling agent in muscle excitation and contraction[1,2], in stimulation of the adrenal medulla and cortex[3], and in vasopressin release from the posterior pituitary[4] Their basic role in membrane reactions has become obvious from many other observations, including that on which we wish to report here

Hydrated Na^+ and Ca^{++} ions are of similar size[5], so that cross-action and synergism in biological phenomena may be expected This applies also to nervous conduction, where calcium behaves similarly to sodium it enters during excitation and leaves during recovery[6], probably playing a key part in this process[7] In view of this interrelation, it seemed unlikely that the melanophore dispersing action of melanocyte stimulating hormone should be specific to sodium[8–10]

In a series of 200 examinations the skin from the back of tree frogs (*Hyla arborea*) was removed, halved longitudinally and flattened on filter paper with its green-coloured side facing upwards The skin was then incubated at room temperature for an hour in frog-isotonic sucrose solution, buffered with 2 mM sodium bicarbonate This procedure was shown to bring extracellular ions down to inert concentrations without significantly affecting the tissue-bound calcium[9] After incubation, half the skin was soaked in different mixtures of frog-isotonic sucrose and isotonic cation chloride solutions, both buffered with 2 mM sodium bicarbonate, the other half was kept in buffered sucrose solution for control, the Na^+ of the buffer being ineffective at this concentration (2 mM)

Osmotic activity was checked by the Fiske osmometer The colour of the skin was recorded every 15 min for 3 h according to the colour scale introduced by us[11] With this method we found that 10 mM calcium chloride not only supported melanocyte stimulating hormone action, *in vitro*, but also caused considerable darkening by itself Higher concentrations caused lightening (yellowish colour) or inhibition of action of melanocyte stimulating hormone Among other bivalent ions, only Mg^{++} behaved like Ca^{++} in supporting action of melanocyte stimulating hormone, but none of them, in concentrations up to isotonicity, led to darkening of the skin.

After soaking the skin in frog-isotonic K^+ solution, the melanophore dispersal could still be induced by 10 mM Ca^{++}, this cation acting as a darkening agent by itself, or in support of action of melanocyte stimulating hormone Melanophore response to Ca^{++} after depolarization by K^+ solution may thus be comparable with smooth muscle response under similar conditions[2]

We accordingly propose as a working hypothesis that Ca^{++} may be the coupling agent between melanocyte stimulating hormone stimulation and melanophore dispersal, having a coupling function parallel to that postulated for muscle[1] and other systems[3,4].

In conclusion we have found that Ca^{++} alone in proper concentrations causes *in vitro* darkening of the skin of the tree frog (*Hyla arborea*)—even in frog-isotonic potassium chloride Melanocyte stimulating hormone may be presumed to act primarily on the cell membrane, its melanophoric effect being mediated by Ca^{++} The melanophore reaction may therefore serve as an easily accessible model reaction for examining coupling phenomena.

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S Dikstein
C P. Weller
F G Sulman

Department of Applied Pharmacology,
School of Pharmacy,
Hebrew University,
Jerusalem

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PHARMACOLOGY

Enhancement by Methyldopa of Tyramine Sensitivity in Man

THE mechanism by which α-methyl-3,4-dihydroxy-L-phenylalanine (methyldopa, Aldomet) lowers blood pressure in man has not been established definitely While the inhibiting activity of the drug on decarboxylation of aromatic-L-amino-acids has been confirmed in human subjects and indeed was our reason for first studying the compound clinically[1], it is doubtful that synthesis of the sympathetic transmitter, noradrenaline, is significantly affected by the drug Thus, investigations in this laboratory and elsewhere[2] have failed to show any decrease in the urinary excretion of the catecholamine metabolite, vanilmandelic acid, in patients receiving the drug Furthermore, depletion of tissue-levels of noradrenaline by methyldopa and α-methyl-*m*-tyrosine in animals appears unrelated to the enzyme-inhibiting effects of these compounds[3] Present evidence[4,5] favours mediation of the depleting effect by amine metabolites formed by decarboxylation (to α-methyl-dopamine) and possibly subsequent β-hydroxylation (to α-methyl-noradrenaline)

While the blood pressure effects of methyldopa in man resemble in part those of sympathetic neuronal inhibition, it is not known whether catecholamine depletion occurs in human subjects An index of this might be obtained by following changes in pressor activity of tyramine since the cardiovascular effects of tyramine are thought to be mediated by noradrenaline and are depressed by catecholamine-depleting agents such as reserpine[6] Accordingly, responses to separate 15-min infusions of tyramine at several dose-levels were determined in five hypertensive patients under three different conditions · (1) during control periods , (2) after 4–20 weeks of oral treatment with 2–3 g of methyldopa daily , (3) during the last three days of treatment with reserpine 1–2 mg/day intramuscularly for eight days Control observations were made at a time when the patients had received no medical treatment for at least 4 weeks

The rate of infusion of tyramine in μg/min required to produce an increase of 30 mm mercury in the systolic blood pressure was calculated from dose response curves Responsiveness to tyramine was expressed as a percentage of the average control values As shown in Table 1, the anticipated decrease in tyramine sensitivity occurred in association with presumed noradrenaline depletion produced by reserpine While a decrease in tyramine pressor activity was also expected during treatment with methyldopa, it may be seen that pressor responses to tyramine were actually enhanced by this agent

Table 1. EFFECT OF RESERPINE AND METHYLDOPA ON RELATIVE SENSITIVITY TO TYRAMINE IN FIVE PATIENTS*

Case No	After reserpine† (1–2 mg/day) (%)	After methyldopa‡ (2–3 gm/day) (%)
1	25	215
2	30	125
3	33	183
4	34	145
5	16	194
6	—	220

* Responsiveness is expressed as percentage of control sensitivity

† Each value is the average of two determinations carried out after 6–8 days of intramuscular reserpine, 1–2 mg/day

‡ Each value is the average of 2–6 determinations done after 2–14 weeks of administration of methyldopa (2–3 g/day) All values were above the average control-level (100 per cent)

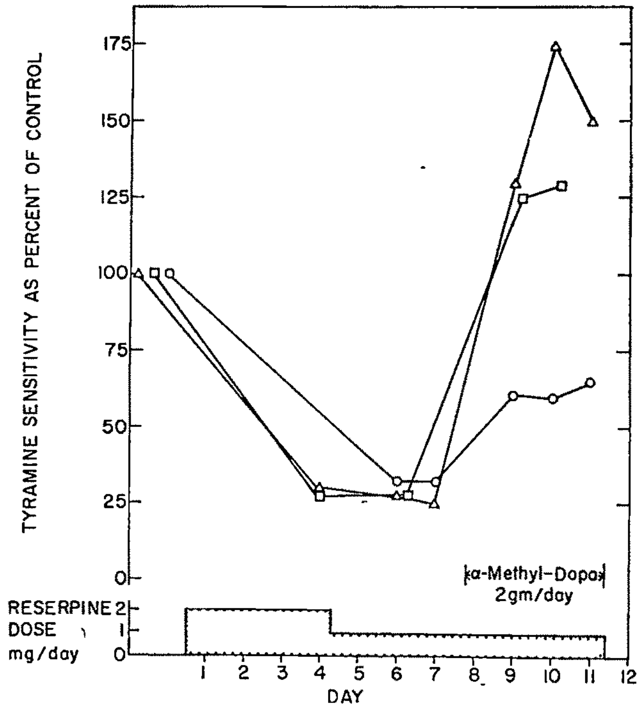

Fig 1 Depression of tyramine pressor responses in three patients by reserpine and restoration by methyldopa

It was of interest to learn whether the depressed tyramine sensitivity due to reserpine could be reversed by methyldopa Three additional hypertensive patients were given 1–2 mg reserpine intramuscularly daily for 11 days ; during the last three days methyldopa 2 0 g/day was given orally Tyramine sensitivity was increased in each of the three patients and in two of them even beyond pre-reserpine control-levels (Fig 1) The restoration of tyramine sensitivity by methyldopa was substantiated further in numerous studies in reserpinized dogs and rabbits[7] α-Methyl-dopamine and α-methyl-noradrenaline were found also to restore tyramine pressor activity Peak restoration was delayed for about 90 min following the amino-acid drug and its decarboxylation product, but was immediate following administration of α-methyl-noradrenaline. Recently, similar work on animals has been reported from two other laboratories While not presenting data, Day and Rand[8] state that they have observed partial restoration of responses to sympathetic nerve stimulation and to tyramine in reserpine-treated animals after infusions of methyldopa and the two amine derivatives Varma and Benfey[9] report that in spinal cats pretreatment with methyldopa prevented the reserpine-induced reduction of cardiovascular responses to tyramine but not to stellate-ganglion stimulation

These findings are interpreted as affording presumptive evidence that the enhanced tyramine response produced by methyldopa is mediated by its amine metabolites, α-methyl-dopamine and/or α-methyl-noradrenaline Both these compounds are pressor substances In human subjects we have found that the pressor potency of α-methyl-dopamine is about 1/100 that of noradrenaline As reported previously[10], α-methyl-noradrenaline is about 1/3 as potent as noradrenaline in increasing the systolic blood pressure in man This information on pressor potency plus the timing of the restoration of tyramine responses in reserpinized animals[7] tends to favour the β-hydroxylated amine as being the mediator in enhancing and restoring tyramine responses The supersensitivity to tyramine of patients treated with methyldopa may be related to the fact that the α-methyl-amines are not substrates for monoamine oxidase This could facilitate their access to receptors following release from a tyramine-sensitive site at the nerve ending

A better understanding of the mechanism of hypotensive action of methyldopa may result from a more detailed investigation of tyramine responses, as well as studies of the interactions of noradrenaline and the α-methyl-amines at receptor sites in the arterioles and in the brain During preliminary investigations we have found that the clinical hypotensive response, in both degree and duration, bears a closer relationship to the magnitude of tyramine response than to any other parameter While this evidence is indicative of a peripheral site of drug action, it must be borne in mind that methyldopa also has central activity Furthermore, frequent lowering of blood pressure in man in the supine as well as erect positions is difficult to explain solely on the basis of a peripheral action on sympathetic mechanisms

WILLIAM PETTINGER
DAVID HORWITZ
SYDNEY SPECTOR
ALBERT SJOERDSMA

Experimental Therapeutics Branch,
National Heart Institute,
Bethesda, Maryland

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Acetylcholine-releasing Material in Neural Tissues

WHILE examining cholinergic substances in sciatic nerve[1], we noted that acetone extracts of nerve caused a slow contraction of the guinea pig ileum (Fig 1*A*) Identical lipid extracts of brain had the same effect (Fig 1*B*) ; similar activity could not be detected in kidney, intestine, lung or liver A salient characteristic of the lipoidal material is that its action is completely blocked by atropine at a dose, 6 ng/ml , that blocked the action of acetylcholine The lipoidal material causes a slower contraction than does acetylcholine (Fig 1) , the material is resistant to acetylcholinesterase, which destroys acetylcholine ; the material retains activity after heating at *p*H 10 for 5 min in a boiling water bath, conditions which destroy acetylcholine, the material does not act on a strip of ileum that had been stored either at room temperature for 1 day or at 4° C for 3 days, whereas acetylcholine elicits the usual contractions , finally, the material is not active on the anoxic ileum, which responds to acetylcholine (Table 1)

Failure of the material to act on the stored[2] or anoxic[3] ileum (Table 1), and the sensitivity of the activity to atropine suggested that the substance may act by releasing acetylcholine from the nerve plexus in the wall of the ileum This inference was strengthened by investigations with a hemicholinium (HC-3), which prevents the synthesis of acetylcholine in nervous tissue[4,5] and prevents the action of the extract on the ileum without inhibiting the effect of either acetylcholine or bradykinin (Fig 2)

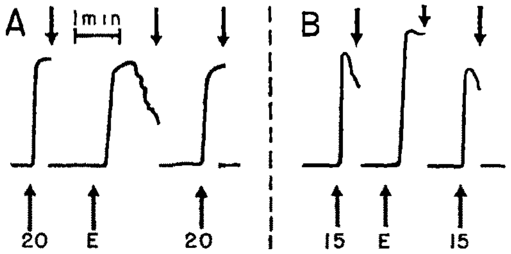

Fig 1. *A*, Action of a lipid extract (*E*) of the sciatic nerve of the rabbit on the guinea pig ileum , *B*, Action of a lipid extract of pig brain on the guinea pig ileum Numbers refer to ng of acetylcholine The ileum was bathed in 5 ml of Tyrode's solution containing mepyramine, 5 μg/l , and lysergic acid diethylamide, 50 μg/l

Table 1 EFFECT OF VARIOUS CONDITIONS AND DRUGS ON THE ACTION OF THE EXTRACT ON GUINEA PIG ILEUM

Treatment	Concentration (μg/ml)	Activity of extract (% of normal)
Storage of ileum		
24 h, 26° C	—	0
3 days, 4° C	—	0
Anoxic ileum	—	0
Incubation with acetylcholinesterase	—	100
Heating extract at *p*H 10 for 5 min	—	290
Lysergic acid diethylamide	0 500	100
Mepyramine	0 005	100
Atropine	0 006	0
HC-3	(2 × 10^{-5} M)	13
Hexamethonium	20 0	100
Tetraethylammonium	40 0	106
Cocaine	4 0	101
Morphine	0 001	0

The action of the material cannot be accounted for by a stimulation of the ganglia in the wall of the ileum, for hexamethonium and tetraethylammonium, in doses that block the action of ganglionic stimulants[6], did not inhibit the action of the extract Since cocaine did not prevent its action, the material cannot be acting through stimulation of the nerve fibres in the ileum[7]. Blockade by morphine (Table 1) in doses that prevent the release of acetylcholine from nerve endings[8,9] strongly implies that the material causes contraction by releasing acetylcholine from the nerve endings in the ileum Direct evidence that the extract causes enhanced release of acetylcholine was obtained by measuring[9] the amount of acetylcholine released in 20 minutes from the resting ileum, before and after exposure to the nerve extract (Fig. 3)

The material differs from substance *P*, the effect of which was not blocked by atropine It also differs from bradykinin not only in its inhibition by HC-3 (Fig 3), but also in its antagonism by atropine The action of 5-hydroxytryptamine is inhibited by lysergic acid diethylamide and cocaine, while the effect of histamine is inhibited by mepyramine , none of these inhibitors influenced the action of the extract (Table 1)

The material may function in a presynaptic event leading to release of the neurotransmitter at cholinergic nerve endings[10-12] and, therefore, may be implicated in the action of botulinum toxin, which prevents release of the cholinergic transmitter, and in myasthenia gravis, in which a presynaptic malfunction has been suggested Perhaps of greater interest is the observation that morphine totally blocks the action of the material on the ileum ; the ileum, in responding to analgesic drugs in a manner that reflects analgesic potency and in developing 'tolerance' to and 'dependency' on morphine, demonstrates a neural network that is analogous to more complicated networks[9]

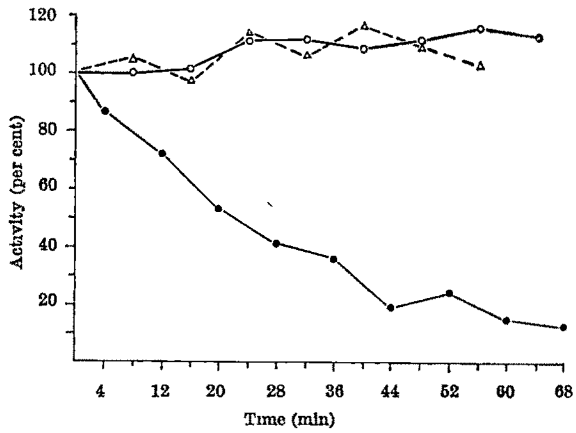

Fig 2 The inhibition by HC-3 of the action of the extract from sciatic nerve Tyrode's solution containing 2 × 10^{-5} M of HC-3 was added to the chamber containing the guinea pig ileum at zero-time At intervals of 4 min, the test substances were added to the bath In one experiment, 200 ng of bradykinin (○) was compared with the extract (●) , in another, 20 ng of acetylcholine (△) The curve showing activity of the extract is the average of both experiments

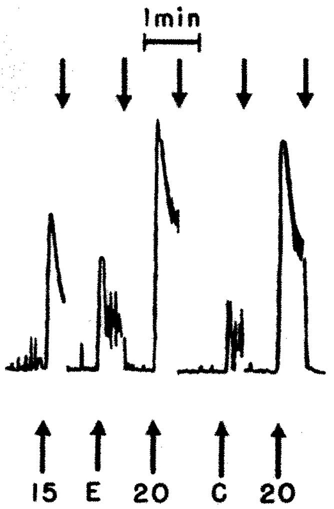

Fig. 3. Enhanced release of acetylcholine from the ileum by the extract of sciatic nerve. Numbers refer to ng of acetylcholine. *E* 1·0 ml. of Krebs–Henseleit solution from the 5·0 ml. in which the ileum (0·35 g) had been immersed with the extract for 20 min. *C* identical with *E*, except that no extract was added to the chamber. In the absence of the extract, 62·2 ng of acetylcholine was released from the ileum in 20 min; in the presence of extract, 87·3 ng was released.

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J. P. Green
E. A. Carlini
J. D. Robinson

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Conn.

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HÆMATOLOGY

Induction of Viscous Metamorphosis in Human Blood Platelets by means other than by Thrombin

Viscous metamorphosis is characterized by the morphological and biochemical changes displayed by the blood platelets during the coagulation of blood. Thrombin is generally thought to be the activating agent. In the course of viscous metamorphosis, vasoactive substances including 5-hydroxytryptamine, adrenaline and histamine, as well as phospholipids (platelet factor 3) essential for intrinsic prothrombin conversion are liberated. Viscous metamorphosis, furthermore, is of major importance for the formation of an efficient hæmostatic plug as well as of the primary white thrombus. In view of all these facts it seemed worth while to investigate whether agents other than thrombin were capable of inducing viscous metamorphosis in normal human blood platelets.

The agglutination of platelets as well as the liberation of vaso-active amines or phospholipid by the action of antigen–antibody complexes[1–5], of endotoxin[6,7], and of anaphylatoxin[2] has been reported. Complement participation has been considered essential at least in some of these reactions. Since complement also depends for its activation on the presence of metal ions, a participation of the blood clotting system cannot be excluded in several of the experiments reported, particularly since the 'plasmatic atmosphere', even of washed platelets, contains most of the factors required for thrombin formation.

Recently it has been reported that a heparinoid (polyanetholsulphonate–'Liquoid', Roche) is also capable of inducing *in vivo* morphological platelet changes resembling viscous metamorphosis[8]. Heparinoids, like heparin, generally display antithrombin and anticomplement activities. In the experiments reported here, the effects of 'Liquoid' and of antigen–antibody complexes on platelets have been studied under conditions which exclude participation of the blood clotting system.

Washed human blood platelets were isolated as described before[9] and used within 24 h after collection. Adenosine triphosphate was determined in samples deproteinized with 3 per cent trichloroacetic acid; commercial reagents were used (Boehringer und Söhne, Mannheim, Germany). 'Liquoid' (Roche) was brought to a 1 per cent solution in 0·15 M saline. Insoluble antigen–antibody complexes were prepared by incubating the antigens (human serum albumin obtained from the Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross, Berne, or chicken serum) with the corresponding rabbit antisera for 30 min at 37° C. The serological precipitates formed were collected by centrifugation and resuspended in an undiluted commercial hirudin solution. ('Hirudex', ANA, Paris.) Hirudin acts as a powerful inhibitor of thrombin.

All incubation mixtures contained the following components: 20×10^6 platelets/µl.; imidazole–hydrochloric acid buffer, *p*H 7·4, 0·03 M; EDTA 0·005 M; $MgSO_4$ 0·008 M; highly purified human fibrinogen 0·05 per cent; and sufficient sodium chloride to bring the mixture to isotonicity (300 µmoles/ml.). To this were added: thrombin (3·8 I.U./ml.), or 'Liquoid' (Roche) (0·05 per cent), or antigen–antibody precipitate (0·3 per cent protein). Values in parentheses represent final concentrations in total incubation mixture.

The addition of 'Liquoid' leads to an immediate aggregation of the suspended platelets, followed by active contraction of the formed aggregate. In fact, speed of contraction and general appearance of the contracted aggregates are indistinguishable from those formed by the action of thrombin (Fig. 1). ATP is consumed in the course of the action of 'Liquoid' on platelets, and the resultant curves of ATP-consumption are identical with those obtained after addition of thrombin (Fig. 2).

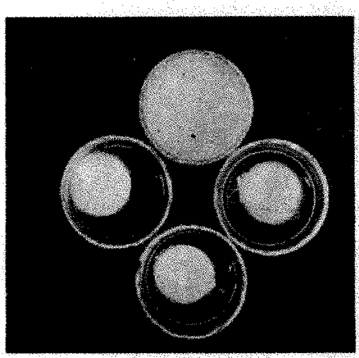

Fig. 1. Top, Non-retracting platelet-rich clot, obtained by the addition of thrombin in the presence of an excess of EDTA; bottom, retracted clot (with thrombin and Mg^{++}-ions); left, retracted platelet aggregate (with 'Liquoid' + Mg^{++}-ions); right, retracted platelet aggregate (with antigen–antibody precipitate + Mg^{++}-ions + hirudin)

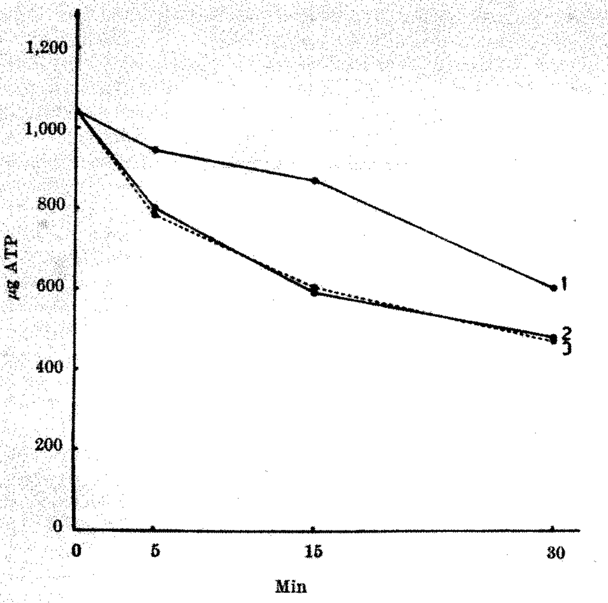

Fig. 2. ATP-levels in platelets incubated with: (1) 0·15 M sodium chloride; (2) 'Liquoid' (Roche); (3) thrombin

Mixtures of human platelets and human albumin–antialbumin precipitates also show contractile activity and ATP consumption, the only difference being that the reaction proceeds at a slower rate. An immune complex formed from chicken serum and rabbit anti-chicken antiserum gave similar results. In Ouchterlony tests, chicken serum proteins did not show evidence of cross-reaction with human serum. This indicates that immune complexes devoid of components which might be present as antigens in the 'plasmatic atmosphere' of the platelets still react in the same way.

These results leave no doubt that typical manifestations of viscous metamorphosis, that is, ATP-consumption and retractile activity, are shown by human platelets under the influence of a heparinoid as well as in the presence of antigen–antibody complexes. In the latter instance, viscous metamorphosis occurred in spite of the presence of hirudin, a powerful inhibitor of thrombin. The release of vasoactive amines and of phospholipid from platelets under a variety of experimental conditions therefore must be considered also as typical manifestations of viscous metamorphosis. A primary activation of the clotting system does not seem necessary. A participation of the complement system, although unlikely, cannot be entirely excluded on the basis of these experiments. Platelets contain a powerful antiheparin factor and a 'plasmatic atmosphere', as well as plasmatic inclusions which could contain complement.

Platelets undergoing viscous metamorphosis, particularly the phospholipids liberated in this process, may act as a trigger for the activation of the intrinsic clotting system[10]. In a variety of experimental conditions, including injections of endotoxins (generalized Shwartzman phenomenon) and 'Liquoid' as well as some reactions elicited by circulating antigen–antibody complexes, the deposition of fibrin in the vascular system dominates the pathogenic picture[8,11]. The evidence presented here suggests that fibrin formation may be a secondary phenomenon resulting from the induction of viscous metamorphosis of the blood platelets by other means than thrombin.

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M. BETTEX-GALLAND
E. F. LÜSCHER

Theodor Kocher Institute,
University of Berne.

G. SIMON
P. VASSALLI

Institute of Pathology,
University of Geneva.

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Effect of Nitrogen Mustard on Erythrocyte Acetylcholinesterase

NITROGEN mustard (HN_2) is known to inactivate certain enzymes, particularly hexokinases and phosphatases[1], in addition to the better-known effects on nucleic acids common to all alkylating agents[1–5]. In the course of examining the effects of nitrogen mustard on circulating erythrocytes, it became important to assay its effect, if any, on erythrocyte acetylcholinesterase. This enzyme is located on the red cell surface, accessible to trypsin inactivation[6].

Determinations were first attempted in the rat, but no erythrocyte acetylcholinesterase activity could be demonstrated in this animal, using Sabine's method[7]. Reports of acetylcholinesterase activity in cellular fractions of rat's blood[8] are undoubtedly due to the presence of platelets, in which it is present in unusually high quantities[9]. *In vitro* determinations of human red cell acetyl-

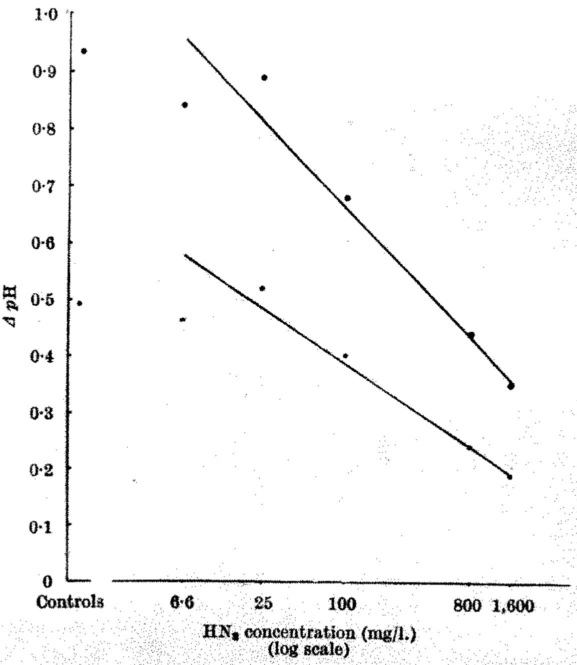

Fig. 1. Observed *p*H change, due to erythrocyte acetylcholinesterase activity, after incubation with 5 concentrations of nitrogen mustard (HN_2) for 1 h at 4° C. Readings were taken after incubation with substrate (acetylcholine) for 30 min (small dots) and 60 min (large dots)

cholinesterase activity were therefore undertaken, using Burman's modification[10] of Michel's method[11]. Twice-washed erythrocytes, resuspended in an equal volume of 0·85 per cent saline, were incubated at 4° C with 5 concentrations of nitrogen mustard:

(*a*) 6·6 mg/l., which, assuming a total blood volume of 60 ml./kg (ref. 12), is equivalent to a therapeutic dose of 0·4 mg/kg; (*b*) 25 mg/l., equivalent to the LD_{50} of 1·5 mg/kg; (*c*) an arbitrary concentration of 100 mg/l.; (*d*) a concentration of 800 mg/l., recommended for chemical sterilization of blood[13]; (*e*) an arbitrary concentration of 1,600 mg/l. Incubation with acetylcholine in buffer at 23° C was commenced 1 h after addition of nitrogen mustard, and *p*H change recorded at 30 and 60 min.

Results are shown in Fig. 1. Activity appears to be unaltered by 1 h exposure to therapeutic or LD_{50} levels of the drug. On the other hand, an effect is apparent in the concentration range from 100 to 1,600 mg/l., and this effect is approximately related to the log of the concentration. These concentrations, however, can only be produced *in vitro*, and it appears that nitrogen mustard has no direct effect on the acetylcholinesterase of circulating erythrocytes at levels likely to be found in biological usage.

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P. C. Vincent *
Nea Brittle
T. S. Reeve

Wellcome Laboratories for Experimental Medicine and Surgery,
Royal North Shore Hospital of Sydney, Crows Nest, New South Wales.
Department of Surgery, University of Sydney.

* British Tobacco Company (Australia) Fellow in Cancer Research.

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HISTOCHEMISTRY

Influence of Adrenocorticotrophic Hormone, Hydrocortisone and Hypophysectomy on the Distribution of β-Glucuronidase in the Adrenal Cortex of the Rat

In an extensive investigation of the activity and localization of several enzymes found in the rat adrenal cortex in different experimental circumstances, striking changes were noticed in the localization and the activity of the enzyme, β-glucuronidase.

Four groups of animals were examined. Each group consisted of 5 males and 5 females weighing 150 g: group I, control animals; group II, animals receiving 2 I.U. ACTH daily for 10 days, subcutaneously; group III, animals receiving 2 mg hydrocortisone daily for 10 days, subcutaneously; group IV, animals killed 10 days after hypophysectomy.

The rats were killed under ether anæsthesia. The adrenals were dissected and stored for several days at −20° C. Sections with a thickness of 10μ were made in a cryostat. The Fishman and Baker[1] method was used to demonstrate the presence of β-glucuronidase.

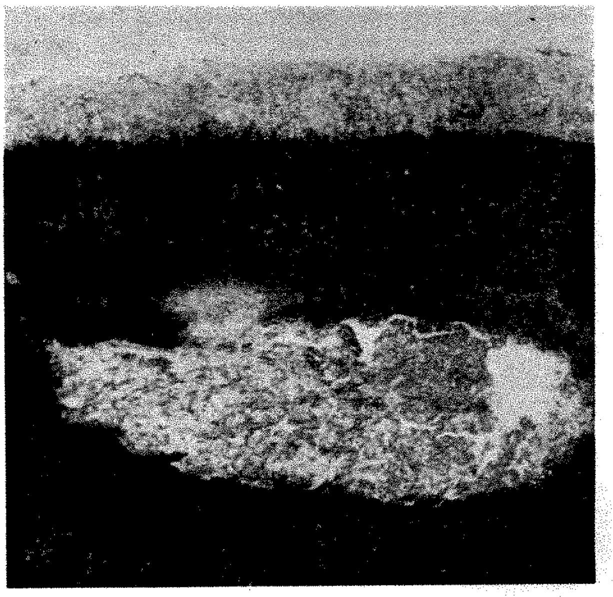

Fig. 1. β-Glucuronidase activity of the adrenal cortex after treatment with hydrocortisone (× 25)

Unlike most enzymes (alkaline phosphatase, acid phosphatase, 5 nucleotidase, adenosine triphosphatase, esterases, aminopeptidase, succinic dehydrogenase and several other dehydrogenases) which are more or less specific for a special zone and correlate with an increase and decrease in the width of these zones, β-glucuronidase showed a completely different localization under the various experimental conditions.

The control animals and the animals treated with ACTH and hydrocortisone showed, apart from changes in the width of the zones, no striking differences. The β-glucuronidase was localized in the zona reticularis and the zona fasciculata (Fig. 1). The peripheral part of the zona fasciculata in particular showed strong activity. No

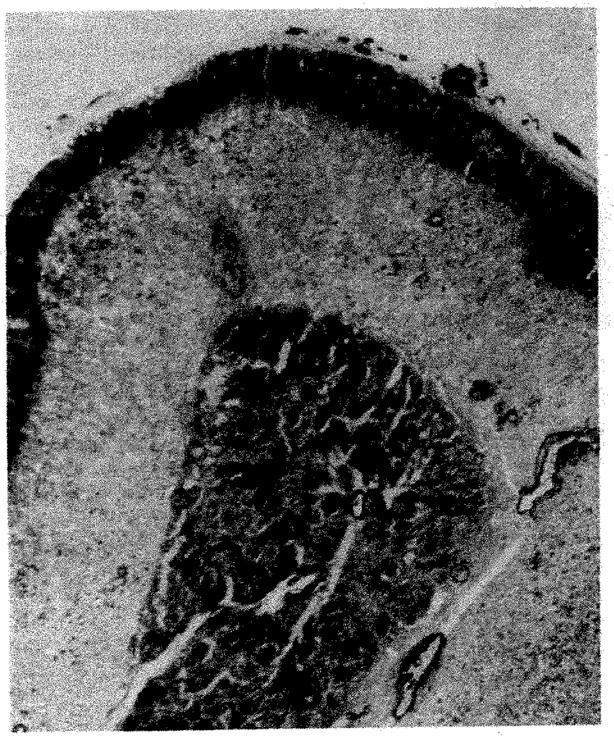

Fig. 2. β-Glucuronidase activity of the adrenal cortex after hypophysectomy × 25)

activity was found in the zona glomerulosa. This enzyme pattern is similar to that found by Fishman and Baker in normal animals. Nayyar and Glick[2], who estimated the enzyme in tissue fragments, also found strong activity in the central part of the cortex, diminishing gradually towards the capsule.

A different pattern was found in the adrenals of hypophysectomized animals. Here the zona glomerulosa and the most peripheral part of the zona fasciculata were strongly positive and the zona reticularis negative (Fig. 2). This observation is in agreement with the quantitative estimations of β-glucuronidase in cortical fragments of hypophysectomized animals made by Nayyar and Glick. These authors found that after hypophysectomy the activity of β-glucuronidase was diminished, the decrease being less than would be expected from the decrease in weight. This slight total decrease can be explained by the increased activity in the zona glomerulosa found in our experiments.

Both hypophysectomy and treatment with cortisone induce atrophy of the adrenal cortex. Our findings suggest that there is a principal difference in functional activity under these two conditions.

Although Janigan and Pearse[3] did not accept Fishman's and Baker's method as specific for β-glucuronidase, the changes found with this method after hypophysectomy suggest such a specificity.

G. TH. A. M. BOTS
R. G. J. WILLIGHAGEN
A. SCHABERG

Pathology Laboratory,
State University,
Leyden,
The Netherlands.

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A Histochemical Technique for the Demonstration of Cytochrome c

HITHERTO no method has been reported for the histochemical demonstration of cytochrome *c* in tissue sections. It has recently been shown, however, that cytochrome *c* behaves like a 'true' peroxidase at low *p*H in a pyrogallol-hydrogen peroxide system[1]. In this communication a method is described based on the peroxidatic activity of cytochrome *c*.

The kidneys of adult albino rats (Wistar strain) were used because they are known to be rich in cytochrome *c*[2]. The rats weighed 220–355 g, and were fed a normal diet *ad libitum*. The kidneys were perfused *in situ* through the aorta with a dextran preparation ('Intradex', Nyegaard and Co., Oslo). After perfusion the kidneys appeared quite pale both on the surface and in sections. The freeze-drying technique was used as the *in vivo* localization of proteins is preserved by this procedure[3]. Transversal blocks of about 1 mm in thickness were prepared, mounted on copper plates and rapidly frozen in isopentane cooled by liquid nitrogen[4]. Thereafter, they were dried *in vacuo* at a temperature between −35° C and −40° C. Next, the tissue blocks were infiltrated for 1·5 h *in vacuo* at 40° C by a mixture of polyethylene glycols (Farbwerke Hoechst AG, Germany), grade 1,000 and 600 (2 : 1, v/v (ref. 5)). Finally, they were embedded in a mixture of polyethylene glycols, grade 2,000 and 1,000 (56 : 44, v/v (ref. 6)). Polyethylene glycol was used because the sections could then be placed into the staining solution without further pre-treatment. Serial sections of 5μ were cut, and put into absolute acetone. The sections sank to the bottom, and the polyethylene glycol disintegrated partly, leaving the cytochrome *c* unextracted. The wrinkles flattened out well, and the sections were transferred to cool glass slides, and air-dried. Persisting small amounts of the wax were dissolved in the staining solution. Alternatively the sections were mounted directly on to the slides by gentle pressure by the finger[6]. The sections always adhered to the slides throughout the procedure.

Serial sections were stained by one of the following methods: (1) Ehrlich's hæmatoxylin/eosin; (2) Beneš's staining for mitochondria[7]; (3) peroxidase staining for the demonstration of cytochrome *c* as described in the following text.

Out of a number of oxidizable substances tested, only benzidine, with sodium nitroprusside present, was found satisfactory. The following method was found empirically to give optimal histochemical results:

Reagents:

A	Benzidine	0·3 g
	Sodium nitroprusside	0·2 g
	Ethanol (70 per cent, v/v)	150 ml.
	Glacial acetic acid	1 ml.
	The *p*H of this solution was about 4·8.	
B	Hydrogen peroxide (30 per cent, w/v)	2·5 ml.
	Glacial acetic acid	2·0 ml.
	Ethanol (70 per cent, v/v)	100 ml.
	The *p*H of this solution was about 3·6.	

The sections were left in solution *A* for 1–10 min and then directly transferred to solution *B* for 1 min. After thorough washing in 70 per cent ethanol, they were counterstained for 1 min in 1 per cent aqueous solution of neutral red. The sections were then dehydrated and cleared through the usual ethanol–xylene series and mounted in 'DePeX' mounting medium (George T. Gurr, Ltd., England).

The quality of the freeze-drying was revealed by staining with hæmatoxylin–eosin, and the mitochondria were visualized by the specific staining according to Beneš. Peroxidase activity attributable to cytochrome *c* caused the appearance of dark blue cytoplasmatic granules (Fig. 1). The nuclei and the cytoplasm, except for the mitochondria, stained deeply red, providing an excellent contrast. The granules, representing precipitates of oxidized benzidine ('benzidine blue'), were confined to the mitochondria; actually, these appeared better defined than in sections stained with conventional techniques. The precipitates of 'benzidine blue' are very stable, and the sections could be stored without special precautions for at least a year.

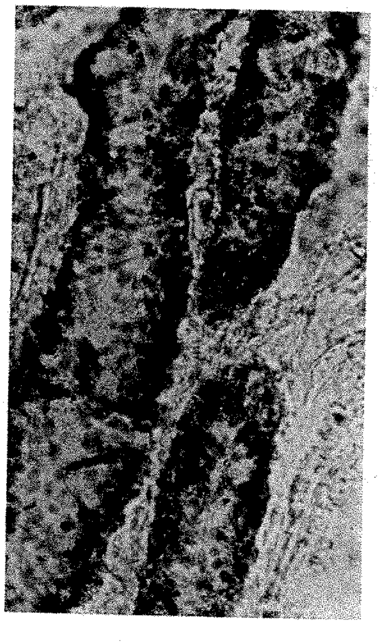

Fig. 1. The intracellular distribution of peroxidase activity, attributable to cytochrome *c*, in the thick, ascending limb of Henle's loop in the outer medullary zone of the rat kidney. Finely coarsed, dark blue precipitates of oxidized benzidine ('benzidine blue') are seen tightly packed basally and perinuclearly, closely confined to the mitochondria. Peroxidase staining (no counter-staining) (× *c*. 830)

The tissue sections showed a well-reproducible and characteristic distribution pattern for cytochrome *c* in the different zones and along the individual nephrons. The activity was greatest in the thick, ascending limb of Henle's loop and in the proximal and distal convoluted tubules. In the glomeruli no activity could be demonstrated. In the collecting ducts, the 'dark' ('intercalated') cells gave a strongly positive reaction, best seen in the outer cortical zone. In the 'light' cells only some scattered blue precipitates appeared in the outer cortical zone.

The specificity of the reaction is of primary interest. The cytochemical localization of the peroxidase activity matches that obtained for the sub-cellular fractions of perfused rat kidney homogenates[8]. By this method the main peroxidase activity was found in the mitochondrial fraction. Other cytochromes (a_3, a_1 and b) do not interfere with the reaction, since the staining reaction is negative when the sections are washed in physiological saline prior to staining. Cytochrome *c* is the only cytochrome in animal tissues known to be extractable by saline[9]. This hæmoprotein could also be demonstrated in the saline washings by means of the peroxidase method[1]. Catalase shows no peroxidase activity in acid solution[10]. Thus, the histochemical method appears specific for cytochrome *c*.

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T. FLATMARK

Institute for Thrombosis Research,
University Hospital (Rikshospitalet),
Oslo.

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PATHOLOGY

Adenosine Triphosphatase in Normal and Scorbutic Wounds

ASCORBIC acid deficiency produces well-known alterations in wound healing. Using histoenzymatic techniques important changes have been described such as a decrease in alkaline phosphatase[1] and a relative increase in acid phosphatase[2] in scorbutic wounds as compared with controls. With the technique proposed by Wachstein and Meisel[3] for the demonstration of adenosine triphosphatase a marked response appears in blood vessels. This investigation was carried out in order to examine adenosine triphosphatase-positive blood vessels in wounds performed in normal and scorbutic animals.

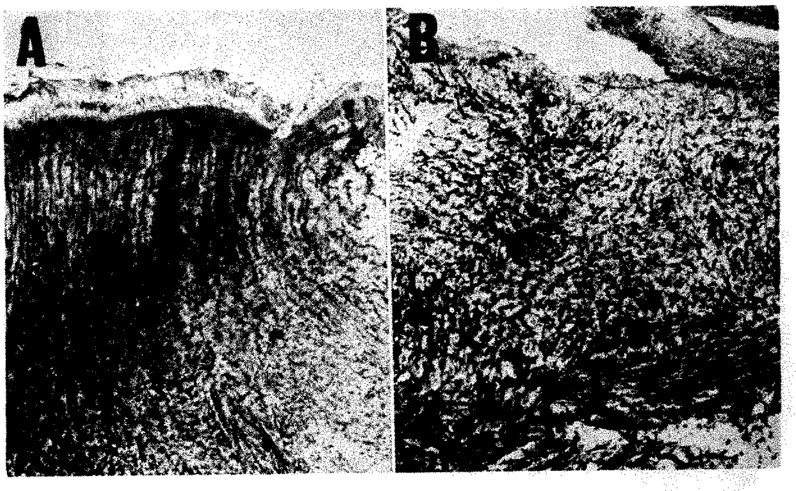

Fig. 1. Seven-day skin wound, Wachstein and Meisel technique, incubation time 3 h. *A*, Control animal; *B*, chronic scurvy

Twenty-five guinea pigs were divided into three groups: total deficiency (6 animals), partial deficiency (9 animals) and controls (10 animals). The diet used was that proposed by Nadel *et al.*[4] in a way similar to that described in a previous paper[2]. Wounds in dorsal skin and tongue were made and animals killed after three days and one week (total deficiency) and 3 days and 1, 2 and 3 weeks (partial deficiency and controls). The wound area with a wide margin of normal tissue was removed, fixed in 6 per cent formalin and processed according to the technique of Wachstein and Meisel.

In control animals an intense response was noted in blood vessels, especially those newly formed, and a moderate reaction in fibroblasts. The granulation tissue area was easily recognized for the abundance in blood vessels which adopted a characteristic pattern perpendicular to the wound surface (Fig. 1,*A*). This vascular orientation was observed until the wound became epithelized; at this time blood vessels appeared reduced in number and lost their orientation.

In vitamin C-deficient animals, adenosine triphosphatase appeared with the same localization and intensity as in controls, but a striking difference in distribution of blood vessels was observed. In as yet unepithelized wounds, a complete lack of orientation of blood vessels was noted, forming a reticular pattern (Fig. 1,*B*). This distribution of blood vessels was seen both in chronic and acute deficiency wounds. When the wound appears epithelized blood vessels were seen reduced in number and with an irregular distribution both in controls and deficient animals.

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R. L. CABRINI
F. A. CARRANZA, JUN.

Department of Pathology,
Hospital Ramos Mejía,
and Faculty of Dentistry,
University of Buenos Aires.

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Effect of 2-Methyl-1,2-*bis*-(3-pyridyl)-1-propanone (Metyrapone) on the Production of Mammary Tumours induced in Rats by Oral Feeding with Dimethylbenzanthracene

THE oral administration to rats of 9,10-dimethyl-1,2-benzanthracene (DMBA) has become an accepted method of producing mammary tumours for experimental purposes[1]. This treatment has also been shown to cause massive adrenal necrosis[2] which can be prevented by simultaneous administration of 2-methyl-1,2-*bis*-(3-pyridyl)-1-propanone (metyrapone)[3]. This communication reports the effects of metyrapone on the incidence in rats of mammary tumours induced by oral DMBA.

Rats descended from stock imported from Sprague–Dawley of Madison, Wisconsin, and commercially available

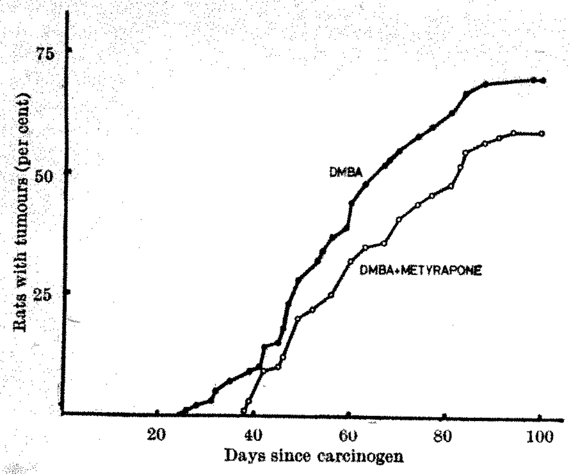

Fig. 1

in Britain, were used. A total of 440 rats was divided into two groups by a formal randomization process. In the first group each rat was given one subcutaneous injection of 40 mg of metyrapone in 4 ml. of corn oil, followed 12 h later by 50 mg of DMBA in 2 ml. of corn oil by intragastric tube. In the second group no metyrapone was given but each rat received the same dose of DMBA as in the first group. The DMBA was given on the 54th day of life. Three days after the administration of DMBA one-fifth of the rats from each group were killed by coal gas. The adrenals were dissected out, fixed in 10 per cent neutral formalin and embedded in paraffin. Sections were cut at 5μ and stained with hæmatoxylin and eosin.

Histological examination of the fixed and stained adrenals showed that only 3 out of 33 rats developed adrenal necrosis in the group receiving metyrapone + DMBA, in contrast to 31 out of 40 rats which developed massive adrenal necrosis in the group receiving DMBA alone. This supports the findings of Currie *et al.*[3], although the dosage of metyrapone in the present experiments differs from that previously reported.

During the 3 months after giving DMBA fewer rats developed tumours in the group given metyrapone + DMBA than in the group given DMBA alone, but the differences are not statistically significant. $P < 0{\cdot}2 > 0{\cdot}1$ (Fig. 1, Table 1). The difference, however, between the total tumours produced in each group by 100 days is just significant at the 5 per cent level (Table 2). It has been shown by Young and Cowan[4] that some of the tumours induced by orally administered DMBA stop growing while others regress spontaneously. The incidence of adrenal necrosis and the relative proportions of growing, static and spontaneously regressing tumours are given in Table 2.

It is clear that whereas metyrapone has almost completely prevented the occurrence of massive adrenal necrosis, it has not materially affected the induction period of tumours, the proportions of rats developing tumours, or the relative proportions of growing, static or spontaneously regressing tumours. It has, however, significantly reduced the total yield of mammary gland tumours in the group of rats to which it was given.

Table 1. NO. OF RATS DEVELOPING MAMMARY GLAND TUMOURS IN GROUPS GIVEN METYRAPONE + DMBA AND DMBA ALONE

	Days after DMBA	60		80		100	
Metyrapone + DMBA	Rats with tumours	22/69		33/69		41/69	
	Total tumours		27		68		90
DMBA alone	Rats with tumours	38/89		57/89		62/89	
	Total tumours		65		137		176
	P	n.s.	n.s.	n.s.	n.s.	n.s.	0·05

Table 2. PROPORTIONS OF GROWING, STATIC AND SPONTANEOUSLY REGRESSING TUMOURS AND INCIDENCE OF ADRENAL NECROSIS IN RATS GIVEN METYRAPONE + DMBA AND DMBA ALONE

	Total tumours	Growing	Static	Spontaneously regressing	Adrenal necrosis
Metyrapone + DMBA	90	24	50	16	3/33
DMBA alone	176	49	70	49	31/40
P	0·05		n.s.		0·0001

JANET E. HELFENSTEIN
STRETTON YOUNG

Pathology Unit,
Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London, W.C.2.

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Incidence of Squamous Cell Carcinoma in Mice fed 9,10-Dimethylbenz(a)anthracene

9,10-Dimethylbenz(*a*)anthracene (DMBA) has been used to induce neoplasms in experimental animals for some years[1]. However, the oral route of administering this compound has received little attention. Engelbreth-Holm and Poulsen[2] increased the incidence of leukæmia and mammary carcinoma at an early age, and Saxén *et al.*[3] induced carcinoma of the forestomach by feeding DMBA to mice of strain Street (then, not an inbred strain). Saxén[4] used irradiated *C57Br* mice for his DMBA feeding experiments. It was considered of interest to extend these observations on feeding DMBA under different experimental conditions and on other strains of mice. This communication reports the successful induction of neoplasms with a low total dose of the carcinogen. It gives the incidence and describes the pathological changes observed when *C57Br* and *C3H* mice were tube fed with a DMBA emulsion.

Two strains of mice, *C57Br* and *C3H*, were used in these experiments with about equal numbers of male and female animals in each group. A 30 mg per cent emulsion of 9,10-dimethylbenz(*a*)anthracene (DMBA) was prepared in 5 per cent 'Triton-100' (alkyl aryl polyether alcohol), a commercial surface-active emulsifier. The carcinogen was introduced into the stomach of mice with a polyethylene tube in 0·3 ml. feedings. A group of *C57Br* mice about 2 months old received 3 feedings a week for 10 weeks, a group of *C3H* mice about 6 months old received 2 feedings a week for 4 weeks and one feeding per week for 7 weeks, a total of 15 feedings in 11 weeks. Another group of *C57Br* mice, about 6 months old, received a single feeding of DMBA emulsion. Two groups of control mice of strain *C57Br* received 0·05 per cent 'Triton-100' in place of drinking water throughout the experimental period. One control group was one month old and the other eight months old at the start of the experiment. All the mice were fed a diet of Purina laboratory chow pellets, and examined at regular intervals to determine their general health, and for signs of developing lesions. An animal was killed when a lesion was suspected or when its general condition was poor. All mice were autopsied and specimens for histological examination were taken from each tumour mass and also from different parts of the stomach, intestine, spleen, kidney, liver and lungs. Tissues were fixed in Zenker-acetic or Zenker-formal fluids and embedded in paraffin. Microscopic sections were stained routinely with hæmatoxylin and eosin.

Tube feeding of 0·3 ml. of DMBA in 5 per cent 'Triton-100', 3 times a week, was well tolerated by mice. Feeding of 0·05 per cent 'Triton-100' in drinking water to control mice for 12–24 months showed no apparent deleterious effect on the general health. Some animals in every group receiving one or more DMBA feedings developed squamous cell carcinoma of the forestomach. In this work only those lesions were diagnosed as carcinomas which penetrated through all the layers of the forestomach wall. Others were diagnosed as precancerous or papillomatous lesions depending on their invasiveness The lesion of the forestomach of the mouse observed in this study is similar to the lesion induced in the mouse forestomach with methylcholanthrene and dibenzanthracene These lesions have been adequately described and illustrated by Stewart *et al* [5] and Firminger and Stewart[6] The incidence of squamous cell carcinoma in the forestomach of mice receiving various treatments and that survived over 100 days after the treatment was started is listed in Table 1 The incidence was 73 5 per cent in *C57Br* mice which received a total of 2 7 mg DMBA in 30 equal feedings, 40 5 per cent in *C3H* mice that received 1 35 mg DMBA in 15 feedings and 8 5 per cent in *C57Br* mice that received 90 μg DMBA in a single feeding There was no appreciable sex difference in the incidence of this tumour, however, 4 carcinomas appearing in the group receiving a single dose of DMBA were all in female mice

Table 1 INCIDENCE OF SQUAMOUS CELL CARCINOMA OF FORESTOMACH IN MICE FED 9,10-DIMETHYLBENZ(*a*)ANTHRACENE

Mouse strain	Sex	No	Doses	Total DMBA ingested (mg)	Duration (days)	Carcinomas No	%	
C57Br	M	22	30	2 7	245	15	68 0	73 5
C57Br	F	27				21	77 8	
C3H	M	24	15	1 35	245	10	41 7	40 5
	F	13				5	38 5	
C57Br	M	28	1	0 09	300	0	0	8 5
C57Br	F	19				4	21 0	
C57Br	M	10	*		256	0	0	3 0
	F	23				1	4 3	
C57Br	M	15	*		632	0	0	0
C57Br	F	36				0	0	

* 50 mg per cent 'Triton-100' in drinking water

One squamous cell carcinoma of the forestomach was observed in a female, in a group of 33 control mice, but there was no incidence of carcinoma in the second group of 51 control mice As animals in the second control group received 0 05 per cent 'Triton-100' for about 400 days more than the mice in the first group, it was concluded that the carcinoma in the first group was probably not caused by 'Triton-100' in the drinking water

Hyperkeratosis of the forestomach was common in most of the mice in both control and experimental groups However, the group of mice receiving a single dose of DMBA showed 3 (6 4 per cent) precancerous lesions and 14 (29 8 per cent) papillomas, while all mice in both control groups showed no precancerous lesions and only 5 9 per cent papillomas

The vehicle, 'Triton-100', used for DMBA administration and fed in drinking water to control mice, is one of a series of compounds with predominantly hydrophylic properties and demonstrated power of strong penetration in the glandular mucosa of the stomach[7] Control mice received a maximum total dose of over 3 ml of the vehicle per mouse during the experimental period This dose of 'Triton-100' did not induce any abnormal changes in the forestomach or glandular stomach of the mouse The maximum dose of 'Triton-100' received by an experimental mouse was only 0 45 ml

In the feeding experiments of Lorenz and Stewart[8,9], the range of carcinogenic hydrocarbon ingested by a mouse during the experimental period was about 100–250 mg of methylcholanthrene or dibenzanthracene in olive oil emulsion[8] and 50–200 mg of the same hydrocarbons in mineral oil emulsion[9] Engelbreth-Holm and Poulsen[2] were the first to tube-feed DMBA to mice They used a glass tube and probably because of trauma to the œsophagus only 68 of 175 mice survived to be 4 months old Total DMBA administered per mouse was 2–6 mg They did not report any forestomach lesions in their mice Saxén *et al* [3] working with Street II strain mice administered a total DMBA dose of 0 3–4 mg by polyethylene tube They found forestomach carcinomas in 3 out of 9 mice receiving 0 6 mg (600 μg) as a total DMBA dose, but no carcinomas in mice receiving 300 μg of DMBA Under my experimental conditions a single dose of 90 μg DMBA administered orally gave undisputed evidence of the induction of carcinoma Induction of neoplasms in 10 per cent of mice by a dose of a fraction of a microgram of carcinogen (0 08 μg of dibenz(*a,h*)anthracene subcutaneously to 12 h old mice) was recently reported by O'Gara *et al* [10]

I thank Dr H L Stewart for help with the histopathology work

AMBADAS S MULAY

Laboratory of Pathology,
National Cancer Institute, Bethesda, Maryland

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Tumour-enhancing Activity of the Active Principles of *Croton tiglium* L.

THE potent tumour-enhancing agent croton oil (from the seed of *Croton tiglium* L) has been used for many years in biological investigations of the cause of cancer[1,2] and several unsuccessful attempts have been made to purify and to describe chemically the active components[3,4] The isolation and chemical structure elucidation of pure compounds which are active tumour-enhancing agents is important since it will facilitate new and definitive studies on the mechanisms of carcinogenesis and co-carcinogenesis

We described recently the chemical constitution of a crystalline product obtained by catalytic hydrogenation of the tumour-enhancing fraction of croton resin[5] This material, which is a fatty acid ester of a polyphenol, was shown to be very similar to the active materials in its chemical constitution

The notable biological activity of some materials obtained from the active mixture of compounds are described here The isolation of these compounds is described elsewhere[5]

All materials were tested by skin-painting on female Swiss Millerton mice. The animals were first given a single application of 7,12-dimethylbenz(*a*)anthracene (DMBA) applied in acetone solution by micro-pipette The dose of carcinogen applied (150 or 300 μg) was at such a level that it did not by itself give any skin tumours The primary treatment was followed 14 days later by three-times weekly applications of promoters

In the first test, 18 animals were used in each group; 150 μg DMBA was used as initiator The promoters were applied three times weekly, 25 μg dissolved in 100 mg acetone per application, applied on the clipped mouse skin with a Number 5 camel hair brush The results are shown in Fig 1 In this experiment the vesicant fraction, croton resin, prepared as described by Cherbuliez[6], is compared with a column-chromatographic fraction derived from it and the whole croton seed extract Concentration of activity was achieved in the croton resin and its sub-fraction Control groups, also with 18 animals per group, included animals which received DMBA only, promoters only, solvent only, and a group which received no treatment One animal with a papilloma was observed in each of the two control groups which received only croton resin or its sub-fraction

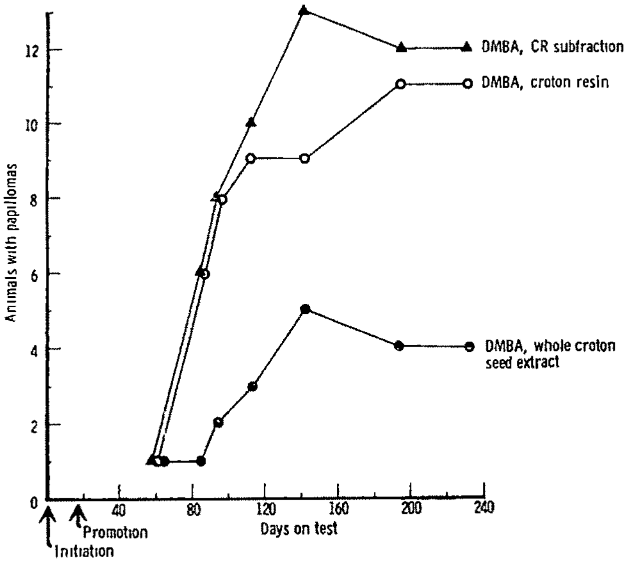

Fig 1 Tumour promotion by croton seed extract and fractions derived from it

Malignant tumours were observed in the promotion experiments with croton resin and its sub-fraction as early as 100 days after initiation At the end of one year, 70 per cent of the surviving animals which had received DMBA followed by croton resin had squamous cell epidermoid carcinomas These were usually situated at the sites of previous papillomas None of the animals in the control groups showed malignant tumours at the end of one year

Histological examination of papillomas showed proliferative changes in the epidermis with marked keratinization, acanthosis and intact basal layer Histological examination of the epidermoid carcinomas showed marked atypia of epidermal cells, mitoses, keratin pearls and break-through of the basal layer Sheets and clumps of tumour cells were present in the subcutis

Progressive purification of the biologically active mixture led to two components, labelled *A* and *C*, which showed single spots on thin-layer chromatograms[5] Both these materials are powerful irritants and showed high tumour-enhancing activity as shown in Table 1 Twenty female Swiss Millerton mice were used in each of the two groups A single dose of DMBA, 300 µg in 0 15 ml acetone, was given to each animal After a 2-week interval the animals in one group received three times weekly paintings of *A* and the other received *C*, 5 µg material was applied in 0 1 ml acetone per application

The control groups, 20 animals per group, were as follows (1) a group which received a single application of 300 µg DMBA in 0 15 ml acetone, (2) a group which received three times weekly applications of promoter *A*, 5 µg in 0 1 ml acetone per application, (3) the same as (2) but with promoter *C*, (4) a group which received applications of acetone three times weekly, 0 1 ml per application, (5) a group which received no treatment Tumours were not observed in any of these control groups

The level of activity of compounds *A* and *C* is much higher than that reported for other tumour-enhancing agents such as phenol[7], the 'Tweens' and 'Spans'[8] With 'Tween 40', for example, 60–80 mg of material was applied per animal, six times weekly[8] This is roughly 30,000 times the weekly dosage of compounds *A* and *C* used in the present work In the interpretation of the work on 'Tween' and related materials the presence of small amounts of active impurities cannot be overlooked since massive doses were applied

Table 1 Tumour Promotion with Compounds *A* and *C* No of Animals with Tumours*

Weeks†	4	5	8	14‡
A	0	0	2	15
C	1	11	17	20
Cumulative weight of promoter applied per animal (µg)	60	75	120	210

* All tumours were benign papillomas and were confirmed microscopically
† After beginning of promotion
‡ All animals were alive after 14 weeks

In another experiment two groups of 18 animals each received an initiating dose of 150 µg of DMBA After one year without further treatment the eleven survivors of one of the groups received paintings three times weekly with 25 µg of croton resin dissolved in 100 mg of acetone per painting The second group of fourteen survivors served as an untreated control Within 37 days from the beginning of painting with croton resin two out of eleven animals developed papillomas and, after 120 days, 4 animals had papillomas and two had squamous cell epidermoid carcinomas Skin tumours were not observed in the control group This experiment confirmed an earlier experiment of Berenblum[2] in which 10 months was allowed to elapse between initiation and promotion.

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B L Van Duuren
L Orris
E Arroyo

Institute of Industrial Medicine,
New York University Medical Center,
New York 16, N Y.

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IMMUNOLOGY

Duration of Antibody Response to Soluble Antigen: Incorporation of Sulphur-35 Methionine into Normal and Antibody Globulin

We recently reported[1] that precipitating antibodies were present in chicken serum more than 300 days after two spaced intravenous injections of bovine serum albumin Antibody remains detectable at 470 days post-injection with no apparent decrease in concentration having occurred over the last 300 days It is of theoretical interest to know if these antibodies represent the product of continued synthesis, approximating a steady state, or alternatively reflect the gradual release from tissue depots of antibodies synthesized at a much earlier time The present communication provides evidence favouring the former hypothesis

Two chickens were injected intravenously with 1 mc each of ^{35}S-L-methionine (12 mc /m mole), 470 days after the second injection of bovine serum albumin Serum samples obtained both before and after injection of sulphur-35 were dialysed against three changes of buffer (0 1 M *tris*, 0 1 M sodium chloride, *p*H 8 0) containing unlabelled L-methionine (0 01 M) followed by dialysis for several days against large volumes of buffer without methionine The antibody content of the samples was then estimated by the method of Talmage and Maurer[2] using bovine serum albumin labelled with iodine-131 to determine where 80 per cent of the added bovine serum albumin was precipitated (*P*-80) Disodium ethylenediamine tetraacetate (EDTA) was added to a final concentration of 0 01 M in order to eliminate, in so far as possible, coprecipitation of non-antibody protein. A *P*-80 for chicken 30 was 0 35 µg bovine serum albumin nitrogen/

ml., for chicken 28, 0.9 µg bovine serum albumin nitrogen/ml. Antibody concentrations of these samples would be (P-80 × 5.5) about 2 and 5 µg nitrogen/ml. respectively. Determinations of specific activity were made on 1 ml. aliquots of serum incubated with the P-80 concentration of bovine serum albumin. Tubes were incubated at 37° for 2 h and then at 5° for 4 days. Precipitates (barely visible in the case of chicken 30) were washed three times with cold buffer containing EDTA and then dissolved in 0.4 ml. of 0.1 N sodium hydroxide. Protein nitrogen determinations[3] were carried out with 0.2 ml. of the solution removed carefully with a Lang-Levy micropipette. The remaining 0.2 ml. was analysed for radioactivity in a liquid scintillation counter (Packard, 'Tri-carb'). Determinations of the specific activity of 'normal' globulins, that is, proteins insoluble in one-half saturated ammonium sulphate, were carried out in similar fashion.

The contribution of non-antibody protein to precipitates was calculated from the radioactivity found in precipitates of a heterologous chicken immune system (pea seed globulin, anti-pea seed globulin). Antibody and antigen were first dialysed against buffer and then added in equivalence proportions (with EDTA) to triplicate 1-ml. serum samples. The antibody content of the precipitates formed was similar to the bovine serum albumin system. Tubes containing serum and buffer alone were also assayed. The latter contained no detectable protein (less than 0.2 µg nitrogen) and had essentially background radioactivity. By contrast, no more than 0.2 µg nitrogen coprecipitated with each µg antibody nitrogen in the heterologous system, despite the presence of EDTA. It is assumed that this applies equally to the bovine serum albumin system.

Even after subtraction of the contribution of non-antibody protein to precipitates, considerable activity remained: this is attributed to antibody (Table 1). At least 50 per cent of the estimated antibody content of precipitates would have to be non-specific protein (having the same specific activity as the normal globulins) in order to account for the total radioactivity present. In addition to the data from the heterologous precipitin system, the close agreement of calculated antibody concentrations (P-80 × 5.5) with protein determinations using the Folin phenol reagent[3] argues against such gross contamination.

Table 1. ANALYSES OF POOLED SAMPLES TAKEN 7 AND 21 H AFTER INJECTION OF SULPHUR-35

Counts* (min. µg anti-BSA nitrogen)		Counts (min. µg anti-pea globulin nitrogen)		Corrected anti-BSA specific activity
		Chicken 30		
$\frac{29.0}{1.4}$	(20.7)	$\frac{30.4}{3.7}$	(8.2)	
$\frac{35.8}{2.2}$	(16.3)	$\frac{29.9}{3.3}$	(9.1)	
$\frac{31.6}{1.7}$	(18.6)	$\frac{29.4}{3.6}$	(8.2)	
Mean	(18.5)		(8.5)	10.0†
		Chicken 28		
$\frac{74.2}{5.0}$	(14.9)	$\frac{21.0}{3.5}$	(6.0)	
$\frac{61.6}{5.0}$	(12.3)	$\frac{16.7}{3.4}$	(4.9)	
$\frac{70.4}{5.8}$	(12.1)	$\frac{18.1}{3.7}$	(4.9)	
Mean	(13.1)		(5.3)	7.8†

* All values based on 10-min counts.
† 21 per cent of the specific activity of normal globulins for chicken 30, 28 per cent for chicken 28.

The ratios of specific activities, antibody to normal globulin, were reasonably constant over the time interval tested, ranging from 25 to 40 per cent for both chickens (Fig. 1). The significance of specific activity differences between normal globulins and antibody is not clear. This is in part because of the heterogeneity of the globulin fraction. However, it has been shown[4] that during the initial phases of antibody responses the specific activity of antibody may be 2–3 times higher than the remaining globulins. If the bulk of the globulin fraction represents antibody synthesized during recently initiated immune responses, then the lower specific activity of late antibody is predictable. The results do not permit a confident estimate of the half-lives of antibody; however, assuming they do not differ markedly from those of normal globulins, values of 60 h (No. 30) and 75 h (No. 28) are obtained. This agrees well with other estimates in chickens[5].

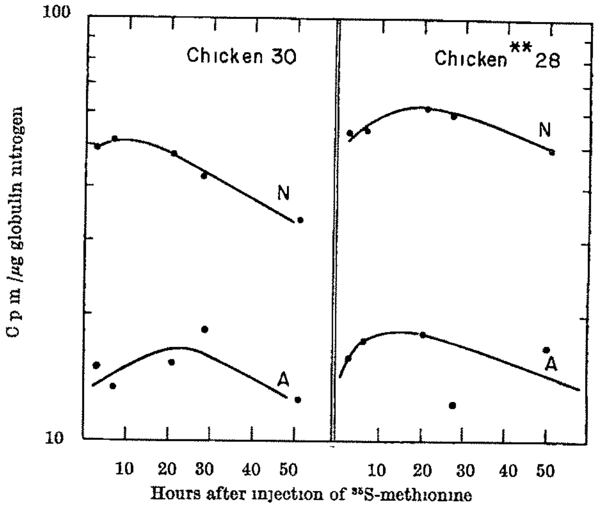

Fig. 1. Incorporation of ^{35}S-methionine into antibody (A)* and normal globulins (N)

* Corrected for non-specific protein

** Original values multiplied by 2

In view of the long-continued active synthesis of antibody, any theory requiring a direct role of antigen in antibody synthesis must obviously require also prolonged storage of antigen or specific fragments in relevant cells.

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R. V. GILDEN*
GRACE L. ROSENQUIST

California Institute of Technology,
Pasadena, California.

* Present address, Wistar Institute of Anatomy and Biology, Philadelphia.

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Radiation Protective Effect and Pharmacologically Changed Activity of the Reticulo-endothelial System

BACTERIAL endotoxins, zymosan and other complex polysaccharides are stimulants of the reticulo-endothelial system[1,2]. This follows from the enhanced phagocytic activity as measured by the rate of removal from the blood of intravenously injected carbon[3]. But these substances also have a radiation protective effect, as has been found with mice, rats and hamsters[4]. Therefore, it is accepted that this radiation protective effect is causally connected with the enhanced activity of the reticulo-endothelial system and that, in general, the radiation resistance of an organism with an augmented activity of the reticulo-endothelial system is greater than with a normal activity state of this unspecific defence system[5]. This concept is in agreement with the conjecture that the prime cause of death after whole-body X-irradiation depends primarily

Table 1 ACTION OF SIMPLE LIPIDS ON THE PHAGOCYTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM (RES) AND ON THE SURVIVAL RATE 30 DAYS AFTER X-IRRADIATION (MICE)

Carbon clearance test and X-irradiation 24 h after intravenous injection of lipid emulsions or suspensions

Lipid	Dose (mg)	Phagocytic activity Mean K values Control	Phagocytic activity Mean K values Lipid	Phagocytic activity Difference (per cent)	Survival rate after X-irradiation Survival/Total Control	Survival rate after X-irradiation Survival/Total Lipid	Survival rate after X-irradiation Difference (%)
Olive oil	23	0 035	0 095	+170	45/194 = 21%	103/226 = 46%	+25
Triolein	11 5	,,	0 078	+123	34/129 = 26%	44/114 = 39%	$+13^{*}$
Tricaprin	20	,,	0 120	+243	2/44 = 5%	10/88 = 11%	$+6^{i}$
,,	40	,,	0 108	+210	2/20 = 10%	8/19 = 42%	$+32^{*}$
2-Oleo-distearin	10	,,	0 098	+180	0/24 = 0%	1/25 = 4%	$+4^{i}$
,,	20	,,	0 057	+ 63	0/24 = 0%	2/25 = 8%	$+8^{i}$
Ethyl palmitate	25	,,	0 015	— 57	30/120 = 25%	70/140 = 50%	+25
,,	50	,,	0 014	— 60	30/120 = 25%	67/100 = 67%	+42
Ethyl stearate	20	,,	0 012	— 66	35/154 = 23%	86/127 = 68%	+45
Ethyl oleate	23	,,	0 018	— 49	12/73 = 16%	45/81 = 56%	+40
Cholesterol oleate	30	,,	0 015	— 57	52/164 = 32%	91/181 = 50%	+18

+ = RES stimulation resp radiation protection , — = RES depression

Significance (χ^2 test) i = insignificant , $* = P < 0\ 05$, all other values = $P < 0\ 001$

on the exhaustion of certain cells originating in the reticulo-endothelial system[6]

It this concept were correct then naturally not only should the mentioned agents alone have the ability to increase the radiation resistance (protective effect) but also other methods of stimulation of the reticulo-endothelial system should act in the same way Likewise, depression of the reticulo-endothelial system would be expected to decrease the radiation resistance This concept is apparently contradicted by the finding that ethyl palmitate, a compound known to depress reticulo-endothelial system phagocytic activity, exerts a protective effect on X-irradiated mice[7] The high doses of ethyl palmitate which were used in these experiments, however, did not affect exclusively the reticulo-endothelial system but also led to a severe damage to the parenchyma of the red and white pulp of the spleen[8] Consequently, they are not suited for the assessment of the relations between the reticulo-endothelial system and the biological radiation effect But this is also the same for the reticulo-endothelial system stimulants already mentioned, because they also are said to exert complex reactions in the liver and the spleen, in which lymphocytes and plasma cells are engaged[2] Therefore, it seems desirable for the elucidation of the relations between activity of the reticulo-endothelial system and radiation resistance to utilize in further experiments only those substances which exert a specific and easily controllable alteration of activity of the reticulo-endothelial system using the already mentioned carbon clearance method[3] These requirements are met by some simple lipids[2] As several substances of this group stimulate the activity of the reticulo-endothelial system and others depress it, their application enables us to compare the influence of stimulation and depression of the reticulo-endothelial system on the biological effect of radiation

I used male white mice of an inbred strain of this laboratory , age 8–10 weeks, weight 18–23 g A small number of the animals was taken to measure the activity of the reticulo-endothelial system at the time of irradiation By far the greatest number was X-irradiated 24 h after the injection of lipids and 30 days later the survival rate was established The reticulo-endothelial phagocytic function was evaluated by measuring the intravascular removal rate of intravenous injected colloidal carbon (phagocytic index K)[3] Whole-body X-irradiation with 610 r , dose rate 90 r /min , 200 kV, 15 m amp , filtration of 0 43 mm copper, half-value layer 0 87 mm copper , focus-target distance of 40 cm For stimulation of the reticulo-endothelial system, I used olive oil, triolein, 2-oleo-distearin and tricaprin , for depression of the reticulo-endothelial system ethyl stearate, ethyl oleate, cholesterol oleate and such small doses of ethyl palmitate which indeed injured the phagocytic function of the reticulo-endothelial system but not the parenchymatous tissue of the spleen[2,9]

Table 1 presents the following experimental results

First Phagocytic activity The statements in the literature concerning alteration of the reticulo-endothelial system by the examined lipids were confirmed with my mouse strain. Olive oil, triolein, 2-oleo-distearin and tricaprin stimulated the phagocytic activity 24 h after injection, but ethyl palmitate, ethyl stearate, ethyl oleate and cholesterol oleate depressed it at this time

Second Survival rate All lipids mentioned, both those which stimulate and those which depress the reticulo-endothelial system, exerted a protective effect against X-rays It is remarkable that the depressing agents were mostly more effective than the stimulating agents

The results of these experiments confirm that with mice it is possible to stimulate or to depress the phagocytic activity of the reticulo-endothelial system by intravenous injection of simple lipids These lipids, furthermore, exert a protection effect against X-rays which differs from one substance to another The degrees of alteration of the phagocytic activity and of the radiation sensibility did not always parallel each other Thus, a radiation protection effect was obtained with lipids whether or not their action on the function of the reticulo-endothelial system was one of stimulation or depression It seems clear that the concept of enhanced activity of the reticulo-endothelial system as a factor of importance for the survival of mice after irradiation is in need of revision Perhaps an alternative idea is that cells of the reticulo-endothelial system engaged in the metabolism of simple lipids as described are more resistant to radiological injury than usual

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KURT FLEMMING

Heiligenberg-Institut, Heiligenberg/Baden and
Radiologisches Institut der Universitat Freiburg

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RADIOBIOLOGY

Cæsium-137 in the Blood of the Finnish Lapps

About 2,500 Lapps live in Finnish Lapland, and, as has been shown by an investigation carried out in October 1961 [1], the elevated radioactivity in them is principally due to the high cæsium-137 content of the reindeer meat which constitutes their main food. In May 1962, a second, wider survey of the body burden of cæsium-137 and of the diet and health of the Lapps was carried out[2]. The subjects, some 200 Lapps of ages ranging from 20 to 50, were selected in advance by the random sampling method from the church register. About 90 per cent of those summoned responded to the invitation. This investigation revealed that the body burden of cæsium-137 in Lapps was on the whole twice as high as in October 1961[1-3], but no correlation between the cæsium-137 content and the findings of medical examination could be observed[2,4,5].

The Lapps provide a unique opportunity to determine the correlation between cæsium-137 content of blood and whole body in a population living in natural conditions. Therefore, 20 ml. blood samples were taken in the foregoing investigation from all adults examined (102 men and 52 women), for cæsium-137 analyses[6]. The samples were transported in ice to the laboratory and ashed carefully at 400° C in order to improve the counting efficiency. The activity was measured in a sodium iodide (Tl) well-type crystal with a Nuclear Chicago model 132 *A* one-channel analyser in the range of the cæsium-137 peak between 612 and 712 keV.

The counting system was calibrated with Radiochemical Centre, Amersham, cæsium-137 reference solution *CDR* 4 in inactive calcium carbonate. A counting efficiency of 9.4 per cent was achieved for cæsium-137 in the range concerned. The background measured in the corresponding range was on the average 14.15 ± 0.15 c.p.m. during the course of the measurements. The number of impulses in the cæsium-137 range caused by potassium in the blood did not differ from the statistical fluctuations of the background during the measuring times used. Therefore, no correction was made. By measuring all blood samples simultaneously placed around a 5 in. × 3 in. Harshaw crystal, with a multi-channel pulse height analyser for 1,000 min, it was seen that they did not generally contain any observable amounts of other γ-emitters (Fig. 1).

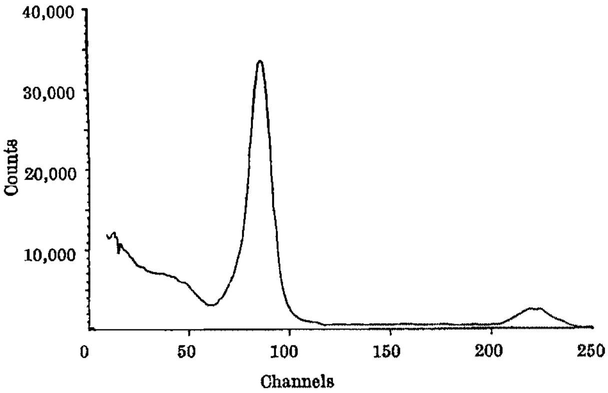

Fig. 1. Pooled blood samples, a net spectrum in 1,000 min

A graphic presentation of the whole-body values[2] in nc (10^{-9} c.) plotted against the pc (10^{-12} c.) values calculated for a litre of blood shows the correlation between them (Figs. 2 and 3). The correlation coefficient differs significantly from zero, the value for the male subjects being $r_{xy} = 0.83$ and that for the females $r_{xy} = 0.84$. By means of regression analyses the best straight line passing through the origin was fitted to the points, and the corresponding ranges of variation at the 95 per cent confidence-level were calculated. Since the graphs in Figs. 2 and 3 showed that there are some values which deviate considerably from the regression line, the ranges of variation for the individual observations were calculated as well. To make the regression line pass through the origin is to assume that when the whole-body value is zero the blood value is also zero and vice versa. However, this is not necessarily true. Therefore, an equation for a regression line which was not made to pass through the origin was derived from the data, also. The equations are given in Table 1.

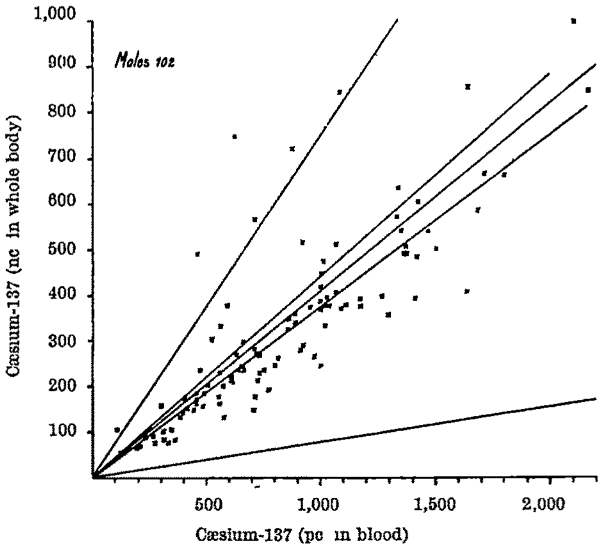

Fig. 2. Graph showing the correlation between the body burden of cæsium-137 (nc) and the cæsium-137 contents of blood calculated in pc./l. (males)

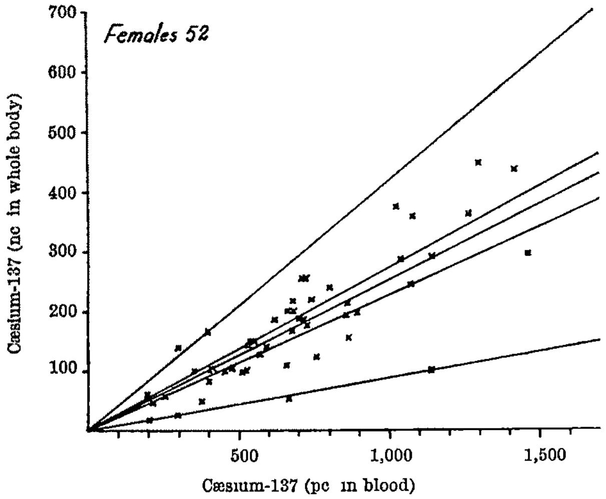

Fig. 3. Graph showing the correlation between the body burden of cæsium-137 (nc.) and the cæsium-137 contents of blood calculated in pc./l. (females)

Table 1. Equations of the Regression Lines

	Males		Females	
	Through the origin	General	Through the origin	General
Regression line	$y = 0.41\,x$	$y = 0.37\,x + 21$	$y = 0.25\,x$	$y = 0.27\,x - 12$
Corresponding ranges of variation at the 95% confidence-level	$y = 0.38\,x$ $y = 0.44\,x$		$y = 0.23\,x$ $y = 0.27\,x$	
Ranges of variation for the individual observations at the 95% confidence-level	$y = 0.08\,x$ $y = 0.74\,x$		$y = 0.09\,x$ $y = 0.41\,x$	

To describe the relationship between the cæsium-137 values of the blood and the whole body a straight line was used in this range, since the possible bending of the describer with higher cæsium-137 values could not be observed owing to the insufficient number of high values

As can be seen from the equations, the slopes of the lines passing through the origin do not deviate greatly from the slopes of the corresponding lines not passing through the origin. Neither can the intercepts be said to deviate significantly from zero when the errors of both activity measurements and the detection limits of the measuring systems are taken into account

It may be noticed that the slopes of the lines for males and females differ from each other This might possibly be ascribed to the differences in the excretion of cæsium in males and females

The subjects, in whom the ratio of whole-body value and blood value deviated from the regression line by more than 30 per cent, were examined in the light of the results of the medical examination and the dietary survey[2] In the comparisons there was nothing exceptional either in the findings of the routine health examination or in the food consumption habits of the subjects concerned In the literature, large individual differences in excretion of cæsium have been reported Half-times are given as 50–160 days A small fraction of the cæsium has been observed to be excreted considerably faster than the remainder[7] These facts could possibly explain the values that deviated considerably from the regression lines

On the basis of the results obtained it can be concluded that the whole-body burden of cæsium-137 can be estimated with sufficient reliability from the blood content of radiocæsium when the cæsium-137 values are 200–1,500 pc /l blood, and when the survey concerns the cæsium-137 burden of a group of people whose recent dietary history is known

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ANNELI SALO

Department of Radiochemistry,

ANTTI ALHA

Department of Forensic Medicine,
Chemical Division,

JORMA K MIETTINEN

Department of Radiochemistry,
University of Helsinki

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Influence of Radio Frequency Heating on Sperm

THE influence of temperature on the survival of spermatozoa *in vitro*[1] and the influence of high temperatures on the reproductive capacity of guinea pigs' spermatozoa have been investigated[2] by many workers

The work recorded here was undertaken to examine the influence of radio frequency heating on sperm in the genital passage of the female rabbit immediately after copulation The number of young born and the average litter size were the criteria used to assess the effect

Radio frequency heating, widely used in diathermy, has no side effects on tissue if the period of treatment and temperature gradient are kept within safe limits In particular, it is known that repeated elevation of the body temperature by radio frequency heating is innocuous to the functioning of the reproductive organs[3–5]. Also, greater uniformity and heat penetration in tissue are obtained than by heating elements or pads

The radio frequency electric field was set up between a plastic-coated metallic probe electrode (¼ in diam × 5 in), which was inserted in the genital passage, and a pad electrode (6 in × 8 in) on which the abdomen of the rabbit was resting Temperatures of 43 2°–45° C could be easily realized, the latter usually in the close neighbourhood of the bony tissue of the pelvic inlet

Six Albino rabbits were used as controls and six were treated in the three series of studies undertaken When the young from the first series were weaned, treated and control groups were reversed and were used in the second series When the young from this investigation were weaned 7 animals that had delivered young in the two previous series were treated and used for the final group The total number of treated animals was therefore 19 Two vigorous males of known fertility were used alternately throughout

Immediately after breeding a vaginal swab was taken to establish the presence of sperm The female was then lightly anæsthetized to facilitate manipulation of the probes and to avoid undue movement and possible injury to the animals during treatment Pre-treatment vaginal temperatures were recorded and then the probe electrode was inserted into the vagina for a depth of approximately 4 in A temperature probe connected to an electronic thermometer was placed in the vagina aside and to the same depth as the electrode The pad electrode was placed so as to be directly under the abdominal and pelvic organs The probe and pad electrodes were connected to a diathermy generator the output of which was then adjusted so that vaginal temperature rose to above 43 2° C as quickly as possible (about 1 min) and treatment temperature was held at 43 2°–45° C for 10 min The generator was switched off whenever a temperature reading on the electronic thermometer was required In this way temperatures devoid of interference from the diathermy were obtained Procedure for control groups was identical to treated groups except that the diathermy machine was not switched on

Three animals did not conceive when used as controls or when treated These animals were discarded and the total number used as controls was therefore 9 and as treated 16

It was found that diathermy treatment reduced the litter size with respect to the total number of animals bred but did not alter the average litter size in animals that reached parturition (Table 1) All animals born appeared normal at weaning time and developed in a manner similar to controls These findings are in accord with the studies by Young in guinea pigs[2]

Table 1 INFLUENCE OF RADIO FREQUENCY HEATING ON PARTURITION COMPARISON BETWEEN CONTROL AND TREATED FEMALE RABBITS

Group	No bred	No of parturitions	% Bred reaching parturition	No of young	Litter size per animal bred	Average litter size per parturition
Control	9	8	88 8	45	5	5 6
Treated	16	5	31 2	27	1 7	5 4

The average litter size over a 1-year period in our breeding colony is 4–5 This average was approximated in the controls used in this study, which would suggest that the manipulations (administration of anæsthetic, insertion of probe, etc) did not contribute to the reduction in litter size in the treated animals

The possibility that reduction in the number of pregnancies was due to effects of heat on ovulation has not been excluded. This work does not permit the assessment of such a possibility.

D. Makow

Radio and Electrical Engineering Division,
National Research Council,
Ottawa.

H. Grice

Food and Drug Laboratories,
Department of National Health and Welfare,
Ottawa.

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Effect of Gamma-radiation on Eggs of the Silkworm

The need for biological information on the effects of extraterrestrial conditions encountered in space environments has recently been presented by Reynolds[1]. Techniques for the conduct of biological experiments in space studies are almost non-existent.

One factor encountered in space flight which is of significant importance to biological material is ionizing radiation. Previous work with *Drosophila* larvæ[2], house-flies (*Musca domestica* L.) and oriental rat fleas (*Xenopsylla cheopis* (Rothschild)) in various stages of development[3] failed to demonstrate any genetic effects after exposure to cosmic radiation at 70,000–82,000 ft. for 16 h. These results may have occurred because no cosmic ray 'hits' on the biological material were made or lethality was induced at low levels which was not measurable. Investigations have since been conducted to find a more suitable organism which could be used in space studies to record accurately the effect of cosmic and other radiations on biological material. Eggs of the silkworm (*Bombyx mori* (L.)), genetically well understood, possess many desirable biological characteristics necessary for investigations of this type. They are extremely light in weight, and require no food or water and only minute amounts of oxygen. In addition, the eggs are laid in a solid, single layer.

Their large size (1 mm), as compared with some insects, makes them a suitable target for 'hits' from heavy nuclei of the primary cosmic radiation. Dutky and Sullivan (unpublished report) have adapted a nuclear emulsion envelope suitable for the detection of such 'hits' on eggs and bacterial spores in space flight. The need for background information concerning the radiation sensitivity of silkworm eggs was recognized.

This communication describes laboratory investigations of the effects of γ-radiation on silkworm eggs in different stages of post-diapause development. The effect of fractionated radiation exposures was also investigated, since the total radiation dose delivered in space flight may not occur in a single exposure.

Freshly laid silkworm eggs (yellow variety) on filter paper were placed in a constant-temperature cabinet (27° C, 90 per cent relative humidity) for 4 days. They were then removed to a refrigerator (5° C), allowed to remain there for about 5 months, and then transferred to a cabinet held at 27° C to break the diapause condition and induce embryonic development. At 1, 4, or 7 days after the termination of diapause, eggs were exposed to a series of γ-radiation doses of 0·8 kr.–51·2 kr. The source of radiation was a cobalt-60 unit, which delivered approximately 320 r./min. Eggs 1 day in post-diapause development were also exposed to fractionated doses of 1, 2, or 4 kr. of total radiation for one exposure period, for two exposure periods of half the total dose each (the second exposure was delivered 90 min after the first), or for four exposure periods of a fourth the total dose, each at 90-min intervals.

After treatment the eggs were returned to the incubator and again held at 27° C. Hatching occurred in 9–12 days and mortality was determined in 14 days. Dosage-mortality curves were derived by the logit method of Berkson[4].

Silkworm eggs in early stages of post-diapause development were more susceptible to γ-radiation than eggs in the later stages of post-diapause development (Table 1). Exposure to 3·2 kr. of γ-radiation killed 100 per cent of the 1-day post-diapause eggs but only 50 and 5 per cent, respectively, of eggs at 4 or 7 days of post-diapause development. Similar results were obtained after exposure to 1·6 kr., but the differences were smaller. Mortality of 1- or 4-day post-diapause eggs increased with increasing exposure to γ-radiation, whereas mortality of 7-day post-diapause eggs did not increase significantly within the 0·8- to 3·2-kr. range.

Table 1. Per Cent Mortality of Silkworm Eggs exposed to Gamma-radiation 1, 4, or 7 Days after Termination of Diapause

Radiation exposure (kr.)	1 Day	4 Days	7 Days
None	8 *ab**	8 *ab*	8 *ab*
0·8	13 *ab*	12 *ab*	18 *b*
1·6	37 *c*	18 *b*	5 *a*
3·2	100 *e*	50 *d*	5 *a*

* Means not followed by the same or common letter are significantly different.

Table 2

Days in post-diapause development	Lower limit	LD_{50} (r.)	Upper limit
1	1,403	1,528	1,664
4	3,350	3,565	3,774
7	22,190	24,840	27,800

The dosage-mortality curves of 1, 4, and 7-day post-diapause eggs are shown in Fig. 1. These results are similar to those discussed. The LD_{50}'s of radiation derived from these results are shown in Table 2.

Total exposures of 0·8 or 1·6 kr. delivered in 1 irradiation period or 2 irradiation periods with half the total dose administered at a time were equally damaging to silkworm eggs in the 1-day post-diapause developmental stage (Table 3). A total exposure of 1·6 kr. delivered in fractions of a quarter of the dose at intervals of 90 min was less damaging than the same radiation dose administered in 1 or 2 irradiation periods. Exposure to 3·2 kr.

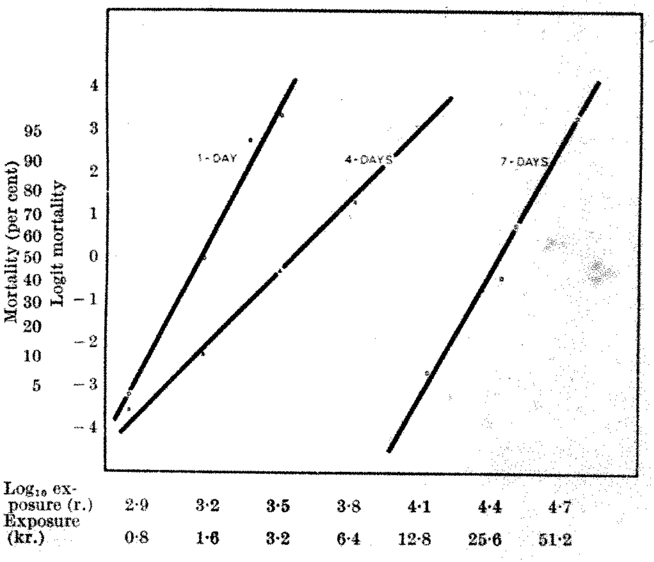

Fig. 1. Radiation dosage-mortality curves of silkworm eggs in 1, 4 or 7 days of post-diapause development

Table 3. PERCENTAGE MORTALITY OF SILKWORM EGGS EXPOSED 1 DAY AFTER TERMINATION OF DIAPAUSE TO SINGLE EXPOSURES OF GAMMA-RADIATION OR SEVERAL EXPOSURES AT 90-MIN INTERVALS

Total radiation (kr.) exposure	1 Exposure	2 Equal exposures	4 Equal exposures
None	8 *a**	8 *a*	10 *a*
0·8	14 *a*	13 *a*	13 *a*
1·6	47 *c*	39 *c*	24 *b*
3·2	100 *d*	100 *d*	100 *d*

* Means not followed by the same or common letter are significantly different.

in 1, 2, or 4 irradiation periods resulted in 100 per cent mortality of the 1-day post-diapause eggs.

T. J. HENNEBERRY
W. N. SULLIVAN

Entomology Research Division,
U.S. Department of Agriculture,
Beltsville, Maryland.

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BIOLOGY

Central Nervous Responses to Chemical Stimulation of a Gastropod Osphradium

THE function of the osphradium, a structure within the mantle cavity of many molluscs, has been the subject of some speculation. It is generally accepted to be a sense organ, but the modality to which it is sensitive remains obscure. Two major theories have been proposed. The first is that it is a chemoreceptor involved in food location. Copeland[1], Wölper[2] and Brown and Noble[3] have shown, by means of behavioural studies, that certain species of carnivorous gastropod depend on an intact osphradium and associated nerve supply for their ability to follow the chemical trail emanating from food material. Extirpation of the organ or section of the innervation leads to the loss of this attribute.

The second theory is that proposed by Hulbert and Yonge[4] and Yonge[5]. They suggest that the osphradium is "a tactile organ concerned with estimating the amount of sediment carried into the mantle cavity". This theory is based on the occurrence and development of the organ in a wide variety of gastropods in relation to their habitats.

In his recent review of gastropod chemoreception Kohn[6] states that Yonge now considers that a chemosensory role of the osphradium may have evolved secondarily from the original particle detecting mechanism.

The only direct physiological investigation carried out so far seems to be the attempts of Kohn[6] to record electrical activity from the osphradial nerves of *Busycon* after stimulating the osphradium with chemicals. This was unsuccessful. In the present work on *Buccinum undatum*, experiments using platinum wire electrodes to record from the osphradial nerves have failed, as have attempts to record from the osphradial ganglion that is situated along the mid-line of the organ. The nerves supplying the osphradium and part of the ctenidium arise from the supra-œsophageal ganglion, whence it has proved possible to monitor nervous activity by means of indium-filled glass micro-electrodes. In this way single units or, more frequently, small groups of units could be located and displayed by conventional means.

It is unlikely that these records represent the response of primary sensory neurones although these may pass directly through the ganglion[7]. It is more probable that they are interneurones or motor neurones. This central activity is, however, modified by stimulating the osphradium when the supra-œsophageal ganglion is isolated from the remainder of the central nervous system, leaving only the peripheral nerves to the osphradium and ctenidium intact.

Manual stimulation of the osphradium by means of a paintbrush or glass rod usually invoked activity although in most cases a large distortion was needed to elicit the response. If the organ does function as a sediment detector it must have mechanoreceptors of high sensitivity. To test this possibility, suspensions of 'Carborundum' particles (20–200μ diam.) in sea-water were run over the organ. This failed to elicit activity. Similarly sea-water adjusted to *p*H values in the range 3–9, and in concentrations between 50 and 150 per cent also failed to elicit nervous responses.

Volleys of action potentials were seen frequently in response to stimulating the osphradium with sea-water in which minced *Mytilus* had been left to stand. Such extracts invoked responses which often lasted for more than 30 sec (Fig. 1*C*). Attempts to mimic these responses with synthetic chemicals were successful only in the case of a neutral solution of 10^{-3} M *l*-glutamic acid hydrochloride in sea-water. A much less prolonged response was noted when a neutral solution of 10^{-2} M trimethylamine oxide hydrochloride was applied to the osphradium. Other chemicals, including betaine, glycine, alanine, glutamine and lactic acid, were also without effect.

Removal of the osphradium, with little damage to the remainder of the mantle, abolished the chemical responses of the preparation. In some cases, however, mechanical distortion remained an effective stimulus, the response

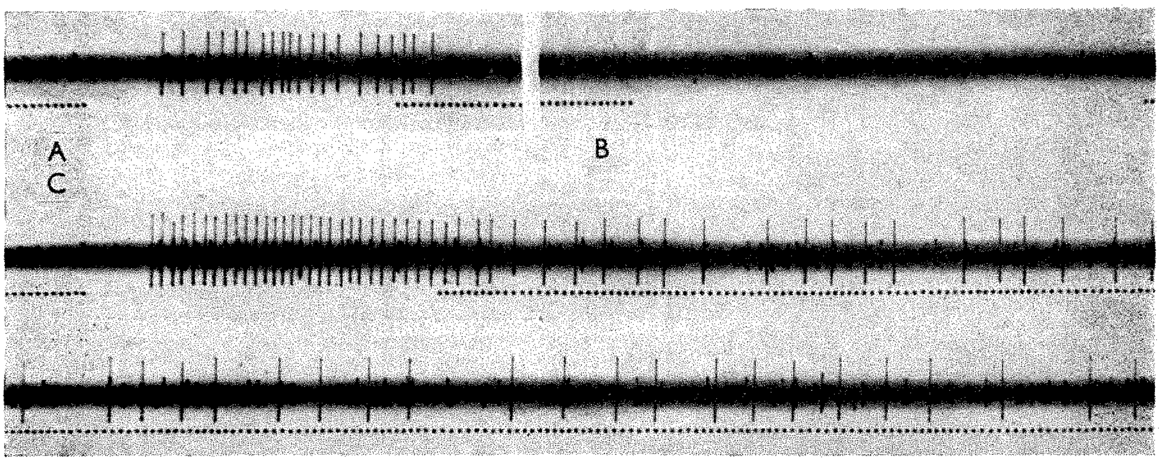

Fig. 1. Record of activity from the supra-œsophageal ganglion in response to stimulation of the osphradium. *A*, Response to touch; *B*, response to the application of sea-water; *C*, response to *Mytilus* extract in sea-water. Lines 2 and 3 are continuous. Time-marks = 10/sec. Duration of the stimulus indicated by the interruption of the time-marks

being mediated by sense organs located in the mantle musculature. This indicates that sensory pathways of several modalities located in the mantle region converge on units in the supra-œsophageal ganglion.

These results support the theory of chemoreception as a function of the osphradium at least in *Buccinum undatum*. Failure to record activity in response to light tactile stimulation suggests that the osphradial mechanoreceptors, if any, are insufficiently sensitive to act as sediment detectors as suggested by Hulbert and Yonge. During the course of the experiments it has been noted that copious production of mucus occurs from the hypobranchial gland, osphradium and mantle surface. This must severely impede the entry of particles into the mantle cavity in the natural situation and their contact with any part of the mantle surface.

D. F. Bailey
M. S. Laverack

Gatty Marine Laboratory,
University of St. Andrews.

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Suppression of Gemma-cups in *Marchantia* by High Humidity

It was noticed that gemma-cups in the New Zealand liverwort *Marchantia berteroana* L. and L. (= *M. tabularis* Nees) were not produced when the pots were covered with glass or polythene sheets. That this effect is the result of increased humidity has been confirmed by growing plants in the saturated atmosphere of a permanent-spray house, but protected from direct falling water. All experiments were carried out on sets of 60 plants, started from disks of *Marchantia* thallus 1 cm in diameter, grown in pots of sterilized garden soil. In all experiments except the second every disk had its counterparts, cut from the same thallus, in the corresponding position in each treatment so that the material under the different treatments was exactly comparable.

In one representative experiment no gemma-cups at all were produced on a unit of 60 plants during nineteen weeks' growth in a saturated atmosphere, whereas the control plants in normal air out of doors had, in the same time, produced a total of 234 gemma-cups (mean $3·9 \pm 0·98$/plant). This difference is highly significant ($P < 0·001$).

A second experiment, using twice as many plants, resulted in a total of 10 gemma-cups (mean $0·08 \pm 0·75$/plant) in saturated air compared with 1,049 cups (mean $8·74 \pm 1·18$/plant) in normal air. The difference is again highly significant ($P < 0·001$).

A similar effect was shown by a third experiment in which units covered with sheet polythene or uncovered were grown in cabinets at three different temperatures, averaging approximately 5°, 10°, and 15° C. The uncovered plants produced 2 (5° C), 9 (10° C) and 175 (15° C) gemma-cups totalling 186, while the covered plants produced no gemma-cups even at 15° C. The total production of gemma-cups was low, probably as a result of the comparatively high ambient relative humidity (about 75 per cent) in the cabinets.

Table 1 presents the results of a fourth experiment, where batches of 6 plants were transferred into the spray house after increasing periods in dry air out of doors. The equation for the regression of number of gemma-cups formed on days of dry conditions is $y = 30·8 + 1·40\ (x - 23·7)$, which is highly significant ($P < 0·001$).

Table 1. Effect of Increasing Exposure to Dry Conditions on Number of Gemma-cups formed. Counts made after 106 Days. Six Plants in Each Treatment

Days in dry conditions	1	2	4	8	16	24	32	40	48	56
Total No. of gemma-cups	1	6	0	1	11	61	36	58	56	78
No. of plants forming gemma-cups	1	1	0	1	4	5	6	6	4	6

After the end of these experiments the plants produced gametangiophores and it was possible to determine their sex, revealing that, in the material used, male plants are able to produce far more gemma-cups than are female plants. For the second experiment, the pooled comparative mean numbers of gemma-cups per plant are $60·13 \pm 7·52$ for male and $4·29 \pm 4·48$ for female plants. This difference is not due merely to the male plants being larger, since the average number of gemma-cups per unit of area of the thallus is male 0·87/cm^2 compared with female 0·07/cm^2. The sex difference also accounts for the rather low numbers of gemma-cups on the plants of the first experiment, for all these turned out to be female.

The precise humidity which is responsible for this suppression of gemma-cup formation is not yet known.

George A. M. Scott

Department of Botany,
University of Otago,
Dunedin, New Zealand.

The Genus *Oceanopapaver*

The monotypic genus *Oceanopapaver* was established for the naming of *O. neocaledonicum* by Guillaumin[1]. Because of certain characters which need not be discussed here he relegated his new genus to Papaveraceae. Friedel[2] cast some doubt on this classification and suggested that the plant showed a number of characters not unlike those of the Cistaceae. Fedde[3], in a review of the Papaveraceae, expressed scepticism about allocating this new genus to that family. Among other criteria he saw some difficulty in finding a papaveraceous plant endemic to the tropical New Caledonia in the southern hemisphere.

Thanks to Prof. H. S. McKee, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia I was able to make an examination of this plant for alkaloids. 400 g of dried material were available, collected when only a few of the seeds had matured. Fortunately this is the stage in most herbaceous plants when the alkaloid content is at a maximum.

The coarsely ground material was thoroughly extracted with methanol, the methanol was evaporated from the extract, the latter extracted with slightly acidified water, the aqueous extract filtered, and exhausted with repeated fresh portions of ether. (The ether extract failed to yield fumaric acid—a common constituent of papaveraceous plants.) A test portion of the aqueous extract did not give a precipitate with potassium mercuric iodide—a test which when positive is an almost certain indication of alkaloids. So far no alkaloid-bearing plants are known which give a negative test with this reagent.

Nevertheless, the aqueous solution was rendered basic with ammonia and extracted with several successive portions of chloroform. The residue from the latter was stirred with aqueous oxalic acid, the filtered solution exhausted with ether, made alkaline again, and extracted with chloroform. The residue from the latter dissolved only partly in dilute acid and the solution did not give a precipitate with the aforementioned reagent.

Inasmuch as all plants of the Papaveraceae, which have so far been examined, have yielded alkaloids, their complete absence in *O. neocaledonicum* can be regarded as sufficient cause for relegating this plant to another family.

R. H. Manske

Dominion Rubber Co., Ltd.,
Research Laboratories,
Guelph, Ontario, Canada.

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A Glossopteridean Fructification from India

About six genera of reproductive organs have been described as belonging to the *Glossopteris*[1-3]. Some other types have also been reported from time to time[4-8]. Only a few of them occur in India[9-12]. The fossil described here came from Pachwara Coalfield, Bihar (basal Barakar—Lower Permian).

The so-called fructification is a mould-like structure on very fine greyish clayey shale. It compares favourably with the structure of the genus *Lanceolatus*[1]. The leaves associated with it cannot be determined accurately because of their bad preservation. They are simple and lanceolate in shape. Their well-developed midribs and crowded anastomosing veins clearly resemble those of some members of the *Glossopteris*.

It is assumed that the two overlapping leaves completely cover the upper half of the leaf to which the slightly raised fructification is attached (Fig. 1). The hollow nature of the midrib bearing the fructification is not clear possibly due to its poor preservation.

The fructification seems to be fully mature, lanceolate and slightly raised (14 × 7 mm). There is a deep groove along the periphery of the fructification. Immediately above the point of attachment of the fructification there is a slightly raised structure, which is perhaps the remnant of the cupular covering. It is almost sessile and grows from the midrib of the leaf; but the actual distance from the leaf-base cannot be determined due to the broken nature of the specimen. The surface shows shallow depressions of many embedded, small, more or less oval bodies (0·9 mm–1 mm), which may be the ruptured seed- (or pollen-) bearing sacs (Fig. 2).

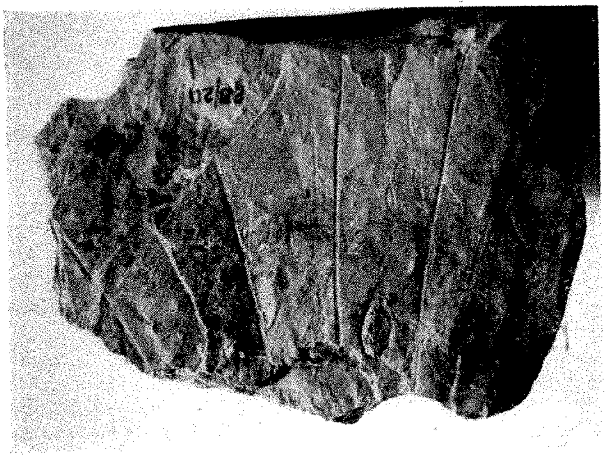

Fig. 1. *Lanceolatus* type of fructification attached to a leaf of *Glossopteris*. The head is slightly raised. Slightly reduced from natural size

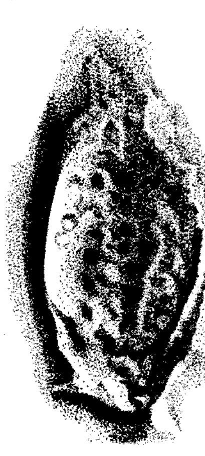

Fig. 2. Magnified view of the fructification pitted with hollow depressions which are believed to be ruptured sacs. (× 3)

It appears, therefore, that this type of fructification can only be compared with that of *Lanceolatus* type. The only other similar record from India comes from Murulidih Colliery in Jharia Coalfield, Bihar (Raniganj—Upper Permian)[11]. This shows that the genus at least occurs from the basal Barakar to Raniganj stage, that is, Lower Permian to Upper Permian of India.

We thank Mr. A. Chowdhury, Department of Geology, University of Calcutta, for the material.

SEBA CHATTERJEE

Botany Department,
University of Calcutta.

J. SEN

Indian Botanic Garden,
Howrah,
Calcutta.

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A Reversal Effect with Pauses on Mixed Schedules of Reinforcement

Fixed ratio (*FR*) schedules of reinforcement typically generate behaviour that occurs with high probability just before reinforcement, but that is unlikely to be repeated for at least a little while afterwards. Also, these post-reinforcement pauses depend on the length of the ratio. Pauses are normally short when the ratio is small and are longer when a larger number of responses is required per reinforcement[1]. In the case of simple *FR* schedules it is impossible to decide whether longer pauses follow longer runs because these runs generate more reactive inhibition than do short runs[2] or because on high ratios organisms have 'a long way to go' between reinforcements[3], because run lengths before and after reinforcements are equal.

But the relative effects of pre- and post-pause response runs on pauses after reinforcements can be investigated by means of alternating mixed *FRFR* schedules of reinforcement in which reinforcements are given at the end of each ratio. The alternating ratios can be made unequal, and if pre-reinforcement run lengths alone are responsible for the duration of post-reinforcement pauses then long pauses should follow long runs and short pauses come after short ratios on the average. If pauses depend on the amount of responding required before the next reinforcement, then the reverse will be true.

Findley[4] has published observations which appear to show that pauses are a function of ratios they precede. Salman[5], on the other hand, found that a rat trained to bar press on an alternating multiple schedule *FR*40*FR*93 consistently made longer median pauses after the longer than after the shorter ratio, and the same was true, but less marked, of two other animals reinforced on *mult. FR*27*FR*73 and *mult. FR*10*FR*57 respectively. (On multiple compound schedules a different external stimulus is correlated with each segment of the schedule. This condition obtained in the Findley and Salman experiments. The present experiment utilized mixed schedules in which the external stimulus is the same for all components.) Salman's findings if compared with those of simple *FR* schedules indicate that it is the ratios they follow that have the major controlling influence over post-reinforcement pauses. However, when the schedules of the latter two animals were changed to *mult. FR*3*FR*31 the effect was reversed: median post-reinforcement pauses became

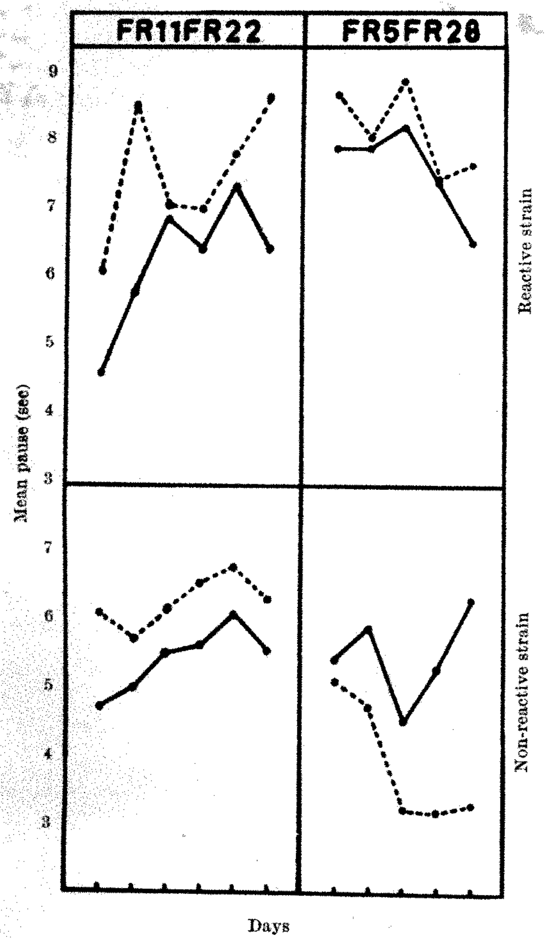

Fig. 1. Average pauses from reinforcement to the second response in the designated ratio made by reactive and non-reactive strains separately. Solid lines connect pauses after the shorter of the mixed ratios, and pauses after the longer ratios are connected by broken lines

longer after the *FR*3 component than after *FR*31. This communication presents further results on this reversal effect.

Six 5-months-old naïve male albino rats, 3 from each of the 19th generation of the Maudsley reactive and non-reactive strains, were trained to bar press for food reinforcement in a Skinner box (Scientific Prototypes type 102). The *Ss* were adapted to a 24-h feeding rhythm and were run 22–23 h food deprived daily for 30 min each, except for days 33–37 when sessions lasted 20 min. Reinforcements were scheduled as follows: days 1–3, *crf*; 4–5, *FR*4; 6–7, *FR*8; 8–16, *FR*111; 7–27, *mix FR*11*FR*22; 28–37, *mix FR*5*FR*28. The results shown in Fig. 1 were collected on days 22–27 and 33–37. These show the mean post-reinforcement pauses after each ratio of the schedules *mix FR*11*FR*22 and *mix FR*5*FR*28 for the two strains separately. The pauses were measured from reinforcement delivery to the second response in the following ratio. The second response was chosen because the non-reactive *Ss* frequently drank after reinforcement and depressed the bar once on moving from food magazine to drinking tube. The reactive *Ss*, on the other hand, typically made a one-bar response quickly after reinforcement, then bit at the bar before running off the rest of the ratio. A count over days 13–16 showed the non-reactives to average 40·9 trips to the water bottle each session against 3·8 trips by the reactives, and there were no overlaps.

Both strains consistently took slightly longer to resume bar pressing after the longer component of the *mix FR*11*FR*22 schedule. From the point of view of simple *FR* schedules, this would mean that post-reinforcement pauses are determined by the ratios they follow: longer pauses follow longer ratios. But when the schedule was changed to *mix FR*5*FR*28 the position was reversed in the case of the non-reactive *Ss*. Pauses after *FR*5 (before *FR*28) remained about the same as those after *FR*11 (before *FR*22), but the pauses after the longer ratio dropped from about 6 sec to a little more than 3 sec when the shorter ratio to follow changed from *FR*11 to *FR*5. Typical behaviour of the non-reactive *Ss* was to run off the *FR*28, pause briefly, run off the *FR*5 and then take a longer pause, showing pauses to be mainly controlled by the ratio to follow.

It is clear that post-reinforcement pauses on ratio schedules are not determined by either pre- or post-pause ratios alone, but by the relationship between them. In all probability, on small simple *FR*'s it is a fact that only a few responses after reinforcement are required before the next reinforcement that causes an animal quickly to resume responding, while on longer *FR*'s post-reinforcement pauses are more under the control of preceding runs. In any event, the Hullian notion of reactive inhibition is not sufficient to account for all the results.

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J. D. Keehn

Department of Psychology,
American University of Beirut,
Lebanon.

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MICROBIOLOGY

Molybdenum and Iron Requirements for Nitrate Reductase in *Photobacterium sepia*

Sadana and McElroy[1] showed that nitrate reductase from the halophytic bacterium, *Achromobacter fischeri*, required iron for its activity, but a deficiency of other micronutrients including molybdenum did not appear to affect the enzyme. In addition, they found that the bacterium would not grow with nitrate as the sole nitrogen source. We now report that this bacterium (kindly supplied by Dr. W. D. McElroy, Johns Hopkins University, Baltimore) has all the characters of *Photobacterium sepia* as described by Spencer[2], who suggests that it should be placed in the genus *Aeromonas*. Moreover, we present evidence that not only will this bacterium grow with nitrate as the only nitrogen supply but also that its growth and nitrate reductase activity are markedly reduced in cells deficient in molybdenum or iron. Thus the enzyme from *Photobacterium* follows the same pattern established for nitrate reductases in other micro-organisms[3–6].

The bacterium was grown in 1·5-l. Erlenmeyer flasks on a reciprocator, and 1, 10 and 20 per cent oxygen in nitrogen gas mixtures were sparged through the cultures. The following culture solutions (units/l.) were used: 30 g NaCl; 5·5 g Na_2HPO_4; 2·1 g KH_2PO_4; 0·2 g $MgSO_4.7H_2O$; 3 ml. glycerol; 2 mg iron (as $FeCl_3$) and 0·1 mg molybdenum (as $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) with either 12·2 g KNO_3 or 6·5 g NH_4Cl. The trace metals used were spectrographically checked for purity. The two micronutrients were removed from the culture medium by the hydrogen sulphide/copper co-precipitation method for molybdenum[7] and by the calcium carbonate adsorption procedure for iron[8]. All cultures were incubated at 24° C for 18 h.

The nitrate reductase activity of the cells was assayed anaerobically in Thunberg tubes using reduced benzyl viologen as the hydrogen donor. The reaction mixture was as follows: in the side-arm, 0·2 ml. 10^{-3} M benzyl viologen (BV), 1 mg palladized asbestos, and 0·3 ml. $2\cdot5 \times 10^{-1}$ M phosphate buffer (*p*H 7·5); in the tube, 0·1 ml. 10^{-1} M KNO_3, 0·1 ml. cell suspension (2 mg nitrogen/ml.) and 0·3 ml. of the phosphate buffer (*p*H 7·5). The tube was evacuated and flushed with high-purity hydrogen, which was treated in a 'Deoxo' catalytic deoxygenator. This was repeated twice, at which stage the dye was fully reduced. After final evacuation, the tubes were pre-incubated for 5 min at 30° C before the reduced dye was tipped in, and after a further 10 min the reaction was stopped by adding 0·1 ml. M zinc acetate and 1·9 ml. 95 per cent ethanol[9]. After centrifuging at 4,000*g* for 5 min, nitrite was determined in an aliquot of the supernatant solution by the sulphanilamide method[10].

Table 1. EFFECT OF OXYGEN PRESSURE, NITROGEN SOURCE AND MOLYBDENUM CONTENT OF THE CULTURE MEDIUM ON THE NITRATE REDUCTASE ACTIVITY OF CELLS

(Specific activity of the enzyme: μmoles NO_2^-/mg nitrogen/10 min)

Nitrogen source	O_2/N_2 (per cent)	Mo μg/l. culture		
		0	10	100
KNO_3	1	700	5,250	7,970
	10	250	3,930	4,360
	20	200	4,240	4,750
NH_4Cl	1	364	4,850	5,130
	10	108	840	850
	20	221	1,365	1,085

The results in Table 1 show that nitrate reductase activity was reduced by a deficiency of molybdenum, irrespective of the oxygen pressure or the source of inorganic nitrogen in the medium. An increase in the oxygen supply markedly depressed enzyme formation in agreement with the results of Pichinoty and d'Ornano[11] with *Aerobacter aerogenes*. It is of interest that the enzyme was formed in cells grown with ammonium chloride, in the absence of its substrate. This contrasts with nitrate reductases in other micro-organisms and higher plants, which are induced by nitrate or nitrite only[3-6] and are inhibited by ammonia as a result of a 'feed-back' mechanism[12].

The enzyme is also dependent on iron for its activity as shown in Fig. 1, in agreement with the results of Sadana and McElroy[1] for the same bacterium. Since the fractionation procedure used to purify nitrate reductase from extracts of cells grown with KNO_3 is also applicable to those from cells grown with ammonium chloride, it is likely that it is the same enzyme.

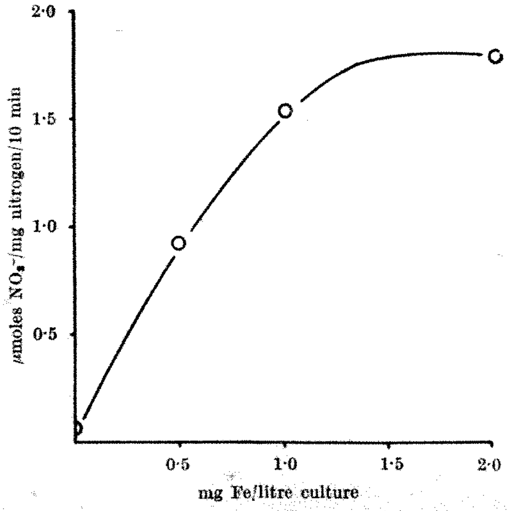

Fig. 1. Effect of the iron content of the culture medium with NH_4NO_3 (5 g/l.) as nitrogen source on the nitrate reductase activity of the cells. Cultures aerated with 10 per cent oxygen in nitrogen

D. J. D. NICHOLAS
W. J. REDMOND
M. A. WRIGHT

Chemical Microbiology Department,
Long Ashton Research Station,
University of Bristol.

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Distribution of a Surface Antigen of *Staphylococcus aureus*

SEVERAL years ago the isolation of a surface antigen from culture supernatants of a mucoid strain of *Staphylococcus aureus* was reported[1,2]. The material, from strain Smith, was designated as SSA (Smith surface antigen). It consisted primarily of an amino sugar which did not behave as the usual 2-deoxy-2-amino-hexose, and which rapidly decomposed during acid hydrolysis. Later, the unknown substance was characterized as 2-deoxy-2-amino-glucuronic acid[3].

The antigen precipitated at high titre with homologous antiserum and with antiserum prepared against an *S. aureus* strain which contained serologically identical material. Other *S. aureus* antisera did not react. Injection of the isolated antigen protected mice against intraperitoneal challenge with strain Smith suspended in mucin; furthermore, the antigen was shown to be a determinant of phagocytosis of this organism.

Several other mucoid strains of *S. aureus*, as well as the Smith strain, have been reported to be more virulent for mice, and more resistant to phagocytosis than the usually occurring *S. aureus* organism[4,5]. The work reported here was designed to determine if these strains contained SSA, and also to test for the presence of SSA in strains of *S. aureus* freshly isolated from routine clinical material.

The mucoid organisms utilized, in addition to strains Smith and Stern[2], were Welwood, Adams, *K*6 and *K*93, which were kindly supplied by Dr. M. G. Koenig. Twenty-five clinical isolates were obtained from New York Hospital through the courtesy of Drs. S. Hadley and H. Shinefield.

Although SSA can be isolated from culture supernatants, it was found that acid extraction of intact bacterial cells also liberated the antigen. Cells from overnight cultures on nutrient agar in Blake bottles were collected in 10 ml. of saline adjusted to *p*H 2. The suspensions were heated at 100° C for 15 min, neutralized, and the bacterial bodies removed by centrifugation. The extracts were then tested for the presence of SSA by capillary precipitin tests using a potent Smith antiserum.

All the extracts of the six selected mucoid strains reacted within 30 min, yielding a heavy precipitate. Reactive material from these organisms was also found in fluid expressed from agar by freezing and thawing after removal of bacterial growth. Thus, the antigen, like many capsular substances, is both bound to cells and liberated into the surrounding medium. None of the acid extracts of the hospital strains precipitated with Smith antiserum, nor could specific serological material be obtained from these organisms by altering the conditions of extraction, freezing and thawing the cultures, or disrupting the cells in a Mickle disintegrator.

Material was also precipitated from acid extracts by the addition of 5 volumes of acetone, dried, taken up in saline, and tested for SSA content. As little as 100 μg/ml. of the *p*H 2 extracts from the six mucoid strains was rapidly precipitated by Smith antiserum whereas as much as 10 mg/ml. of material from the other strains was unreactive.

The specificity of the immune precipitation was examined in gel diffusion studies. Smith antiserum reacted with 1 mg/ml. solutions of the acid extracts which were positive in the precipitin test to produce a single band which in turn showed a reaction of identity with purified SSA (Fig. 1). The diffusible substances obtained by freezing and thawing the agar were also immunologically identical with SSA. There was no reaction with 10 mg/ml. of acid extracted material from the other *S. aureus* organisms. Furthermore, antisera prepared against the six selected mucoid strains reacted with SSA whereas antisera against the other strains did not.

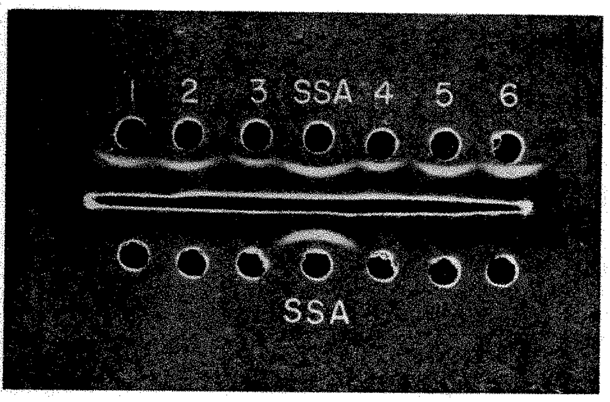

Fig. 1. Reactions of acid extracts of *S. aureus* strains with Smith antiserum. Top row: 1 mg/ml. of extracts of strains (1) *K*93, (2) *K*6, (3) Smith, (4) Stern, (5) Adams and (6) Welwood. Bottom row: 10 mg/ml. of acid extracts of other *S. aureus* strains. Concentration of SSA is 100 μg/ml.; Smith antiserum is in centre slit

The 25 strains obtained from clinical sources therefore did not contain SSA. These organisms included 7 strains in Phage Group I, 6 of which were type 80/81; 5 in Group II; 6 in Group III; and 7 which were not typable. Virtually all the strains were resistant to penicillin and isolated from active lesions. All produced both free and bound coagulase. The strains containing SSA produced free but not bound coagulase and were non-typable. It is of interest that strain Smith at one time was phage-type 44*A*/42*E*, but during the course of laboratory sub-culture it has become untypable.

It has been suggested that a determinant of pathogenicity of *S. aureus* might be capsular material such as SSA[6]. As indicated in this report, SSA was not demonstrated in routine clinical isolates of *S. aureus*; nor have other specific surface antigens been found in these organisms. It should also be noted that some of the selected strains containing SSA were obtained from normal flora rather than from active lesions[7]. In addition, two of the SSA positive strains have been carried in this laboratory for a number of years, suggesting that the antigen is not readily lost on sub-culture on artificial media.

Recently, material has been isolated from a mucoid *S. aureus* organism similar to strain Smith[8,9]. The compound bears many similarities to SSA: it is present in acid extracts of intact cells; it protects mice against intraperitoneal challenge with mucoid, but not 80/81, *S. aureus* strains; and it contains the same fundamental carbohydrate unit as SSA, aminoglucuronic acid.

This substance was described as a 'new' staphylococcal antigen and called SPA (staphylococcal protective antigen). There is no reason for believing that SPA is significantly different in chemical or immunological properties from SSA. It would be unfortunate, as well as misleading, if two identical antigens were denoted by two separate designations. Since strain Smith is the prototype of the unique mucoid *S. aureus* strains, the original designation of Smith surface antigen, or SSA, would seem to be preferable.

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STEPHEN I. MORSE

The Rockefeller Institute,
New York 21.

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Control of Growth and Morphogenesis in some *Myxococcus* Species

THE influence of nutrition on growth and (particularly) on morphogenesis of some fruiting myxobacteria was observed in this laboratory some two years ago. As recently reported[1], a number of *Myxococcus* strains grow luxuriantly on an agar (2 per cent w/v) medium containing enzymatically hydrolysed casein (Difco 'Tryptone', 1 per cent w/v). However, only vegetative cells are present; neither the formation of microcysts (cellular morphogenesis) nor of fruiting structures (colonial morphogenesis) takes place. Microcyst and fruiting body formation does occur, however, when the 'Tryptone' concentration is reduced to 0·1 or 0·2 per cent (w/v).

It seemed possible that these morphogeneses might result from a depletion in the 'low' 'Tryptone' medium of some essential nutrient(s) which would be present in the 1 per cent 'Tryptone' medium in amounts sufficient to support continued vegetative cell growth. An experimental test of this starvation hypothesis was carried out by transferring replicate vegetative cell populations to fresh 'low' 'Tryptone' medium at intervals as short as 4 h. All such cell populations, including those control populations which were not transferred, encysted and fruited at nearly the same time. Thus, even those cell populations which were continually in a fresh 'fruiting' medium, and which had little, if any, opportunity to deplete their nutritional environment underwent morphogenesis in the normal fashion. Cell populations similarly transferred to the non-fruiting medium (1 per cent 'Tryptone') did not encyst or fruit. Very little, if any, growth or morphogenesis occurred on a non-nutrient water agar.

A number of non-encysting, non-fruiting spontaneous mutants of several myxococcal strains have been isolated. None of these could be stimulated to encyst or fruit when grown on rabbit dung pellets, or on an agar medium containing *E. coli* cells, or on 'low' 'Tryptone' medium supplemented with either killed or viable microcysts or with extracts made from wild-type cells in the process of fruiting, nor with supplements of vitamins, amino-acids, purines or pyrimidines.

In view of these failures to implicate nutritional deficiency as a regulatory factor in encystment or formation of fruiting body an alternative explanation was considered. Previous work in this laboratory had demonstrated that addition of acid-hydrolysed casein to the 'low' 'Tryptone' medium would inhibit the morphogeneses, thus suggesting that an inhibition or repression mechanism of control might be involved. An auxanographic approach used to determine if any single amino-acid, purine, pyrimidine, or vitamin could substitute for the presumed inhibitory component(s) in the acid-hydrolysed casein. Of some forty compounds tested, only phenylalanine or tryptophan inhibited the morphogeneses. Dworkin[2] has since noted that phenylalanine affects the morphogenesis of his strain of *Myxococcus xanthus* when grown on a defined medium.

It seems likely that the non-encysting, non-fruiting mutants are characterized by an increased synthesis and excretion of similarly inhibitory compounds; combined plating of mutant and wild-type cells on the 'low' 'Tryptone' medium results in inhibition of encystment and fruiting of the wild-type. With auxanographic methods it has been possible to demonstrate that some, but not all, of these mutants will fruit near the areas containing the amino-acid analogue β-2-thienylalanine, presumably because this analogue has interfered with amino-acid metabolism.

This series of observations suggests that phenylalanine and/or tryptophan are essential for both growth and morphogenesis of vegetative cells but, when present above certain concentrations, are effective in inhibiting encystment and fruiting body formation. They do not appear to inhibit microcyst germination. Their mode of action is unknown.

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Note added in proof. A more detailed account of Dworkin's studies have appeared (*J. Bacteriol.*, 86, 69; 1963) since this communication was submitted for publication.

E. R. LEADBETTER

Biology Department,
Amherst College,
Amherst, Mass.

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ENTOMOLOGY

A Chitinase from the Gut of the Cockroach *Periplaneta americana*

CHITINOLYTIC activity has previously been demonstrated in the gut contents of *Periplaneta americana* (L.)[1], and a fraction, chitinase I, has now been separated and purified from this source by ammonium sulphate precipitation followed by fractionation on DEAE cellulose. Two additional peaks with acetylaminodeoxyglucosidase and another with chitinase activity were also observed in the effluent from DEAE cellulose columns. The terms chitinase and acetylaminodeoxyglucosidase as used here refer to enzymes No. 3/2/1/14 and No. 3/2/1/30 of the International Union of Biochemistry nomenclature[2]. Chitobiose, a substrate of chitobiase (enzyme No. 3/2/1/29), is the disaccharide of acetylaminodeoxyglucose, and the other oligosaccharides used form a series[3]. For convenience the oligosaccharides are referred to as biose, triose, etc. The chitin used was a suspension prepared by reprecipitation of purified crayfish (*Jasus verreauxi* H. M. Edw.) chitin from sulphuric acid. The products of digestion, up to hexaose, were satisfactorily separated by paper chromatography using a solvent of pentan-2-ol/pyridine/water (1:1:1)[4]. Oligosaccharide spots were located by chlorination followed by a starch-iodide spray in a modification of the Rydon and Smith[5] method for peptides. The oligosaccharides gave similar colour densities per unit weight, unlike the results given by other methods, such as silver development, in which the colour varies considerably with the size of the oligosaccharide.

Chitinase I (0·028 mg/ml.) digested 35 per cent of chitin in 5 h. The amount of digestion was determined by the change in optical density at 420 mμ (Beckman spectrophotometer, model *DU*) of a chitin suspension (2 mg/ml.) in 0·005 M citrate buffer, *p*H 4·5. The enzyme preparation was free of acetylaminodeoxyglucosidase activity using phenyl acetylaminodeoxyglucoside and chitobiose as substrates.

Reaction mixtures containing chitinase I (0·001 mg/ml.), oligosaccharides (1 μmole/ml.) and citrate buffer *p*H 4·5 (0·005 M) were incubated at 35° C for periods up to 5 h. The disappearance of the substrate spots on the chromatograms proceeded in order of increasing rate tetraose < pentaose < hexaose. The products from hexaose were biose, triose and a small amount of tetraose; from pentaose-biose and triose; and from tetraose only biose. No acetylaminodeoxyglucose was observed in these mixtures, and biose and triose were apparently not attacked by the enzyme. Under otherwise similar conditions but with increased enzyme concentration (0·028 mg/ml.) and time (18 h), biose was not affected, but about 40 per cent of triose was digested producing biose and acetylaminodeoxyglucose. This was confirmed by estimation of acetylaminodeoxyglucose[6]. The results are recorded in Table 1, in which the original amount of each substrate was assigned 6+ according to the colour of the chromatogram spot and the amount of each product was assessed similarly. Mixtures of chitin suspension or purified chitodextrin (2 mg/ml.) and chitinase I (0·028 mg/ml.) under conditions similar to the foregoing produced biose, triose, a little tetraose and a trace of acetylaminodeoxyglucose.

Table 1. DIGESTION OF CHITIN OLIGOSACCHARIDES BY CHITINASE I

Substrate	Enzyme (μg/ml.)	Time (h)	Products 1	2	3	4	5	6
Biose	28	18		6+				
Triose	28	18	1+	2+	3+			
Tetraose	1	1		1+		5+		
	1	5		3+		3+		
Pentaose	1	1		1+	2+		3+	
	1	5		3+	3+		0	
Hexaose	1	1		1+	2+	1+		2+
	1	5		2+	3+	1+		0

Reaction mixtures contained 1 μmole/ml. substrates and 0·005 M citrate buffer *p*H 4·5. Incubated at 35° for times shown. Products are labelled 1, 2, etc., in order of molecular weight of oligosaccharide, and amounts are based on colour of chromatogram spots.

Chitinase I is incapable of splitting chitobiose, splits chitotriose very slowly and higher oligosaccharides at a considerably faster rate. The production of biose and triose from hexaose shows that random splitting occurs and that chitinase I is an endoenzyme. Two chitinases from *Streptomyces*[7] acting on chitin produce considerable amounts of acetylaminodeoxyglucose and the only other product is biose, thus differing from chitinase I, which produces varying amounts of higher oligosaccharides but very little acetylaminodeoxyglucose.

The action of chitinase I resembles that of salivary α amylase[8], which produces the higher oligosaccharides but little or no glucose from starch. It appears that, because of the preference for the second or subsequent bonds in the oligosaccharides, the production of acetylaminodeoxyglucose by chitinase I is very slow, and in practice may occur only when triose remains in relatively high concentration.

R. F. POWNING
H. IRZYKIEWICZ

C.S.I.R.O. Division of Entomology,
Canberra.

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VIROLOGY

Interference between Polioviruses and the Effect of Ultra-violet Light

INFECTION of animal cells with one type of poliovirus interferes with the growth of another, superinfecting, type added 30–60 min later[1,2]. On the other hand, simultaneous infection with type 1 and type 2 polioviruses can result in both types of viral RNA growing in the same cell, and in a considerable degree of phenotypic mixing in the progeny[3,4] This communication describes a case of interference between two strains of poliovirus after simultaneous infection at equal multiplicities

ERK cell monolayers were trypsinized and maintained in suspension overnight in growth medium at 36° C (ref 5) Suspended cells were infected or superinfected at 0° C (ref 5), and incubated in cell-growth medium at 36° C Samples were lysed with 0 2 per cent deoxycholate and diluted through either type 1- or type 2-specific antiserum before plaque assay[6]

In the first experiment (Table 1), cells were infected at 0° C with a mixture of type 2, *MEF*–1 (*MEF*) and either (*a*) type 1, Brunhilde (*BH*), or (*b*) type 1, Brunenders (*BE*), 5 adsorbed plaque-forming units of each type per cell After incubation for 4 h at 36° C, the progeny in flask (*a*) could be neutralized to the extent of 70 per cent by type 1 antiserum and 73 per cent by type 2 antiserum, indicating the presence of about 43 per cent of doubly neutralizable infective particles[3,4] In flask (*b*), however, the progeny were almost exclusively type 2, although a subsequent experiment demonstrates that the inoculum of type 1 (*BE*) alone was sufficient to infect all susceptible cells Separate one-step growth curves of *MEF* and *BE* were constructed under identical conditions, but no differences could be found between latent periods, rates of maturation, or final yields of the two viruses

Poliovirus which had been irradiated with ultra-violet light before adsorption to cells was not able to interfere with the growth of a later-infecting strain[1,7] In order to investigate the effect of ultra-violet irradiation on the ability of *MEF* (type 2) to interfere with the growth of simultaneously adsorbed *BE* (type 1), cells were infected (3 original plaque-forming units of each virus per cell) with a mixture of untreated *BE* and irradiated *MEF* (7 per cent survival of plaque-forming units) After incubation for 5 h at 36° C, the progeny were predominantly type 1 (Table 1, Exp 2), thus irradiation of *MEF* had much reduced its capacity to interfere with simultaneous *BE* The total yield of plaque-forming units was no less than in a control culture infected with the same two viruses without irradiation, confirming that the inoculum of *BE* was sufficient to infect all the cells In a further experiment it was found that in cells infected with *MEF*, incubated for 30 min at 36° C and then irradiated (sufficient to reduce the 5-h yield of *MEF* to 8 per cent of normal), the yield of superinfecting *BE*, added immediately after irradiation, was only 10 per cent of that in control cells infected with *BE* alone after similar irradiation (Table 1, Exp 3)

Table 1

Exp No	Treatment* of cells/time of infection or ultra-violet irradiation	Time of lysis (h)	Total yield plaque-forming units/cell	Percentage of infectivity neutralized by antiserum Type 1	Type 2
1	(*BH* + *MEF*)/0 h	4	40	70	73
	(*BE* + *MEF*)/0 h	4	38	10	92
2	(*BE* + *MEF*)/0 h	5	160	15	92
	(*BE* + U-V'd *MEF*)/0 h	5	160	94	23
3	*MEF*/0 h	4 5	52	0	90
	MEF/0 h, U-V/0 5 h	4 5	4 0	0	90
	MEF/0 h, U-V/0 5 h, *BE*/0 5 h	4 5	5 6	25	76
	— U-V/0 5 h, *BE*/0 5 h	4 5	14	99	0

* *BH*, infection with type 1, Brunhilde, *BE*, infection with type 1, Brunenders, *MEF*, infection with type 2, *MEF*-1

The fate of the (non-infective) RNA of ultra-violet-irradiated virus in susceptible cells is uncertain Unpublished experiments with poliovirus labelled with phosphorus-32 have shown that irradiated virus still adsorbs to cells, and that there is no gross change in the sedimentation rate of its RNA But there is no evidence that it achieves any of the early results of normal virus infection. irradiated virus, for example, does not cause an early inhibition of cellular RNA synthesis[8] as does normal virus[8–11].

However, as described here, irradiation of infected cells 30 min after adsorption of an interfering virus did not eliminate the interference, although it stopped the growth of the interfering virus It may be that irradiation of virus so alters the configuration of its RNA as to prevent its attachment to a specific site within the cell, but that RNA from infective virus once attached to the site cannot be dislodged by ultra-violet light Two strains of virus infecting simultaneously would compete with each other if they required the same or overlapping sites

An alternative possibility is that an early virus-induced protein is responsible for the interference Experiments with puromycin have suggested that protein synthesis is essential in the very early stages of infection[8,12,13] Perhaps the same early protein causes inhibition of cellular RNA synthesis and interference with superinfecting virus It would be interesting to know whether puromycin prevents this type of interference which is established within 30 min or less Interference between simultaneously adsorbed viruses (*MEF* and *BE*) by such a mechanism would infer that one (*MEF*) produced its interfering protein before the other, although this was not reflected in a measurably shorter latent period after single infection

M L FENWICK*

Virus Research Unit,
Medical Research Council Laboratories,
Carshalton,
Surrey

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* Present address Department of Pathology, University of Colorado Medical Center, Denver 20

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PSYCHOLOGY

Relative Retention in Immediate Memory determined by the Missing Scan Method

STORAGE in immediate memory is often evaluated through retrieval, by requiring subjects to recall or recognize previously presented material The serial error curve generated by retrieval, which shows the distribution of errors of omission in retrieval according to the serial position of presented material, appears to reflect characteristics of retrieval as well as of storage

Some effects of retrieval on the serial error curve have been summarized by Broadbent[1] in his discussion of order effects in recall When subjects are asked to recall material in the order presented, the usual finding is that most errors occur in the second half of the series presented However, Kay and Poulton[2] found that when subjects were asked to report the second half of the list first, an equal number of errors occurred in both halves of the list Investigations by Brown[3], by Lawrence and LaBerge[4], and by Broadbent[1] have shown that items recalled first are more likely to be correct Because the first items recorded were most likely to be correct even if subjects knew in advance which to record first, Broadbent concluded that items are not recalled first because they are correct but are correct because they are recalled first Such order effects appear whenever a subject retrieves information in some order

These considerations indicate certain limitations of retrieval methods for the investigations of storage in short-term memory These limitations may be overcome by the use of sampling methods which require only partial retrieval, as Sperling[5], Averbach[6], and Coriell[7] have shown This report presents the serial error curve generated by another method which may have certain advantages for the investigation of short-term retention

The missing scan' method for the examination of retention in immediate memory[8] does not require retrieval by recall or recognition It depends on knowledge of class membership, requiring the subject to report only that member of the class which was not presented For example, nine numbers randomly chosen from the class 1–10 inclusive may be presented serially in random order the task being to report which of the 10 numbers was not presented To report the missing item correctly, all information presented must be retained, therefore the missing scan offers a sensitive measure of storage capacity If an error is committed it takes the form of reporting as missing some item that was presented, indicating relatively poorer retention of an item presented in that particular serial position The serial error curve generated by the missing scan in this way may more clearly delineate retention, uninfluenced by any recall order effects

It has been shown that most missing scan errors are in the first half of the list[8] The present experiment examines the missing scan serial error curve in detail to show relative retention in short-term storage The twenty male subjects were patients on the Neurology Service of the Palo Alto Veterans Hospital, without clinically demonstrable mental deficit, who ranged in age from 26 to 71 with a mean of 44 1 years Subjects were tested individually and instructed to report that number missing from a known set of thirteen after the examiner had read the other twelve at the rate of one each second The missing numbers were chosen at random so that each of the thirteen was equally often missing, and the remaining twelve numbers were presented in random order The sets of thirteen numbers used were 1–13 and 4–16 inclusive, all lists from one set were read before proceeding to the other Each subject received 156 such twelve-item lists in counterbalanced order

The proportion of correct reports given by all subjects clearly exceeded chance, ranging from 20 to 50 per cent with a mean of 33 per cent correct responses The missing scan error curve given here is based on the serial distribution of the 2,091 errors committed by these twenty subjects Fig 1 shows the 95+ per cent joint confidence intervals on error probabilities for each of the twelve serial positions Given the occurrence of an error, Fig 1 indicates the relative probability of 'forgetting' an item as a function of its position in a serially presented list The cross bars show in decimal form the proportion of total errors in each serial position, from which the probabilities are derived

These results confirm that most missing scan errors are found in the first half of the list, and extend independent analysis of relative retention in short-term storage to show, at the 95+ per cent level of confidence, that (1) the first item is better retained than the second, the probability of error in serial position 1 being less than that in serial position 2, (2) separate consideration of serial positions 4–12 inclusive shows a generally decreasing probability of error from position 4–12, since the probability of error in position 4 > 5, 6 > 7, 8 > 9, 10, 11 > 12

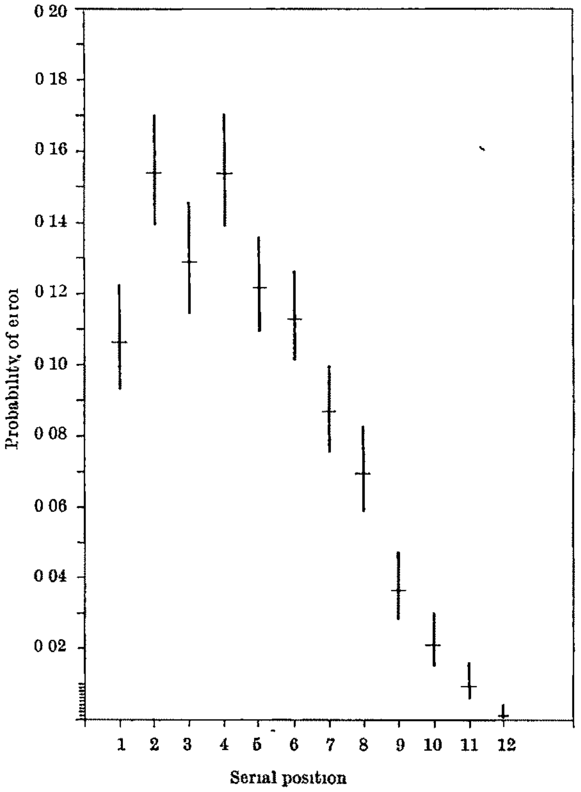

Fig 1 95+ per cent joint confidence intervals on error probabilities with respect to serial position, given that an error is committed The cross bars show in decimal form the proportion of 2,091 errors in each serial position

This missing scan serial error curve deserves further investigation because it would appear to offer more adequate independent characterization of short-term storage, for the following reasons (1) Since the missing scan does not require retrieval by recall or recognition, the serial error curve generated by this method is not influenced by any retrieval order effects (2) The missing scan offers a stronger determination of retention because the missing scan error is an error of commission while the comparable recall or recognition error is an error of omission (3) The missing scan may specifically estimate relative retention, since, in the case where there is only one missing item to be reported, the forced choice of a single item should select that item least well retained The independent analysis of storage thus available should enhance understanding of both retention in and retrieval from immediate memory

I thank Dr Helena Chmura Kraemer, research associate in the Department of Biostatistics of Stanford University School of Medicine, for the statistical analysis

HERMAN BUSCHKE

Division of Neurology,
Stanford University School of Medicine,
and Palo Alto Veterans Hospital,
Palo Alto, California

[1] Broadbent D E, *Perception and Communication* (Pergamon Press, New York, 1958)

[2] Kay, H, and Poulton, E C, *Brit J Psychol*, **42**, 34 (1951)

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[6] Averbach, E, and Sperling, G, in *Information Theory* (Fourth Lond Symp, 1960), edit by Cherry, C, 196 (Butterworth, Inc, Washington, D C, 1961)

[7] Averbach, E, and Coriell, A S, *Bell System Tech J*, **40**, 309 (1961)

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 16

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W C 2), at 5 30 p m —Mr W F Gibbons "Ceramic Sealing Techniques Applied to Electron Tubes and Semiconductor Devices"

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S W 1), at 5 30 p m —Mr A Stevenson "Analysis of Dithiocarbamates and Thiuram Disulphides Used in Agriculture A Collaborative Study of Macro-Methods Available for the Evaluation of Technical and Formulated Products"

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W C 2), at 6 30 p m —Sir Albert Mumford, K B E "Communications and Radio"

Royal Geographical Society (at 1 Kensington Gore, London, S W 7), at 8 15 p m —Dr L S B Leakey "Exploring Man's Prehistory in East Africa"

Monday, December 16—Tuesday, December 17

British Biophysical Society (at the Institution of Electrical Engineers, Savoy Place, London, W C 2)—Symposium on "Control Mechanisms"

Monday, December 16—Wednesday, December 18

Institute of Physics and Physical Society, Electronics, Electron Microscopy and Analysis, and Low Temperature Groups (at Imperial College, London, S W 7)—Conference on "The Electrical and Magnetic Properties of Thin Films in Relation to Their Structure"

Tuesday, December 17

Institution of Civil Engineers (at Great George Street, Westminster, London, S W 1), at 5 30 p m —Mr E C Hall and Mr D R Russell "The Malta Underground Oil Fuel Installation"

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W C 2), at 5 30 p m —Discussion on "Teaching of Electrical Measurements Abolition or Reform"

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m — Dr A J Buller "Mammalian Fast and Slow Skeletal Muscle" (Last of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Wednesday, December 18

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 15 p m —Dr F R Oliver "Inter-Regional Migration and Unemployment, 1951–1961"

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W C 2), at 5 30 p m —Mr S J Emerson, O B E "Protective Earthing of Consumers' Installations"

Institution of Mechanical Engineers, Lubrication and Wear Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m — Discussion on "The Role of Metals in Lubrication"

Thursday, December 19

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Dr W Domzalski "Some Trends in Mineral Resources Exploration", followed by a film—"The West Wits Line" introduced by Mr A R O Williams

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W 1), at 5 p m —Dr B J Birch "Elliptic Curves"

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S W 1), at 6 p m —Sir William Glanville, C B , C B E "Materials and Construction Problems Bearing on Road Safety"

Friday, December 20

Institute of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S W 1), at 5 30 p m —Mr J Bentkowsky "Bridge Console Designs"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Senior Library Assistant (with appropriate professional qualifications and/or experience), for cataloguing duties—The Librarian, University of Essex, Wivenhoe Park, Colchester, Essex (December 19)

Assistant Librarian (Chartered Librarian preferably with a university degree), for duties which will include classification and cataloguing and some help with reference and information work—Clerk to the Governors, Woolwich Polytechnic, London, S E 18 (December 20)

Assistant (preferably graduate, with a wide interest in books and reading, some experience in publishing and/or the editorial side of journalism) in the Reviews Section of the Science Department, for the selection and distribution of books and other publications (mostly scientific) to overseas reviewers—The Director, Recruitment Department, The British Council, 65 Davies Street, London, W 1, quoting Ref A9/64/G/1 (December 20)

Lecturer in Bacteriology (Science)—The Secretary of University Court, The University Glasgow (December 23)

Lecturers (2) in Pure Mathematics—The Registrar, The University, Manchester 13 (December 28)

Lecturers (3) (with research or industrial experience in electronic applications and circuit techniques, in automatic control or in engineering systems) in the Department of Electrical Engineering—The Registrar Queen Mary College (University of London), Mile End Road, London, E 1 (December 30)

Fellow and Tutor in Engineering Science—The Rector, Lincoln College, Oxford (December 31)

Imperial Chemical Industries Research Fellows—The Secretary, The University, Aberdeen (December 31)

Lecturer in Clinical Science within the Department of Surgery —The Secretary, The Queen's University, Belfast, Northern Ireland (December 31)

Lecturer (Physicist or Physical Chemist) in the Department of Physics, to assist with the teaching of the new degree course in the physical properties of materials—The Registrar (Room 22, O R B), The University, Reading (December 31)

Lecturers (2) (preferably with a special interest in the study of synthetic high polymers or in the application of radioactive tracer techniques) in Physical Chemistry—The Assistant Registrar (Science), The University, Birmingham 15 (December 31)

Unilever Research Scholar (with a first- or upper second-class honours degree in a relevant subject or able to provide evidence of research aptitude and experience) in the Department of Biological Chemistry, to assist in work on the chemistry, biochemistry or cytology of mammalian intestine —Dr J W Porteous, Department of Biological Chemistry, Marischal College, University of Aberdeen, Aberdeen (December 31)

Junior Lecturer (preferably interested in the cytological or physiological aspects of vertebrate reproduction) in Zoology—The Secretary, The Medical College of St Bartholomew's Hospital, West Smithfield London, E C 1 (January 4)

Lecturer in Geography—The Secretary of Faculties, University Registry, Oxford (January 6)

Lecturer in Chemistry at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 10)

Senior Lecturer/Lecturer in the Psychology of Education, University of Cape Town—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (January 10)

Senior Tutor-Demonstrator (with a university degree in pharmacy (or its equivalent) and teaching experience since graduation) in Pharmaceutics at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 10)

Professor of Soil Science—The Registrar (Room 22, O R B), The University, Reading (January 11)

Chair of Virology at St Mary's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W C 1 (January 13)

Lecturer or Assistant Lecturer in the Department of Pure Mathematics—The Registrar, University College of North Wales, Bangor, North Wales (January 13)

Professor of Mathematics at Newcastle University College, The University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (London and Australia, January 13)

Professor of Physics at Newcastle University College, University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 13)

Lecturer or Assistant Lecturer in Physics at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 15)

Lecturers (2) in Psychology at the University of Sydney, Australia —The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 15)

Lecturer in Histology and Embryology, and a Senior Tutor/Demonstrator in Histology and Embryology at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 16)

Lecturer (with an honours degree in physics and some teaching and/or research experience) in Physics at the University of Queensland, Australia —The Secretary, Association of Commonwealth Universities (Branch Office) Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, January 17)

Senior Lecturer and a Lecturer (with an honours degree in mathematics, and preferably a higher degree and some experience of research) in Mathematics at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, January 17)

Senior Lecturer (with a higher degree in biochemistry and considerable teaching and/or research experience in this field) in Biochemistry at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, January 17)

Chair of Biology (in the field of animal biology) at the University of Sydney—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 31)

Chair of Mathematics—The Secretary, University of Lancaster, Bailrigg House, Lancaster (January 31)

Lecturer in Educational Psychology—The Registrar, The University, Manchester 13 (January 31)

Second Chair in Applied Psychology at the University of New South Wales, The Agent General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 31)

Senior Lecturer (preferably with medical qualifications) in Pharmacology at the University of Hong Kong—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Hong Kong and London, January 31)

Foundation Chair of Mathematics (either Pure or Applied) at Wollongong University College—The Agent-General for New South Wales, 56–57 Strand, London W C 2, and The Appointments Section, The University of New South Wales Box 1, Post Office, Kensington, New South Wales, Australia (February 28)

Professor (with high academic qualifications in chemical engineering or related fields, considerable research experience, and preferably industrial experience in the fields of petroleum refining and/or petrochemicals) of

CHEMICAL ENGINEERING at the University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (March 31)

BIOLOGIST (aged 24–35, with an honours degree in zoology with post-graduate experience in animal ecology) with the Government of Tanganyika, to carry out investigations connected with the management of wild life and to advise the Chief Game Warden—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, London, S W 1, quoting Ref RC 346/145/03/T

MASTER TO TEACH MATHEMATICS, including Sixth Form work—The Headmaster, Bolton School, Bolton, Lancashire

RESEARCH ASSISTANT (graduate in zoology, physiology or biochemistry), to work under the Professor of Zoology on the physiology of coelenterates and/or the water and ionic balance of invertebrates using radioactive isotopes—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W 8

RESEARCH ASSISTANT IN THE DEPARTMENT OF EDUCATION—Department of Education, The University, Old Aberdeen

TECHNICIAN FOR THE ZOOLOGY DEPARTMENT—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey

REPORTS and other PUBLICATIONS

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The Empire Cotton Growing Corporation Progress Reports from Experiment Stations, Season 1961–1962 Tanganyika—Coastal Regions By R Smith and T Bradley Pp 13 2*s* 6*d* Survey of Reports By H E King Pp 9 (London The Empire Cotton Growing Corporation, 1963) [269

Nuffield Foundation Science Teaching Project—Progress Report Pp iii+40 (London Nuffield Foundation, 1963) [269

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Science Museum, London Science Library Bibliographical Series, No 788 Flight of Birds Pp 18 (Supplement to No 295) (London Science Museum, 1963) [269

University of London. University College Calendar, 1963–1964 Pp lxxxiii+482 (London University College, 1963) [269

General Register Office Census 1961 England and Wales Population, Dwellings, Households Leaflet No 15 Cornwall and the Isles of Scilly, Devon, Gloucestershire, Somerset and Wiltshire Pp ii+26. 2*s* 6*d* net Leaflet No 16 Berkshire, Buckinghamshire, Oxfordshire Pp ii+11 2*s* net Leaflet No 17 Norfolk, East Suffolk, West Suffolk Pp ii+16 2*s* 6*d* net (London H M Stationery Office, 1963) [269

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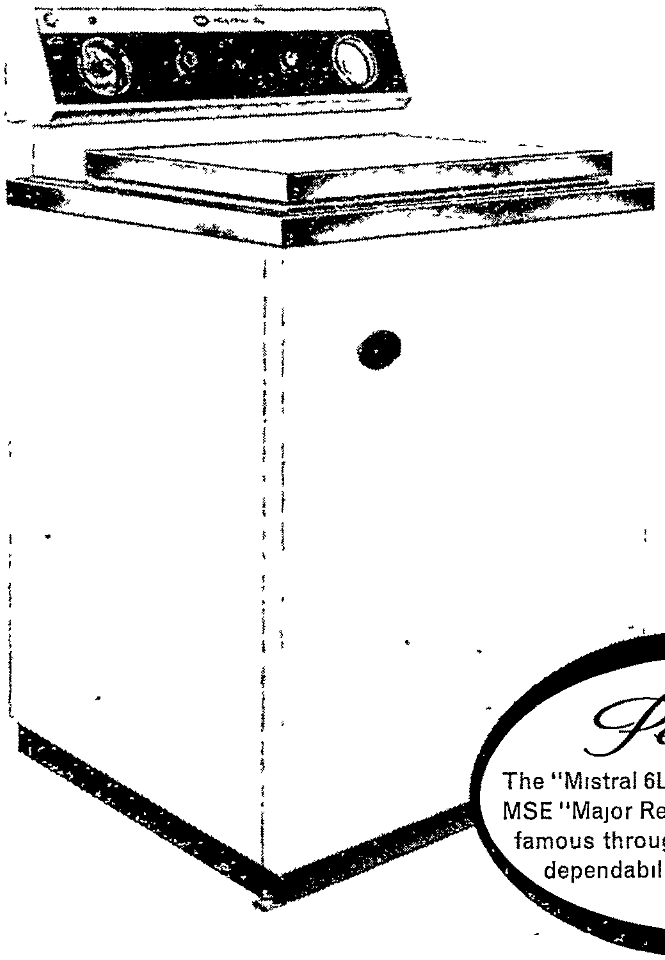

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UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for two Lectureships in Physical Chemistry in the Department of Chemistry Preference may be given to applicants with a special interest in the study of synthetic high polymers or in the application of radioactive tracer techniques Initial salary in the scale £1,250 by £60 to £1,670 by £80 to £1,830 , £1,830 by £80 to £2,150, according to age, qualifications and experience, together with F S S U and family allowance benefits

Applications (three copies), naming three referees, should be sent by December 31, 1963, to the Assistant Registrar (Science), the University, Birmingham 15, from whom further particulars can be obtained

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF SOILS

PLANT PHYSIOLOGIST OR MICROBIOLOGIST

Applications are invited for appointment to a position of Research Officer (Plant Physiologist or Microbiologist) with the Organization's Division of Soils, located in Adelaide, S A

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The Microbiology Section of the Division is well equipped to undertake studies in soil microbiology, biochemistry, organic chemistry, and plant physiology Equipment includes normal microbiology and biochemical requirements and in addition fluorescence microscope recording oxygen analyser, gas chromatograph infra-red and ultra-violet recording spectrophotometers, mass spectrometer, radioactive isotope counting equipment and glasshouse The Section is currently engaged on the oxidation of sulphur in soil, mycorrhizal associations on pine trees, phytotoxic substances from soil and from decomposing plant residues, microbiological prospecting for oil, microbiology of the rhizosphere, bacterial inoculation of crops, and the chemistry of soil organic matter

The successful applicant will investigate the chemical nature of plant root exudates, mechanisms of exudation under different environmental conditions, and the importance of exudates in the nutrition of micro-organisms in the rhizosphere

Applicants should possess a Ph D degree in plant physiology, biochemistry, or microbiology, or should have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability

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THE AUSTRALIAN NATIONAL UNIVERSITY

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Applications should be forwarded to the undersigned at Box 4, G P O , Canberra, A C T , Australia by December 20, 1963

W S HAMILTON,
Bursar

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Applications are invited for two posts of Lecturer in Pure Mathematics in the Department of Mathematics in the Faculty of Science Salary scale £1,250 to £2,150 per annum, initial salary according to qualifications and experience Membership of the F S S U and children's allowance scheme

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Secretary of the University Court

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AUSTRALIA

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invites applications for appointment to the position of

PROFESSOR OF INDUSTRIAL ENGINEERING

SCHOOL OF MECHANICAL ENGINEERING

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Details of conditions of appointment, including superannuation, travel arrangements, study leave, housing scheme, may be obtained from the Agent-General for New South Wales, 56-57 Strand, London, W.C.2. Four copies of applications should be typed and forwarded, together with the names of three referees and a recent photograph, to the Agent-General before February 17, 1964. A copy of the application should be forwarded by airmail to the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., to reach there before the above-mentioned date.

AUSTRALIA

THE UNIVERSITY OF NEW SOUTH WALES
SYDNEY

SENIOR LECTURER/LECTURER
in
INDUSTRIAL CHEMISTRY
NEWCASTLE UNIVERSITY COLLEGE

Salary: Senior Lecturer, £A2,755 range £A3,255 per annum. Lecturer, £A1,905 range £A2,605 per annum. Commencing salary according to qualifications and experience. Applicants must hold an honours degree in science, applied science or chemical engineering or have equivalent qualifications. For appointment as Senior Lecturer a higher degree is essential. Teaching or research and industrial experience would be advantageous. The successful applicant, if a Senior Lecturer, will be responsible to the Head of the Department of Chemical Engineering and Industrial Chemistry for undergraduate instruction in industrial chemistry to the fourth year honours level and for the supervision of postgraduate research in the same subject.

Details of conditions of appointment may be obtained from the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, with whom four copies of applications and names of two referees, should be lodged before February 17, 1964, and a copy of the application forwarded by airmail to the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, to reach there before the above-mentioned date.

AUSTRALIA

THE UNIVERSITY OF NEW SOUTH WALES
SYDNEY

invites applications for appointment to the
FOUNDATION CHAIR OF MATHEMATICS
(either Pure or Applied)
WOLLONGONG UNIVERSITY COLLEGE
(An associated College of the University of New South Wales)

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Details of conditions of appointment may be obtained from the Agent-General for New South Wales, 56-57 Strand, London, W.C.2. Four copies of applications should be typed and forwarded, together with a recent photograph and the names of three referees, to the Agent-General before February 28, 1964, and a copy forwarded by airmail to the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales.

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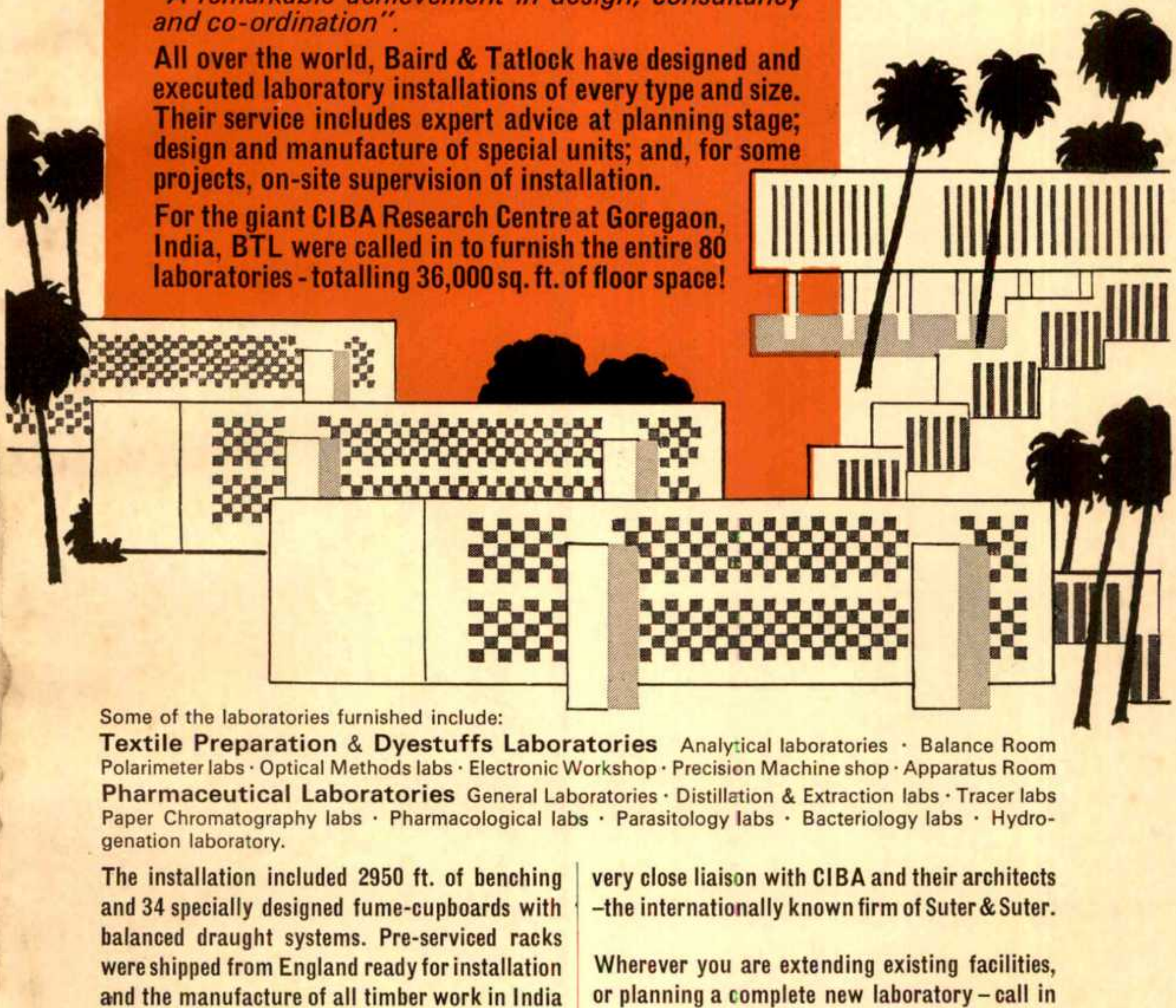

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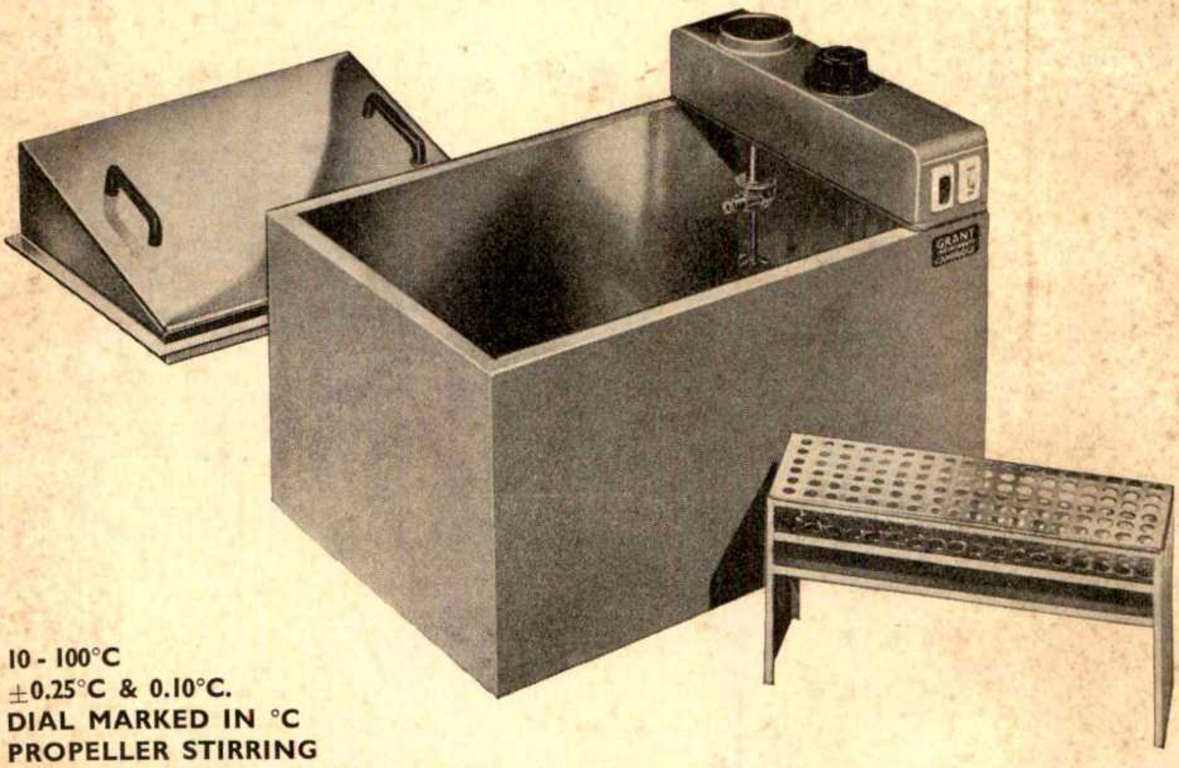

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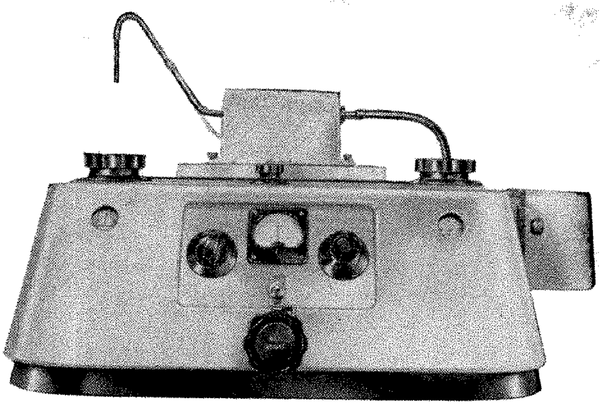

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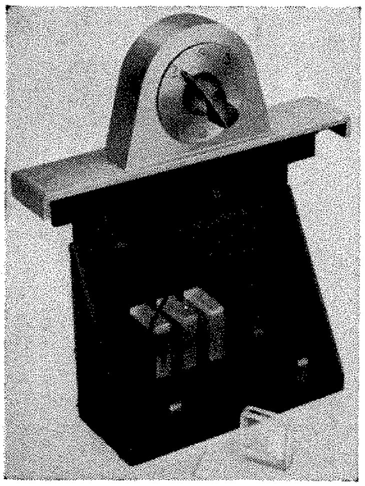

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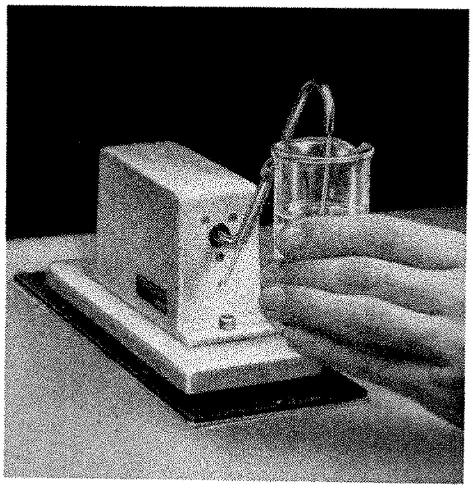

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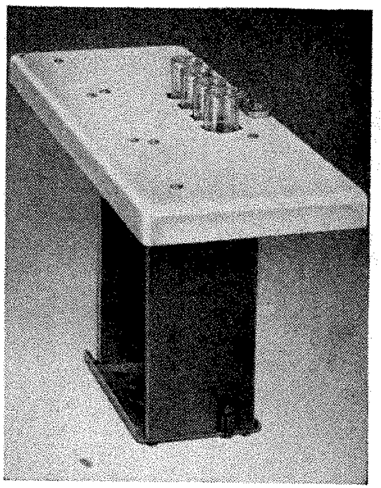

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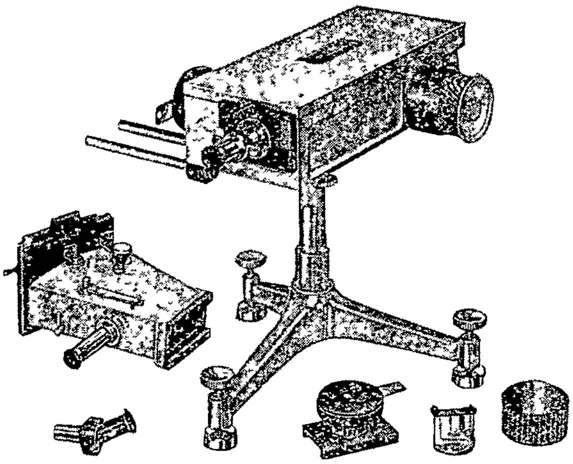

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A STIMULANT FOR BRITISH SCIENCE

THE sixteenth annual report of the Advisory Council on Scientific Policy *, covering the year 1962–63, was published shortly after the report of Sir Burke Trend's Committee on the organization of civil science †, in which the report of Sir William Slater's Committee on Research into National Resources appended to the Advisory Council's report, is discussed Even in that respect, the Trend Committee, while anticipating, does not lessen the interest of the Advisory Council's own report, which may indeed be scrutinized even more closely in the light of the Trend Committee's proposal to reconstitute the Advisory Council itself as a more authoritative and independent body Apart from this, however, the Advisory Council's report has features of particular interest in its comments on the European High-Energy Physics Programme, on scientific documentation and on research in oceanography and marine biology A brief note on the emigration of scientists is supported by a copy of a memorandum on the subject submitted to the Minister for Science last May

The report of the Advisory Council on Scientific Policy opens with a discussion of the report of the Committee on Scientific Manpower on *Scientific and Technological Manpower in Great Britain*‡, published early in October This has recently been discussed in these columns (*Nature*, 200, 297; 1963), and no more need be added here than that the Advisory Council reiterates its concern over the preference for science over technology which continues to be shown by the more promising entrants to the universities and the need for greater emphasis on post-graduate research in technology The Council's continuing concern over an adequate supply of supporting staff in the shape of technicians has now led it to institute an enquiry, under the chairmanship of Sir Willis Jackson who has now succeeded Sir Solly Zuckerman as chairman of the Scientific Manpower Committee, into the supply of and demand for such staff (see also p 1145 of this issue)

During the year, the Advisory Council was invited to advise the Minister for Science on the attitude the United Kingdom should adopt to the proposals in a report from a representative body of nuclear physicists, with Prof B H. Flowers as chairman, set up by the Joint Consultative Panel for Nuclear Research on the Department of Scientific and Industrial Research and the National Institute for Research into Nuclear Science The Advisory Council does not dispute this working group's belief that physics is to-day on the threshold of far-reaching developments which will come to fruition with a new theory of the nature of matter and of its fundamental interactions It is possible that, either at this stage or later, gravitation, the creation of matter and the problems of cosmology may be unified with high-energy physics Nor does the Council deny that Britain's national facilities for high-energy physics are now, or will be in the near future, bettered only by those of the United States, while through the European Organization for Nuclear Research, Britain has opportunities second to none The working group accordingly wishes Britain to continue to play a full part in European collaboration in high-energy physics even in the enlarged programme now proposed, which envisages *inter alia*, capital costs of £120 million over an 8 5 year constructional period, followed by operating costs of £20 million upwards, compared with the capital costs of the existing installation at the European Centre for Nuclear Research of some £20 million and current operating costs of £8 million, of which Britain's contribution has been slightly less than 25 per cent

The Advisory Council recognizes that nuclear physics is much more expensive than most other types of scientific work, but it recognizes also that Britain's expenditure on other scientific research is too low There is also a serious deficiency in provision for university research, the expenditure for the Research Councils is expected to increase at more than 10 per cent per annum In the circumstances, unless the Government is prepared to provide the relatively small sum needed to satisfy the legitimate needs of general scientific research in the universities by supplementing departmental research budgets, increasing the supply of technical assistance of all grades and creating more senior posts, the Advisory Council considers that it will not be justified in supporting the expenditure required to further these research proposals, good as they are in themselves It stresses, instead, the urgent need for international co-operation in this area and in that of space research

It is impossible not to believe that the Advisory Council is right to insist that these needs of the universities are primary and must be met first, not merely to avoid distortion of Britain's research effort in general but equally to avoid serious consequences in the universities and other institutions of higher education Nor is this opinion invalidated by the effects of the National Institute for Research in Nuclear Science and of the European Organization for Nuclear Research in attracting the return of physicists from the United States to use the new opportunities—which is noted in the Advisory Council's report, though not in its memorandum to the Minister for Science on the emigration of scientists That memorandum, after reviewing the report of the Royal Society's committee and other evidence, made six specific proposals for Government action, all of which are under consideration and on some of which action has already been taken

A review of salaries in universities by the National Incomes Commission is already well advanced Research Councils are already authorized to pay the university scientific departments an additional £200 per annum in respect of each holder of a postgraduate award, and the responsible bodies have already taken steps to minimize delay in the award of student grants and grants for specific research projects Besides increasing the number of senior posts in universities, the memorandum also suggested increasing the scale of technical assistance at all levels for university research, and awarding post-doctoral fellowships, to be held on their return, to outstanding men before they leave for research experience overseas, or placing a member of such fellowships at the free disposal of universities and governmental research laboratories

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* Sixteenth Annual Report of the Advisory Council on Scientific Policy, 1962–1963 (Cmnd 2163) Pp 53 (London H M S O , 1963) 3*s* 6*d* net

† *Committee of Enquiry into the Organization of Civil Science* Pp 59 (Cmnd 2171) (London H M S O , 1963) 4*s* net (See *Nature*, **200**, 1029, 1963)

‡ Advisory Council on Scientific Policy Committee on Scientific Manpower *Scientific and Technological Manpower in Great Britain*, 1962 (Cmnd 2146) Pp 60 (London H M S O , 1963) 3*s* 6*d* net

In these two matters alone, on the evidence of the present report, the Advisory Council is displaying a vision and an energy that has been lacking in some recent reports and which should preclude any hasty acceptance of proposals for its reconstitution—though not for strengthening its authority On scientific documentation, too, the Advisory Council shows a greater awareness of its responsibilities and opportunities, and although during the year the Department of Scientific and Industrial Research made its first grants to universities and colleges for research into problems relevant to scientific documentation, there is no doubt that the Advisory Council does, as it is already constituted to do, take a wider view than the Department The proposal to transfer such responsibilities to the Office of the Minister for Science, which will presumably be in close touch with the Advisory Council, is fundamentally sound and should also stimulate that essential wider conspectus of library resources and responsibilities which embraces the contribution of the National Central Library, the public library service, the university libraries and others, not to mention the new National Reference Library of Science and Invention, which is not mentioned in this report

This aspect could well be illuminated by the survey, which has already been put in hand, of the sources of information used by a representative sample of research chemists and physicists, and those working on the borders between these two subjects The survey asks for critical appreciations of the limitations of these sources and suggestions for improvement, and it also covers the storage and retrieval of information The Institute of Physics and the Physical Society, the Chemical Society and the Royal Institute of Chemistry have co-operated in selecting the sample and ensuring that it is representative

The Advisory Council also refers to the recommendation in 1963 by the British National Committee for Astronomy, following discussions between British and Australian scientists, that the urgent need for British astronomers to have access to a large-diameter optical telescope in the southern hemisphere could best be met by a joint British–Australian project for a 150-in instrument, sited in Australia, and working in close association with radio-astronomy. In reviewing in its 1959–60 report the balance of scientific effort in Britain, the Advisory Council noted the neglected position of optical astronomy and the interest and importance of the relatively unexplored southern sky No firm proposal had, however, previously emerged which could be put to the Government for decision, but the Advisory Council now strongly supports the view of this Committee and recommends that a design study should be undertaken as soon as possible

In view of the Trend Committee's recommendation regarding a National Resources Research Council, based essentially on the proposals of Sir William Slater's Committee, which has already been fully discussed in these columns (*Nature*, **200**, 1031, 1963), there is little to add by way of comment on the present report The Advisory Council agrees with the main conclusions of the Slater Committee but disagrees with the majority's view that responsibility for research in terrestrial ecology should be separated from responsibility for the establishment and management of nature reserves It considers, rather, with Sir Solly Zuckerman, that the whole of the Nature Conservancy's work should fall within the ambit of the proposed Council

Apart from this, it should be added on the subject that, besides its general discussion of the case for a new Research Council for Natural Resources, the Slater Report gives separate sections to the discussion of fisheries research, oceanography, hydrology, land use, terrestrial ecology, forestry research, soil science, and to the work of the National Resources (Technical) Committee The latter Committee was set up in 1950 in succession to the Imports Substitution Panel of the Committee on Industrial Productivity, and at present advises the Minister for Science and the Chancellor of the Exchequer on technical problems of the development, use and conservation of natural resources arising from considerations of the United Kingdom import and investment programme This Committee, the Slater Committee suggests, should come under the new Research Council and, with its staff, form an operational research unit in this field While agreeing that the laboratories of the various fisheries departments should also be transferred to the new Research Council, the Advisory Council was undecided whether the responsibility for the Torry Research Station should be transferred to the new Research Council or to the Agricultural Research Council, though it was satisfied that it should no longer rest with the Department of Scientific and Industrial Research Moreover, in view of the blindness of the Trend Committee regarding overseas research, it should be noted that the Slater Committee recommends that the proposed Research Council should be empowered to support or undertake research overseas, and it directs attention to the urgent need of many developing countries for expert advice in this wide field It is also to be noted that it visualizes the resultant important contribution to technical aid as being of benefit to British scientists as well as to the developing countries themselves

The Slater Committee, as just noted, discussed the organization of research in oceanography although this was not included in the list of subjects which it was originally asked to consider The final section of the present Advisory Council's report considers at some length the question of research in oceanography and marine biology However, it does not comment specifically on the recommendations of the Slater Committee in this field In these recommendations it was proposed to place the administrative responsibility for the National Institute of Oceanography with either the Admiralty or the new Natural Resources Research Council, which should, in any event, take over the scientific and financial interest of the Development Commission in the Institute and should be authorized, directly or through the Institute, to undertake or support oceanographic research in any part of the world

The Advisory Council on Scientific Policy has not, however, completed its own survey of oceanography and marine biology, and its comments in the present report are limited to the work of the institutions already considered In 1961, the Council was requested to consider the Royal Society's proposals concerning Britain's participation in the International Indian Ocean Expedition and for general expansion in the marine sciences. Twenty nations and some forty research vessels are participating in the Indian Ocean Expedition, in which Britain is playing a full part in the form of a well-diversified investigation of the north-western area of the Indian Ocean The principal geophysical aim is to establish the crustal structure of the Arabian Sea and to determine its relation to the surrounding continental land masses, while the biological programme, besides covering the descriptive biology of the Arabian Sea, aims at investigations of the cycle of events in a typical upswelling region, and of variations of fertility in relation to present-day boundaries and crater layering

Commenting specifically on the National Institute of Oceanography, the present research programme of which covers a wide range of physical, chemical and biological investigations, the Advisory Council points out that its grant-in-aid has increased from £175,000 a year in 1961–62 to £360,000 for 1963–64, and that in the period 1961–63 the new R R S *Discovery* was built at a cost of £800,000 The Institute's staff of 25 in the scientific officer class on April 1, 1960, is expected to total 35 on March 31, 1964 On the work of the Development Commission, the Advisory Council points out that total grants by the Commission to the independent marine biological institutions at Plymouth, Edinburgh, Millport on the Clyde, Port Erin and Cullercoats, have risen from £209,500 in 1960–61 to an estimated £257,900 in 1963–64, while contributions to university research in oceanography in 1962–63 exceeded £4,000, some £90,000 being allocated for this latter purpose by the Department of Scientific and Industrial Research There were 654 students in 1962–63 taking special subjects related to marine biology as part of honours courses compared with 450 in 1959–60 and 81 postgraduate students compared with 39, while six universities were providing undergraduate courses in oceanography

The Advisory Council is much impressed with the progress which has been made in what it regarded in 1959–60 as a relatively neglected field, but, as already indicated, its full comments are reserved until it has completed its survey of other institutions supporting work in this field, on which the aggregate research expenditure in 1962–63 was a further £725,000 Meanwhile, here again it seems clear that the Advisory Council is fully competent to discharge the responsibilities that are already placed on it for co-ordination and advice over the whole field of science It would seem that given the adequate staff stressed by the Trend Committee it should be able to meet any further responsibilities which may fall to it from the expansion of those of the Minister for Science

There may be real substance in the recommendations of the Trend Committee that its authority should be strengthened and that its value would be enhanced by widening the basis of appointing its members and ensuring that they were independent rather than official representatives Nevertheless, there is much in the Advisory Council's present report to restore confidence in the judgment and independence of the Council which some of its recent reports have shaken and to demonstrate that it can be an effective instrument if wisely and firmly used The Advisory Council is to be congratulated on a fine report, which should be carefully studied by all responsible for taking decisions on the future organization of civil science in Britain If in the event this report should prove to be the 'swan song' of the Advisory Council as at present constituted, it is not one of which the Council need fear reproach

THE WONDERS OF PSYCHOTHERAPY

A Study of Brief Psychotherapy

By D H Malan (Mind and Medicine Monographs, No 8) Pp xiv+312 (London Tavistock Publications (1959), Ltd , Springfield, Ill Charles C Thomas, 1963) 35*s* net

Therapist–Patient Expectancies in Psychotherapy

By Dr Arnold P Goldstein Pp xvi+141 (Oxford, London, New York and Paris Pergamon Press, 1962) 30*s*

Dependency in Psychotherapy

A Casebook By Prof William U Snyder Pp viii+424 (New York The Macmillan Company, London Collier-Macmillan, 1963) 70*s*

RESEARCH in psychotherapy has for long been the despair of experimentally minded psychologists, and while there are some gleams of light contained in the books under review these are not really sufficient to consider the gloom any less inspissated than before The crucial question that confronts psychotherapists is a very simple one, does it work ? Curiously enough this is precisely the question that psychotherapists have always avoided, relying on assumptions, persuasion and sheer accumulation of clinical anecdotes, rather than scientific demonstration Gradually the repeated criticisms of this unverified assumption have caused more and more analysts to acknowledge the sad deficiencies of their discipline, and now even the Tavistock Clinic, previously among the most reluctant to admit the point, joins the majority by admitting, in the words of D H Malan, the senior hospital medical officer "that there is not the slightest indication from the published figures that psychotherapy has any value at all" If the acknowledgment of ignorance is the beginning of the acquisition of wisdom, then we may perhaps regard this as one of the few rays of light mentioned above

For the rest, alas, Dr Malan's book falls into all the traps which have beset the task of previous workers who have tried to produce evidence in favour of psychotherapy in general or a special type of psychotherapy in particular Malan tries to show that brief psychoanalytic therapy is possible even with relatively severe cases of neurosis; he does this on a very small group of about twenty cases, which he admits to have been very highly selected indeed There was no control group and consequently no assessment of the likely role of spontaneous remission was possible Malan argues, not entirely without reason, that the short duration of the treatment and the chronic nature of the disorders militate against an argument based on spontaneous remission, in view of our lack of detailed knowledge of the occurrence of spontaneous remission in the type of case treated, this argument will appeal more to the converted than to those whose minds are still open

Malan's reasons for accepting the efficacy of psychotherapy appear to be based on the belief that the standards of cure adopted by analysts are higher than, and different from, those used by behaviour therapists This may be so, the example he gives does not suggest, however, that this is very likely, and the fact that many behaviour therapists started out as trained psychoanalysts would also seem to speak against it In any event, if psychoanalysts adopt different criteria we must ask for some evidence that these criteria are relevant and refer to something which can be demonstrated to exist, if a patient complains of impotence, it would require a good deal of demonstration to show that to relieve him of this impotence is not to cure him, and that what is required in addition (and possibly in lieu) is to give him insight into his Oedipus complex !

Malan gives some statistical treatment of his data, undeterred by the small number of cases (which he acknowledges as making this enterprise rather hazardous) or by the fact that the very curious method of selection does not make them representative of any conceivable universe of patients, thus making the application of statistical methods relatively unrewarding Altogether Malan is clearly not very sophisticated in the use of statistical methods, his assertion on p 173 that two-tail tests must be used when a prediction has been made but the correlation found is of the opposite side to that predicted is quite fallacious The reader obviously cannot rely for statistical guidance on the author of this book, and this makes it all the more difficult to evaluate his conclusion that brief psychotherapy is useful in certain types of cases, or his attempted demonstration of some of the correlates accompanying improvement However, when all is said and done this is nevertheless an interesting book, sophisticated in many ways, and more aware than most of the pitfalls and difficulties attending rigorous demonstration in this field It is a pity that Malan has fallen prey to the prevailing belief among psychotherapists that to acknowledge a deficiency in a research design makes it unnecessary to improve the research design in such a way that the deficiency is removed

Goldstein's book is a purely argumentative one containing no new material It has an unusually high ratio of references to text, there are 121 pages of text and 342 references In parts the book reads as if the author had handed his secretary his reference cards with a few comments scribbled on each, with the instruction of typing it all into one consecutive text The theme that seems to run through some of this display of erudition seems anchored in the placebo effect—and it is characteristic of the author that he cannot even tell the reader what the word means in English without having to put in a reference to somebody else

What Goldstein says in brief is that the notion of spontaneous remission is erroneously labelled and that expectations on the part of the patient aroused by such things as registering for assessment, being administered personality tests, etc , have an important part to play in his recovery These placebo effects are indeed important, but it is difficult to think of anybody who has written on spontaneous remission who has not held a similar view Obviously while spontaneous remission occurs in time it is not effected through the passage of time alone, it has always been regarded as a task for further research to establish just precisely what are the agencies responsible for spontaneous remission Learning theorists, for example, have implicated extinction as a possible mechanism, but this whole school is completely neglected in Goldstein's discussion What the notion of 'spontaneous remission' maintains is essentially that the remission occurs without benefit of explicitly formulated and planned psychotherapy on the part of a trained psychotherapist, by using his own notions of what might be meant by spontaneous remission, and by disregarding the accepted usage, Goldstein manages to build up a man of straw which he then demolishes amidst universal merriment It is not obvious what useful purpose is served by this exercise

Snyder's book rouses one's worst fears, indeed, in some ways it had better be called a non-book Recent developments in American psychology had led one to a nightmare of patients' outpourings under psychotherapy being taken down on a tape recorder, typed out by a secretary and immediately rushed to the publishers without intervention of any form of critical reasoning This book approaches the ideal although not quite reaching it, the two cases recorded are edited rather than being quoted in full, but nine-tenths of the book is still made up of the tape recordings as they came from interview They are meant to illustrate the principle of 'dependency' in psychotherapy which I myself found too nebulous to state in brief, their purpose, apart from acting as an illustration, appears to be to help in training future psychotherapists It is difficult to see what they can possibly learn from this agglutination of verbiage, or what relationship, however tentative, there can be between what apparently goes on in this 'dependency' relationship and science Strictly speaking this book cannot be reviewed at all, not being a book in the ordinary sense, it will presumably be studied and acted on by Snyder's students, but it is unlikely that anyone else would want to read it or derive any benefit from doing so There is, it should be added, a brief introduction dealing with some research accounts in this field, but these do not enlighten at least one reader as to the meaning and usefulness of this book H J EYSENCK

NUCLEAR INSTRUMENTATION

Proceedings of the Symposium on Nuclear Instruments, Harwell, September 1961

Edited by J B Birks Pp vii+251 (London· Heywood and Co , Ltd , 1962) 60s

Nuclear Instruments and Their Uses

Vol 1 Ionization Detectors, Scintillators, Cerenkov Counters, Amplifiers Assay, Dosimetry, Health Physics (National Academy of Sciences—National Research Council) Edited by Arthur H Snell Pp 494. (New York and London John Wiley and Sons, Inc , 1962) 57s

WE have moved a long way from the ionization chambers and Geiger counters which were used with the 37-in cyclotron at the time of Rutherford's death in 1937 Progress has been far more rapid in the sophistication of the experiment and the saving of the physicist's time than in almost any other branch of human affairs, and the meeting in Harwell which succeeded the Rutherford Conference reviewed the armoury of techniques now available for a renewed attack on the outstanding problems of nuclear physics There were three main sessions two on particle recording devices, operating either by the light emitted or, in the case of the semiconductor, by the conversion of particle energy into an electrical pulse, the third session was on recording of complex data

Prof McGee described the construction and output of photo-electric image intensifiers to record the faint light emitted by high-energy particles passing through scintillation chambers, some show a light gain of a thousand, some have been developed as gates shutting in a microsecond, phosphors may be chosen to store for a microsecond or a minute, but these devices are at present restricted to a few special experiments—a typical use is given in the recording of weak interactions of mesons There is some useful information on the statistical properties of scintillation counters, the time taken to deliver a signal from one photo-electron, and the total time of operation of the photo-multiplier Recently liquid helium has been shown to be a good scintillator, emitting light in the far ultra-violet which is not seriously attenuated in the liquid and may be absorbed by a phenyl to give a visible light pulse

The semiconductor particle counters are being developed at high speed, but the Conference did not devote much time to descriptions of these, one paper discusses the effect of imperfections of a liquid-air-cooled silicon crystal on the current pulses released by fast ions passing through the crystal in different directions, but energy resolution can be disappointingly small Very thin counters can be used to distinguish between the ionization of particles of different mass but of the same total energy before they enter a detector to measure their total energy Neutron counters depending on neutron induced reactions in the counter have been developed in Harwell, they can be so compact that they cause little modification to the neutron flux, the proton recoil counter has a polythene layer in contact with a silicon surface barrier detector, the helium-3 counter is a gas layer of helium between two silicon detectors

There are several papers on the spark discharge chambers used for tracking multiple events arising from cosmic rays or high-energy particle accelerators, one simple application is as a speedy check on the exact location and shape of the beam emerging from a machine operating at hundreds of cycles per second High efficiency of discharging is essential if a multiplicity of events is to be recorded over a large volume, yet spurious sparking must be suppressed Neon/helium mixtures have been shown to be the best with methane to quench the discharges, and these have an efficiency of nearly 100 per cent and are markedly superior to the earlier argon-filled chambers For work involving merely deflexion in a magnetic field thin-plate electrodes are used with a $\frac{1}{4}$-in spacing, but absorption experiments may be performed with thicker plates of various elements There seems to be a difference in experience with chambers sealed, or flushed continuously with gas

The third and fourth sessions were devoted to improvements in the recording of complex electrical signals derived from the detection of nuclear events Three papers from Saclay review the conditions to be satisfied in setting up automatic experiments in nuclear physics, and extreme reliability of components is emphasized with repeated checks on the operating accuracy of the computors used An ingenious improvement in the storage of information on a magnetic tape by the use of a multitrack recording head was described and papers were given on sophisticated multi-channel pulse height analysers

The second book is a really comprehensive text-book on nuclear instruments compiled by seven authors, mainly from the Oak Ridge Laboratory, and each chapter is extremely well supported by bibliographical references so that the book will serve the advanced student and also the research scientist very satisfactorily Ionization chambers proportional counters, Cerenkov counters and scintillation counters are described in great detail with full accounts of the theoretical considerations on which they are based, and these chapters are followed by a detailed account of the construction and operation of electrometers and amplifiers The last three chapters concern the measurement of radioactivity, dosimetry and personnel monitoring, chapters invaluable to the scientists in many disciplines using trace elements and responsible for the safety and health of operatives in laboratories, factories, or at nuclear power stations The seven authors and the editor have generously contributed their efforts on a royalty-free basis, and the 500-page book is commendably cheap for the wealth of information it contains

The fundamental processes involved in creating ionization in gases by fast and slow electrons and charged atoms are described in detail in the opening chapters, with attention directed specially to the importance of gas purity if true distinctions are to be drawn between ionization in different gases Fast counters only collect the electrons produced in the gas. ignoring the positive and negative ions which have far lower mobility, so gas purity is also important to avoid electron capture by electro-negative gases, a full account is given of the factors including geometric which affect electron collection The importance of purity is enhanced in chambers operating at high pressure, for example for γ-ray absorption experiments, and details are given of methods to purify the gases commonly used The proportional counter which operates by amplification of primary ionization in the high field surrounding the collector electrode has found its greatest application in recording quanta of low energy, for example from 250 eV upwards, and the chapter discusses resolution, proportionality and calibration of counters operating on this principle

The application of the scintillation counter to the efficient detection and spectroscopy of nuclear radiations arises because charged and uncharged particles and gamma-rays having a wide range of energies can be wholly absorbed in the scintillator and the light output is nearly proportional to the absorbed energy Moreover, the light is emitted in an extremely short time, so resolutions in the millimicrosecond range can be obtained Brief accounts of the growing of inorganic crystals are given together with the scintillation properties of the most important with their activators, why some halides are efficient scintillators and others are not is still a mystery Organic crystals of the aromatic hydro-carbons are efficient scintillators if very pure and plastic scintillators can be made by dissolving in polystyrene made from the very pure monomer freed from dissolved air· as they can readily be made very large they are efficient detectors of fast neutrons A second solute may be used to convert the fluorescent radiation to a spectrum more acceptable to the photo-cathode utilized in the multiplier For very large volume detectors for very fast particles organic liquids may be used, the solvent acting as the stopping medium, the solute, the fluorescent or wave-length shifter Extreme purification is necessary, oxygen must be removed for reasons not fully understood Many solutes have been tried and the solvent may be loaded with boron or cadmium salts if neutron detection is required or with heavy elements for γ-ray detection

The latest addition to the scintillation detector is the family of noble gases, but they need to be very pure and then to be mixed with wave-length shifters Their use is specially for the heavier charged particles, the light output per unit energy being insensitive to the charge or mass of the particle stopped in the gas

The optical coupling between scintillator and photomultiplier tubes is briefly treated, together with characteristics of many tubes, and there is a long and informative account of many types of experiments using these counters

Cerenkov counters find their application in detecting particles of extremely high energy They use the shock wave emitted when particles travel through them with speeds higher than the velocity of light in them, and the angle of emission depends on β n (refractive index, *n*) They therefore at once discard all particles below a threshold velocity, and typical of their use is the detection of mesons in a high background flux of neutrons and knock-on protons Focusing and non-focusing types of detectors are well described, transparent liquefied gases. and water may be used to record electrons from 300 keV upwards and heavier particles in the multi-MeV range Wave-length shifters may be used, and then the light emission is isotropic A careful analysis of the efficiency of collection of light in the counter concludes an invaluable chapter

The chapter on d c measurement of the outputs from ionization chambers and the pulse amplification for spectral analysis of the output from the many counters is very comprehensive, advanced theoretical analysis is coupled with many practical details to be observed in the choice and assembly of components

The last three chapters deal with the techniques used in radiation measurements and will be of value to a wide range of users who may not necessarily be specially concerned with the physical principles of particle detection and the circuits of recording devices, other physicochemical methods of radiation measurement are not dealt with β-Counting is dealt with first, as the many disturbing factors such as absorption. back reflexion, sample covering and geometry are perhaps more important than for α or γ-rays The application of the 4-π counting technique has been greatly enhanced by the crystal and liquid scintillation counters, as samples may—in certain cases—be placed in the scintillator or in a cavity within it The points needing extreme care for absolute measurements are most clearly stated with adequate references to original work where the greatest precision is expounded For α-counting the problems are similar, though less onerous than for β- and for γ-counting the scintillation method is rapidly replacing all others due to its high (nearly 100 per cent) efficiency There are important corrections to be made for geometry and γ-escape, and the 'total intrinsic efficiency' of a scintillation counter is exhaustively treated

The chapter on dosimetry is confined to fundamental concepts and present trends when dealing with physically (physiologically) significant doses, and in Chapter 7 most explicit details and instructions are given for people working with sources of and in excess of microcuries There is a short historical account of the way the 'safe' exposure dose has been arrived at over the past thirty years culminating in the 1959 recommendations for operatives exposed to different kinds of radiations, it is good to note that the author and most health physicists dealing with people continuously employed in a radiation field divide even these conservative figures by a further ten The improved film meter is advocated for personnel monitoring, and many instruments for surveying are described General guidance is given for waste disposal and accidents The health physicist—a new and rapidly growing profession—needs to know every word of this account T E Allibone

WORKING UNDER PRESSURE

Solids Under Pressure

Edited by William Paul and Douglas M. Warschauer (McGraw-Hill Series in Materials Science and Engineering) Pp xvii + 478 (New York McGraw-Hill Book Company, Inc ; London McGraw-Hill Publishing Company, Ltd , 1963) 116*s* 6*d*

A WIDE variety of investigations, which have been conducted at high pressures, is reviewed in this book, but descriptions of high-pressure techniques are excluded almost entirely—apart from a special bibliography at the end

Having chosen a connecting thread, the editors would seem to have pulled it, but the result is a book which is unusually easy and entertaining to read This is in part due to the fact that, with a new parameter to be varied, the simplest properties of the simplest solids are the first objects of exploration The bases of familiar theoretical models are, therefore, probed afresh, and most of the chapters in this book involve re-statements of these basic models from slightly unfamiliar points of view (hence the entertainment)

My guess is that many physicists have the notion that the results of measurements at high pressures are only rarely of much significance, and that the experiments themselves are always of the brute-force varieties This book would dispel such views There are great difficulties in the way of achieving hydrostatic conditions at pressures in the megabar range, but up to 30 kilobars there is standard and not too elaborate equipment now available and measurements of high resolution and of several different kinds can be made The same is true of pressures up to 100 kilobars in specialist laboratories Pressure might not rival temperature as a parameter, the variation of which can produce large and significant changes in the behaviours of solids, but it is clearly catching up This is a timely situation, because there is an increasing need to separate those parts of a change in behaviour with temperature which are explicitly temperature-dependent, on one hand, and implicitly temperature-dependent (for example, via lattice expansion) on the other Ancillary measurements at high pressures make this possible and there are several examples in the book The present-day theoretical concern with interactions between quasi-particles (lattice-waves and spin-waves in particular) makes this separation particularly desirable

Eighteen authors contribute fourteen chapters on equilibrium, transport and activation properties, and phase transitions, involving descriptions of optical, magnetic elastic, thermal and other measurements The whole book is addressed as a tribute to Prof Bridgman, whose death regrettably preceded the publication He contributed the introductory chapter, and the rest of the book illustrates the first steps in his vision of the future of high-pressure research as a progressive delocalization of electrons in successive atomic shells

D H Martin

TEXTILE PHYSICS

Physical Properties of Textile Fibres

By Prof W E Morton and Dr J W S Hearle Pp xxi + 608 (Manchester The Textile Institute, London Butterworth and Co (Publishers), Ltd., 1962) 105*s*

A COMPENDIUM of information, dealing with a subject wide enough for no one to be master of all of it, tends to be received with opposite comments according to the self-control of the reader He is tempted to look first at the part dealing with his own speciality The result will be a criticism—none of us approve of encyclopædia articles on our own subjects (unless, indeed, we have written them ourselves) But this is to misuse the book, its proper role is to help us to look up other people's subjects as may be necessary, even if superficially, so that we do not have to pester our colleagues Who, then, could safely write a book like this ? Obviously a teacher, for he alone in our sadly specialized world has to maintain more than a passing interest in all branches of his subject *The Physical Properties of Textile Fibres* is just such a book, and it is fittingly written by a professor and a senior lecturer at the Manchester College of Science and Technology It made a good first impression, and was accordingly subjected to the stringent test of being lent to various colleagues of mine with a request for their opinions on parts dealing with their own topics In no case was there an adverse report—this is high praise

After an introductory chapter on fibre structure, which may be regarded as setting the background for the rest of the book, there is a long section on fibre dimensions and their measurement, followed by sections on density, moisture relations and effects, mechanical, electrical, optical and thermal properties, and, finally, one on friction Throughout, the treatment preserves a good balance between methods of measurement, results specific to particular fibres, and discussion of their meaning The accounts of methods are critical where required, and attention is given to that especial plague of textile physics —variation in results—and how to deal with it The methods of measurement described are not only those used in research laboratories, methods used in industry are given a fair share of attention, though in all cases the emphasis is on principles rather than on practical details (which are available elsewhere)

The book is well supplied with diagrams, and data are presented by graphs rather than tables wherever possible Here arises one of the few criticisms that can be made where data are to be read from graphs in a book, it is convenient if the scale of reproduction is such that the units are simply related to centimetres or inches so that a ruler can be used without paper calculations This is not often done, but it would be a welcome novelty In the present book some 50 per cent of the graphs do comply with this requirement, but the remaining 50 per cent are reproduced just a little too big or too small When the book comes to a well-deserved second edition, may we have these adjusted ?

The lists of references, always an important part of a book like this, are adequate so far as can readily be seen, where faults are usually those of omission

Occasional blemishes occur, for example, at the bottom of p 105 some of the instrumental parts referred to by letter are not present in the appropriate diagram, and there are a few rash statements such as that in the last sentence on p 522, where the authors speak of " the hygroscopic salts which are the most effective anti-static agents . ", but these lapses are few In general, the book can be warmly recommended to all textile physicists who can afford to pay the price P S H Henry

THE CRIPPLED MIND

The Biology of Mental Defect

By Prof L S Penrose Third edition, completely revised with the assistance of J M Berg and Helen Lang-Brown Pp xxiv + 374 + 8 plates (London Sidgwick and Jackson, Ltd , 1963) 42*s* net

THIS book is a new edition of Penrose's classic revised by the author and two of his assistants, J M Berg and Helen Lang-Brown It is an excellent book, and gives the latest knowledge of this difficult subject

Forty years ago mental deficiency was regarded as more or less an impenetrable mystery Not that many felt it was worth while spending their time trying to investigate it Indeed, it was the most disregarded waste-land of a neglected area of medicine Psychiatry was considered unworthy of the orthodox physician's interest and mental deficiency more or less hopeless from the start Imbeciles were herded into vast buildings which were little better than prisons, and idiots put into special hospitals where they were merely kept alive There was practically no attempt to understand the condition, and any idea of treatment or prevention would have aroused ridicule

Naturally, the literature devoted to such a condition was on a similar level Text-books were few and devoted mainly to anecdotal cases on which were built theories based on conjecture These were copied from one text-book to another and dated back to such theorists as Lombroso with his celebrated ideas of 'degeneracy', which was an inherited state and condemned the sufferer not only to a hopeless life but also any progeny he might have to a similar fate Indeed, according to Mott, any children of a defective were due to an even worse end because of his theory of 'anticipation' which implied that once the germ plasm was damaged it tended to deteriorate more and more through the generations Another theory of that time was 'blastophoria', or damage to the germ tissue, put forward so enthusiastically by Forel He believed that this damage was caused by alcohol and similar poisons, in spite of the fact that in many cases there was no particle of evidence that the parents had ever used alcohol or suffered from any form of poisoning

These theories became modified by time, and it was thought that mental defect was due to injury to the brain through trauma or infectious disease This was usually attributed, since it was possible to detect that the child was abnormal at birth, to some intra-uterine condition The alternative view was that defect was caused by 'neuropathic diathesis' which was inherited, and was only a more scientifically sounding name for Lombroso's 'degeneracy

All these theories were blown away by the patient work of Penrose, who produced statistics which shattered them In 1933 he published his book, *Mental Defect,* and suggested that the condition was due to a multiplicity of causes He followed this up with statistical proof in his *Colchester Survey* for the Medical Research Council in 1938 His *Biology of Mental Defect,* published in 1949 and revised in 1953, was soon a classic, and the new edition will be unchallenged In it he reviews the new developments The great discovery that such conditions as mongolism are due to cytological abnormalities, the commonest being the extra chromosome Again the revelation of the biochemical factor, phenylketonuria, is likely to lead to other advances

J B S Haldane, who writes a foreword to the new edition, suggests that these discoveries will finally lead to the elimination of a quarter of the defectives This is true, but the discovery of where to look and the sort of advances which can be made will inevitably lead to other points of progress

One can sum up by saying that this excellent book should be in the possession of every psychiatrist, geneticist and research worker interested in mental defect He will find in the descriptions valuable ideas, and in the forty-one pages of references material for a great deal of future work

The book is well bound in dark blue cloth, has clear print, and a full index Clifford Allen

ARCHÆOLOGY IN THE TRANSVAAL

Prehistory of the Transvaal

A Record of Human Activity. By Revil Mason Pp. xxiv + 498 (257 figures) (Johannesburg Witwatersrand University Press, 1962) R 6; 60*s* , 8 70 dollars

DR MASON'S book is unfortunate in that it is published at a time when inter-disciplinary studies of archæological remains are opening up new possibilities of understanding prehistoric peoples. The application of scientific techniques, such as pollen analysis and radiocarbon dating and the statistical analysis of assemblages of artefacts, are together forming a basis for future studies of the relationship of man to time and environment

It is, therefore, disconcerting to read that in Dr Mason's opinion little reliable work has been done in the Transvaal on phenomena which indicate climatic change and that prehistoric remains are also not likely to provide an accurate reflexion of environment In consequence, environment is discussed in terms of a simple territorial division for descriptive purposes only Elsewhere, he states that it seems doubtful if the radiocarbon dates from the Transvaal can be accepted and also that geological interpretations so far put forward are often unsatisfactory It is, therefore, apparent that this book does not offer the fuller picture of prehistory to which archæologists in recent years have become accustomed and that, by and large, Dr Mason has not been able to apply in full the correctives of available scientific techniques to typological hypotheses

There is, however, an analysis of the artefacts from many sites in the Transvaal For the most part this is a simple numerical analysis of defined tool categories In defining these categories Dr Mason is faced with the dilemma of whether to make them so wide that essential differences may be obscured or so numerous and narrow that they may be ill defined He has chosen the former alternative and on this basis has attempted even an inter-continental comparison between Transvaal sites and two Mousterian sites in France Without geographical limitation or environmental comparison it is doubtful whether or not comparisons based on such a small sample are of value Indeed, simple numerical or metrical analyses of tool types are not likely to be regarded in future as sufficient, in view of the more complex statistical analyses now used in Europe and the United States There is, however, a body of information of use to research workers

There are, in addition, many drawings and photographs of artefacts, some imaginative drawings of life in the Stone Age which may have some popular appeal and which have been executed with attention to the known facts, and some excellent reproductions of rock art There is a good bibliography

On the whole the book may appeal more to the layman than to the professional archæologist, but Dr Mason's resolute refusal to accept ready-made conclusions drawn from evidence which he believes to be inadequate or wrongly interpreted is of value to African archæology, and so are his attempts to solve debatable field problems by direct personal action E S Higgs

La Mesure des Températures au Laboratoire et dans l'Industrie

By M Terny Pp xv+320 (241 figures) (Paris Dunod, 1962) NF 48

THE emphasis in this book is much more on the industrial than on the laboratory problems of temperature measurement. For example, though the interpolation formulæ used for standard resistance thermometers are given, the only thermometers described are industrial types with which these formulæ are not normally used Moreover, it is implied that the potentiometric method is the only way in which an accuracy of 0 001° C can be attained, whereas this method is being steadily displaced by bridge methods in all parts of the world.

The book has much too large a number of errors, ranging from typographical slips to definite mis-statements On p 11, for example, it is stated that the maximum difference between the International Practical Scale and the thermodynamic scale up to temperatures of the order of 500° C is 0 01° C In fact, measurements by different workers with the gas thermometer at 444 6° C show differences ranging from 0·07° C to 0 10° C We are told that the wire method for the determination of melting-points is used for silver and gold in practice, it is used for gold and palladium, and it would certainly be difficult to use it for silver.

From p 9 one infers that the International Scale dates from 1948 (when it was revised) instead of 1927, though the correct date is given on p 117, where the lower limit of the scale is given as −190° C. This was in fact true in 1927, but it was soon found that extrapolation below the oxygen boiling-point was unreliable, and the scale was limited to −182 97° C at the earliest opportunity On p 293 the triple point of benzoic acid is incorrectly given as 112 36° C, while the correct value (122 36° C) appears on p 110, but is ascribed to the melting-point

It is a pity that a book which contains a good deal of information that might be useful to the industrial user of temperature-measuring equipment should have so many errors (those cited here are only a representative sample) that one can have little confidence in it J. A. HALL

Chemical Calculations

An Introduction to the Use of Mathematics in Chemistry

By Prof Sidney W Benson Pp xii+254 Second edition (New York and London John Wiley and Sons Ltd , 1963) 23*s*

CHEMICAL Calculations contains problems covering the Advanced and Scholarship Levels of the General Certificate of Education A large number of these are realistic and interesting; those on atomic and molecular dimensions are particularly attractive and are introduced early in the course—a most valuable idea Unfortunately they are mixed up with mere arithmetical exercises such as: "What is the weight in tons of 120 mg of radium?" Chemistry teachers often find it necessary to teach sixth formers a little arithmetic, but to do it by purchasing books like this would prove very expensive

There are sections dealing with basic mathematical techniques such as using indices, logarithms or obtaining approximations These are quite unnecessary for pupils studying mathematics for Advanced Level examinations, and would prove inadequate for the person who needed to read them A serious attempt is made to help those who have difficulty with the notion of proportionality, which after all is involved in most calculations at this level The rigorous use of units is to be recommended

The laws and theories of the subject are stated clearly and are illustrated by worked examples Laws are often derived and the whole appears to progress in a logical manner Often the derivations are unsatisfactory and there is insufficient stress on the experimental nature of chemical laws

The book seems to fall between two stools On one hand it is a most expensive source of problems, on the other it is a very inadequate physical text

D C FIRTH

Interfacial Phenomena

By Prof. J T. Davies and Prof. E K Rideal. Second edition Pp xiii+480 (New York Academic Press, Inc ; London. Academic Press, Inc (London), Ltd , 1963.) 107*s* 6*d*

THE second edition of this excellent and stimulating work has appeared barely two years after the first (see *Nature*, 193, 408, 1962) this is of itself a striking tribute to its value and importance Changes from the first edition are not great, the sections on circulation in moving drops and on wave-damping have been re-written, and some corrections and clarifications have been made The pagination is practically the same, except for the subject index which has been rearranged and enlarged In the heading of one chapter, on transport of material across interfaces, the word 'diffusion' has been altered to 'mass-transfer'

The great merit of the book is in its clear and original presentation of modern developments, not a few of which are advances made by Davies The electrical properties of interfaces are introduced quite early in the book· the treatment of adsorption at liquid interfaces is largely original There is a very good chapter on chemical reactions at interfaces, a subject very largely due to Rideal and those inspired by him Two topics of much practical importance, to which Davies himself has contributed a great deal, are the movement of material across interfaces, and surface viscosity and its importance in wave-damping All these topics are put into proper perspective against the classical, older parts of surface chemistry, which is concisely and adequately given, though in less detail than in some other books

This book is the result of some forty years' pioneering research by the senior author, and about twenty years' by the junior author it is a very well-considered exposition of much of their work, put into clear relation with the work of many others N. K ADAM

Five Years' Work in Librarianship 1956–1960

Edited by P. H Sewell Pp viii+559 (London The Library Association, 1963) 180*s* , non-members, 135*s* , members

LIKE its predecessor for the years 1951–55, this volume covers developments in librarianship over a period of five years and follows much the same pattern It draws to a large extent on sources not readily accessible, and in the large section on archives almost entirely on material not previously published Moreover, the value of this assessment of developments in Britain during the period derives largely from the standing and experience not only of the contributors but of other librarians who have read and commented on different chapters. As a result, the report is an authoritative as well as a comprehensive and concise source of reference on numerous subjects, and the value of the information thus made accessible is enhanced by full references to each chapter Besides the section on archives already mentioned, there are six others between which there is some, but not excessive, overlapping These deal with national and academic libraries; special libraries; public libraries, library practice and library service; bibliography and documentary reproduction, and the library profession and professional organizations In the final section, separate chapters are now given to the Library Association, to the Association of Special Libraries and Information Bureaux and the International Federation for Documentation, and to international librarianship There are also new chapters on public relations and on the acquisition and use of periodicals in libraries

HISTORICAL RELATIONS OF SCIENCE AND TECHNOLOGY*

By Prof RUPERT HALL

Department of the History of Science and Technology, Imperial College of Science and Technology, University of London

IT is splendid that a great scientific and technological institution, such as the Imperial College of Science and Technology, has found room among the many facets of its expansion for a branch of historical investigation which has been, until lately, too often neglected I am happy also to acknowledge the great contributions to the study of the history of science which have come from our sister College (University College, London) over a period of more than forty years, and recently not only from the ancient universities in Britain but from the more newly founded ones also In Britain there is the nucleus at least of a proper professional body, so that the time for pleading the cause of history of science, and perhaps even for justifying its study, has already passed. Perhaps even in Britain, as already in the United States, the obstacles to the further increase in the number of university posts arise less from failure of demand than from insufficiency of supply

The history of technology, however, is in little better case now than the history of science was half a century ago—indeed, it is rather worse off since it cannot even to-day produce a scholarly eleven equal to that made up by Tannery, Sudhoff, Duhem, Heath and the rest, all at the wicket in the years before 1914 Yet societies have been formed, specialist journals have been founded, and within the walls of the great national museums the work of conservation and research goes steadily forward Here in Britain—with such leading figures as H W Dickinson and his successors—the Science Museum, South Kensington, has carried almost the whole burden of stimulating interest in the history of technology In France the Conservatoire Nationale des Arts et Métiers has been almost equally pre-eminent, and I think the situation has been much the same in Germany and Italy also, as indeed in the United States until very lately Despite spirited efforts to the contrary, this infant subject has not flourished in academic thickets, and in a measure less in Britain than elsewhere, perhaps because here (with the residua of the Industrial Revolution so thick on the ground around) the problems of conservation have seemed especially pressing and the tendency towards antiquarian enthusiasm peculiarly strong Just as Imperial College has its Queen's Tower, so Britain (I suspect) will never lack its last devoted tram-road and consecrated slag-heap The Flemings have Brueghel we have Lowry Not to push the point too hard, let me illuminate it in another way· on the Continent and in the United States small groups of scholars—altogether, admittedly, a very small band of medieval historians, archivists, palæographers and so on—have begun to take a constructive interest in the crucial, though slow, evolution of techniques which occurred in Europe during the Middle Ages It is perhaps strange that the pre-history of Western civilization is to be reconstructed from Lombard charters or the disputed meanings of words of obscure etymology, but so it is It would be pleasant to see more British scholars joining in this work, since it is necessarily in their province rather than that of the engineer or technologist The economic historians have already done a good deal, especially for modern times, but the particular alliance of interests and skills I am thinking of has scarcely yet occurred in Britain.

Perhaps I should try to explain why this kind of investigation into medieval technology (or one might as well say any technology which prevailed much before 1850) seems to me significant It might be condemned by applying to it any of those pejorative epithets which are attached to activities neither obviously utilitarian nor flattering to the speaker's particular tastes dull, esoteric, academic and so forth. Yet I think that it is broadly agreed that universities should provide something more than courses in survival, if life-jackets are to be the only wear and the only music that of the alarm-bell, then we should indeed end all frivolities That was the attitude of the medieval university But I presuppose a society which has leisure, surplus production, and free intellectual energy in such a society, it seems to me, at least the naive question, "How did things come to be as they are ?" is natural, widespread, and of general interest among men It is answered in a variety of ways, including the mythical, the poetic, and the religious scrupulous historical enquiry is that way which most closely resembles the scientific exploration of the natural world It is difference in logical structure, not (as is commonly supposed) difference in the methods used for the establishment of facts, which causes the expression of historical enquiry to be distinct from the expression of scientific enquiry, it is this (for example) that renders the discussion of historical 'laws' very different from that of scientific laws But this contrast is one which I need not pursue

Perhaps it is still worth emphasizing again that, if necessity is the mother of invention and altruism its maternal aunt, nevertheless the social conscience by itself has not proved a powerful spur to creative innovation. Pity may impel a man into medicine or inspire a humanitarian invention, but in general the creative faculty has been, so to speak, more highly introverted than extroverted Whatever may be the case now—and I do not really believe it to be different—certainly in the past the feelings in this respect of a literary investigator were not very different from those of a scientific research worker You may recall G H Hardy's observation that "A physiologist may indeed be glad to remember that his work will benefit mankind, but the motives which provide the force and inspiration for it are indistinguishable from those of a classical scholar or a mathematician" To which I would add, "or a historian" and I would further say that (setting aside the direct usefulness of applied science) Hardy's point can be extended to the appreciation of the work of physiologists and classical scholars, mathematicians and historians That is, the ultimate private intellectual satisfactions—disregarding altruism, which of course has no necessary nexus with the intellect at all—have profound common qualities, for the intellectual satisfaction of understanding something, of having intellectual command over something, is much the same whether the thing be differential equations, or the Romantic Movement, or Norman feudalism I take it to be in part the object of life (after the needs of survival have been met) to enlarge and enrich in a kind of Benthamite calculus the sum of intellectual satisfactions, and this has traditionally been considered a major function of education I have no wish to involve myself in a discussion of the philosophy of education, and so I shall simply assume that the traditional view is a just one, and if it is, then I think it follows that an interest in our past, in all its manifold aspects, is proper and deserving of encouragement

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* Substance of the Inaugural Lecture delivered at the Imperial College of Science and Technology on November 19 (*Nature*, **196**, 319, 1962)

To be more specific, and to return to my point about medieval technology, its fascination is that of potentiality. It is possible—though I do not think this has yet been proved—that we can push back to a very early stage in the evolution of European society after the collapse of the Roman Empire (which was a Mediterranean civilization) those changes in both the quality and quantity of technological innovation that were to prove decisive notably, of course, the effective use of power It is a question of discerning whether a growth-curve is linear or exponential If early medieval technological development possessed only linear characteristics it is not terribly exciting If it possessed an exponential quality as the origin of the curve that was to shoot almost vertically upwards in the nineteenth century, then the technology of the early Middle Ages truly contains the historical mainspring of our own world, and the origin of the challenging events of our own time

The history of technology, like the history of science, is a study so multifarious in its demands that the proportion of scholarly to technical or scientific content is almost infinitely variable I have just mentioned an area where the scholarly skills are much in demand, but modern scientific and technical knowledge less so For in historical studies of the period before the past two centuries the 'language barrier' is as important as the 'mathematics barrier' in modern education Not to mention the most ancient languages in which the beginnings of all civilization are shrouded, nor the languages through which alone the oriental civilizations may be reached, nor Greek, there is the obstacle of Latin The Roman tongue, of course, was never nearly so important, intellectually speaking, as it became after Latin had ceased to be a living language The last major scientific contribution to be described in Latin which springs readily to mind is that of Karl Ernst von Baer, the discovery of the mammalian ovum, announced in 1827 There may be others of later date yet, and Latin certainly remains, to-day, a feeble vehicle of scholarly communication In spite of the immense volume of translation into English and other modern vernaculars, he who is ignorant of Latin must still see the thought of the past darkly and partially only the Babel of the past two hundred years is accessible to him This is the result of that eclipse of Latin as a universal language which the seventeenth century lamented and the growth of science, I fear, accelerated When the first *History of the Royal Society*, compiled by Thomas Sprat seven years after its foundation, was presented to Cardinal Leopold dei Medici in Florence, he complained (as did nearly all Continental scholars) because it was printed in English, to him a barbarous and unknown tongue But there was a ready retort to hand Leopold himself had permitted the *Saggi di Naturale Esperienze* of his own Accademia del Cimento to go abroad in Italian only a few months before As with us all, so with the French too they disliked the publication of the *Philosophical Transactions* in English, but they themselves, in their new Royal Academy of Science, adopted the vernacular

The story of the first phase in the battle between man and Nature, of course, is not recorded in language at all, the archæologists, with all the force of the scientific aids they can muster, must reconstruct it for us The story of the second phase is dominated linguistically by Latin, and operationally by the techniques of historical scholarship. The transition from the end of the second phase into the beginning of the third, long as two or three hundred years seem to us, has been brief on this historical time-scale Since we ourselves have but just now embarked as protagonists on phase three it is impossible to say how its history will be related We can only hope that the archæological methods used for the first phase may prove unnecessary, as we may guess that the scholarly ones used for the second phase will seem inappropriate For those terrifying statistics about the 90 per cent of all scientists being those now living, and so forth, will render that virtually certain

Historians of science and technology have so far been chiefly concerned with the second phase, and with the earlier part of the transition period signalized by the 'scientific revolution' of the seventeenth century The latter stages of this transition in the nineteenth century and the 'scientific revolution' in Sir Charles Snow's sense (introducing the third phase in the twentieth century) they have so far done little to explore It is easy to see why when we reflect that the kind of problem which has interested such historians into very recent times was actually determined about the end of the nineteenth and the opening of the present century I do not mean that there has not been great change in methods and ideas, and that in detail the modern academic conspectus of the history of science is not altogether different from that prevailing about 1890 but the overall shape is much the same, and indeed what we are doing now is built on what was done then, and that in turn was conditioned, I believe, by late-Victorian optimism and security, in the world of the solid atom, the steam-engine and (curiously enough) the horse

Although Wells and a few others foresaw the catastrophic potentialities latent in scientific and technological development, historians took a complacently progressive view Despite episodic retrogressions, sometimes lasting for centuries, the course of human history in the West seemed to show neither a Fall from a Golden Age (as men had once supposed), nor that stagnation which was attributed to the oriental empires, but, by and large, a steady increase in verifiable (or positive) knowledge, and constant enlargement of man's dominion over his environment What the seventeenth century had aspired to, through the mouth of Bacon and others, the late nineteenth century European had at last secured Not a few scientists of stature proclaimed that no more momentous discoveries were to be expected, historians on the whole concurred in regarding the views of their own times as the truths to which their predecessors had approximated It is needless to say that the same anticataclysmic attitude prevailed in the study of the history of technology the steam-turbine was simply a wonderfully more efficient Watt engine as the Watt engine in its time had been a wonderfully more efficient horse No one—or very few—who admired the work of Parsons and Edison and the rest regarded even the social forms of their own society as being under severe pressure

All that has been changed Speculation about what was progress and what was retrogression has been overwhelmed by the fact of change The secure world of the Victorian historian has disappeared He could tolerate—though I do not think he quite understood—the swiftness of scientific evolution in the seventeenth century because it could be accommodated the notions of Aristotle, Ptolemy and Galen were 'wrong', and the contradictory notions of Galileo, Copernicus and Harvey were 'right' With false truths dethroned, the enthronement of truth occurred once for all But it was impossible to repeat this—or at least it became very repugnant to repeat this—when it became a question of declaring that Galileo and Newton were wrong because Planck and Einstein were right For to admit this was to destroy the conception of truth's progressive revelation and the idea of complete developmental consistency

As my friend Tom Kuhn* has considered this matter of small jumps and big leaps in the history of ideas, I need not pursue it further I want to point out that there are now a few people—a very few—who are getting to grips with the start of the third phase, at least so far as pure science is concerned This means tackling, very directly, this problem of intellectual quantum-jumps on one hand and their immense cultural repercussions on the other For technology the beginning has still to be made, and should be made while change is still vivid in the minds of many men I think it important that the beginnings made, or to be made, should be expanded by many willing hands, for exactly the same reason that I believe political or social historians should interpret for us not only the fall of Troy or the wool-trade of medieval England, but also the events that middle-aged people like myself never properly understood and have already half-forgotten I hope something of that will be done in Imperial College, itself a product of the nascent third phase in the natural history of man

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* Kuhn, T S , *The Structure of Scientific Revolutions* (Chicago, 1962)

But I must confess that, as an ingrained historian of the great transition, the story of this present scientific revolution is beyond me and I must leave it to younger men I have found some incursions into the first scientific revolution, that of the seventeenth century, sufficiently absorbing and complex I am not sure that on the purely intellectual plane (leaving aside the impact of science on technology and hence on society) there are not significant parallels between them For example, the Pascal syndrome—the reluctance to depart from an ordered, familiar world of the intellect to one that appears inchoate and strange—was apparent after Copernicus as after Einstein The shift from macrophysical modes of explanation to microphysical ones was a feature of the seventeenth century as of the twentieth The assertion that a new science demanded a new philosophy was common to both, and so on not least, there is to-day a revival of that appeal to a technological social conscience which was a marked feature of the seventeenth century I hope this evokes no absurd notion of 'history repeating itself'. nothing is further from my mind, what I mean to indicate is simply that the kind of intellectual gear-changing which was so constructive (and destructive!) three hundred years ago has analogies of form—not of content—with what has happened more recently Certainly—as I have already hinted—I believe the twentieth century revolution to be enormously helpful in understanding the seventeenth

In this earlier period my own chief interests have lain I had the advantage of setting to work under one of the finest English historians of that period, Sir George Clark, investigating the then quasi-mathematical science of ballistics and the interesting case of interaction between theory and craft that its early development presents I was seeking to vindicate neither the contention that the scientific revolution was the result of a drive for technical mastery, nor the opposed opinion that science is purely an intellectual game for scientists, the more beautiful as it is the more useless, this rivalry of argument was not my main concern Nevertheless, I learned something about this debate (to which I have again returned quite recently), which was a matter of warm concern until about twenty years ago, but has died down since Other issues have come to the forefront, rightly so, I think, because the attempt to assess the relations of science and technology in the seventeenth century on the basis of a still inadequate examination of the inward history of both the sciences and the techniques was necessarily premature

Historians are allowed, and indeed encouraged, to be a good deal less strict in their allegiance to one type of enquiry than scientists I have ranged fairly widely in the matters I have looked into here and there over the whole period from the early sixteenth to the early eighteenth century, with a fairly strong concentration on the English scientists associated with the first years of the Royal Society May I just say here how generous the officers of that venerable institution are to all who delve into their superb collections of books and manuscripts? My wife and I together have done a fair amount of work on the greatest of all that group, Isaac Newton, who is at last receiving the attention he deserves, rather than the praise which was much easier to give, and yet—if I may digress again—it is astonishing how little national pride we seem to have in the Englishmen who belong to all humanity except, apparently, to their fellow-countrymen

Perhaps I can again illustrate what all this is about by dwelling on Newtonian studies for a moment Into Laplace's time, ± 1800, Newton's was still a living influence much (one might say, because time moves faster now) as Bohr's is with us His printed works, but little more, were piously collected in one edition by Bishop Horsley a decent half-century or so after Newton's death—a Latin edition, of course Although the Bernoullis, and Leibniz, and Euler, not to say Laplace himself, had found some pretty weak spots in the Newtonian fabric, it was only the work of Young and Fresnel in optics that really upset a major piece of Newton's scientific work This fact won general recognition at a time when there was, for other reasons, a rather wide interest in the process by which the tremendous changes in thought between (say) Leonardo's day and Newton's had been brought about Some people, believing that early nineteenth-century science had lost this pristine vigour, sought to discover its origins Naturally, Newton was a focus of renewed, critical interest How had he been able to do so much? What was the history of his discoveries, and the nature of his thought? For whatever Wordsworth might say, Newton accompanied is much more interesting than Newton alone One result was the best of our early scientific biographies, Sir David Brewster's life of Newton—an important source-book even to-day

There, essentially, the matter rested until our own time Brewster, with Edleston, Rigaud, and De Morgan, had done much to make Newton a human and intelligible figure, neither a god nor a computor But they had scarcely scratched the surface of the mystery of intellectual power, of which Newton is a supreme and, as it happens, a well-documented example Brewster had been privileged to dip into that great mass of Newton's personal papers which had passed through the husband of his niece to the noble family of Portsmouth By a generous gift, the then Earl presented the scientific portion of the Portsmouth Collection to the University of Cambridge in 1872 A Cambridge mathematician and historian of mathematics, W W. Rouse Ball, was the first to make use of this open treasure in a little book that is still valuable, after that, I regret to say, it was almost totally neglected until some twenty-five years ago For long no writers on Newton attempted to go beyond his works and other printed sources Unfortunately, just as this position was about to change—and the event indeed stimulated the change—that part of the Portsmouth Collection which the family had retained was put up for sale by auction and dispersed That was in 1936 Only the energetic personal action of Lord Keynes prevented a complete tragedy by securing much for Newton's University The loss was the greater because the dispersed papers, though indeed containing little enough relating immediately to Newton's achievements in science and mathematics were capable of throwing much light on his mind and thought Fragments in Newton's hand now command high prices on the open market, and they will never again be reassembled for study

Meanwhile the intact portion of Newton's documentary legacy preserved at Cambridge has been subjected in the past few years to very intensive examination it has proved full of riches We can now know Newton's thoughts more intimately than any of his contemporaries knew them, and discern his personality with a clarity obscured by the rival partisanships of his friends and enemies We can see, as those could not for whom Newton was a miracle, more precisely what he owed to the giants on whose shoulders he stood, and how his own thought was interwoven with that of his age We can see, too, and perhaps most fascinating of all, a good deal of the development of Newton as a scientist from his very early twenties, as a student, to his last defences against the ghost of

Leibniz. There are still many gaps and problems, but it is possible to begin to reconstruct—as only study of copious and intimate manuscript material permits a historian to reconstruct—the movement of Newton's mind from his reading in Galileo, Descartes, Boyle and so on to the brilliant but partial truths of 1665–66 when he was "in the prime of his age for invention" and so to the full maturity of his major writings.

What is the historian after in dealing with such materials, both printed and manuscript? Certainly not just the human story of a life, fascinating though this may often prove to be. In the first place, he is after information; at this level he can share the satisfaction of a biologist in the discovery of a new species or that of a chemist in recognizing the intermediate steps in a reaction; the more information the more complete the picture, and the greater the verifiability of interpretation. But, above all, the historian of science and technology, like the research scientist, seeks for order or logic among the facts. He seeks to frame an intellectual scheme which is equivalent to, but less formal than, a theory in science. The object of such a scheme is to reduce apparent paradox, to show that the seemingly incompatible is compatible, and to relate the previously disparate. What did Newton mean by condemning hypotheses, when he himself made statements that he qualified as hypotheses? How could Newtonian space be a vacuum and yet action at a distance be a manifest absurdity? How did Newton come to be the author of both the *Principia* and the *Observations on the Prophecies of Daniel and John*? This is the kind of question that immediately suggests itself. Clearly the perfect scheme resolving these and other problems must be logically consistent, all-inclusive, and sequentially exact; it will remove out of the arbitrary realm and into the pattern of order what Newton pronounced here, the error he committed there. The essence of pattern, of course, is that it extends this side and that in space, or forwards and backwards in time; if it is true, as Karl Popper has taught, that explanation and prediction are logically equivalent, then perhaps I may add that one has a sense of historical understanding—the desideratum of historical enquiry—precisely when one achieves this Janus-like unity of the forward and backward view. That is, when we take our stand (say) at the year 1680 and see, on one hand, Newton's early thoughts as entailing his future achievements, and on the other these great achievements as no less accounted for by his early intellectual development.

No doubt history will never attain such perfection—not for Newton, nor for Galileo, nor Lavoisier, nor Darwin, to mention only a few of the giants intensively examined in recent times; and perhaps I seem to be expressing too personalist a view of history. I confess that to me it does seem that in the history of science and technology the history of men and their activities must figure largely; the anonymous idea yielded by a fictitious 'spirit of the age' is pretty much an excuse for inadequate research. One can usually find someone who pushed it. Of course, the man whose idea enthralls posterity may, like Mendel, have seemed dim to his contemporaries (though this is rare enough); but it is broadly true that to trace an idea, an experiment or a technique one must discover and comprehend the relevant individuals.

There is, however, another side to this coin. The fate of ideas and methods is no less interesting than their early evolution. Hence it is instructive to put the giants in their local settings: to discover the significance of Newton's philosophical naivety as Leibniz saw it, or how it was that Johann Bernoulli could accuse Newton of not knowing how to take a second differential. This is a type of case that is especially interesting just because one cannot simply say that men like Leibniz or Bernoulli were unable to adjust their conservative habits of thought to Newton's newer views—as we might at first feel inclined to say of Priestley and Cavendish with respect to Lavoisier—because it was rather the case that, to his opponents, it was Newton who was insufficiently *avant-garde*. This is the sort of situation which forces one to look particularly closely at both the historical and the logical relationships between ideas.

Gravitation furnishes but one example of the confrontation of an idea and a universally prevailing conviction: it would be easy to instance others. I should emphasize that in such confrontations the prevailing conviction is not just an opinion, however dignified—like one's preference, say, for neo-Georgian as against Bauhaus, or Bartok against Beethoven—but a psychological rock. Max Planck said that opponents of scientific innovation were never converted: they merely died. For Darwin, acknowledging his belief in evolution was like confessing a murder. So much of one's intellectual make-up, one's ego, can be involved in the falsehood of one idea, or the rectitude of another.

In a sense we may call this a social phenomenon, for it is the community through its educational system, its religion, and other more subtle influences that conditions the degree of flexibility or rigidity that governs a given area of scientific discussion, and moulds attitudes towards it. By 'community' I mean here essentially the scientific community, though one would sometimes need to take the word in the widest sense, too. So that if one side of the historical coin is the genetic evolution of scientific ideas, as I have already mentioned, this other side involves the sounding and surveying of the scientific community to see what sort of a culture it is in which the ferment of new ideas works. Perhaps I may just mention the activity which we are pursuing here at this level, namely, the editing of the correspondence of Henry Oldenburg, the active secretary of the Royal Society from its origins in 1660 until his death in 1677. Oldenburg was in touch with an immensely wide range of correspondents from the most sophisticated scientists like Newton or Malpighi to bucolic doctors and rural deans. In this great collection of letters one can see not only how science lived from day to day and year to year, but also who the men were by whose efforts science lived. Here, too, as in the study of such a giant as Newton, through his manuscripts, it seems to me that the adequacy of the whole picture depends on the sharpness of microscopic images. We hope that when our work on Oldenburg is finished we shall have done something to increase the resolution of our picture of the early Royal Society in action, of the relations between British and Continental science, and of the scientific movement in Britain. But I do not wish to dwell on this so much as to direct attention to the even greater relevance of this kind of treatment to the early progress of technology.

It is easy to overlook the smallness of the scientific community in the past. In seventeenth-century England the average number of men elected in each year to the College of Physicians was five or six; one more physician on the average took a degree abroad each year. Thus the number of men living in and about London with a keen, active interest in the medical sciences can never have been much more than a score, a number so small that each could be intimately acquainted with the others. It is doubtful whether, in Newton's day, the number of competent mathematicians in the whole country was so large as that, and certainly there were not half as many capable astronomers. I am conscious of the relatively large numbers of amateurs and of those who (like opticians or distillers) practised empirical science in a commercial way: yet, in fact, the average number of elections to the Royal Society before 1700 (neglecting the 115 original Fellows) was only about ten, and so on. That means that the impact of any question of abstract science on a human brain was exceptionally infrequent—it could only happen to, say, one individual in a hundred thousand. It is therefore easy for the historian to know this tiny fraction of the population quite intimately—

better than one can know, say, the composition of the contemporary House of Commons But in the history of technology the situation is very different, the proportion of human beings who have been very well acquainted with hand-mills and ploughs and textile- and horse-gear has always been very large indeed, until the last century or so in the West Very few of these ever effected the slightest variation in any technique but the potentiality for effecting a variation was virtually universal It is only when the use of relatively uncommon machines or techniques is introduced that the potentiality for innovation becomes restricted Moreover, we have not much reason to believe that in the early stages, at any rate, learning or literacy had anything to do with it, on the contrary, it seems likely that virtually all the basic techniques of civilization up to a couple of hundred years ago were the work of men as uneducated as they are anonymous. Moreover, while it is one thing to examine, say, the reaction of a small group of medical men to the notion of the blood's circulation, it is quite a different thing to investigate the attitude of a whole population to a new plough, or a new military technique, on which, nevertheless, its economic or political survival may depend Hence, to choose yet another example from our own continent, it is often difficult to determine whether delay in change is due to ignorance or inertia Were the Saxons defeated at Hastings because they just had no knowledge of the new continental ways of fighting, or because they had obstinately refused to practise them ? Without wishing to be utilitarian, it is obvious that discussion of this kind of issue is by no means lacking in contemporary relevance we certainly do not know much about the factors that cause a society either to make good use of its technical potential or to neglect it History cannot solve such problems as these, but it can make us better informed

I shall conclude with a question "What is the role of historical studies in a scientific and technological college ?" The Robbins Report expresses the opinion that such colleges should develop "related subjects such as social studies, operational research and statistics on a significant scale, and languages will be needed at least as auxiliary subjects" The Report does not firmly pronounce on the much-debated question of recent years, whether or not steps should be taken to break down the segregation between arts and sciences that is so typical of first-degree work in British universities as it is untypical in the United States Nevertheless, its view is warmly in favour of greater educational breadth than we now have, and it specifically mentions history of science as an appropriate combining subject Again, I avoid deliberately opening that issue, though perhaps I may state my personal view that early specialization either way is indeed productive of intellectual sterility I remember a good many natural science students from Cambridge days who found history and philosophy of science a relief from memory-loading, and for whom discussion of ideas was a revolutionary experience The sheer expansion of numbers now approved will itself enforce a change in methods of teaching—in the schools as well as in the colleges—and a slowing down of the tempo The alpha-plus student, given books, can virtually teach himself or herself any subject, large numbers will demand the meticulously worked-out, highly-organized and very thorough course-work that is commonplace in the United States but not in Britain—at any rate, it was not a few years ago No one dare predict, but it is on the cards that when this happens the pair of contradictory arguments one has so often heard—according to which students of science (1) acquire culture by osmosis and (2) have no time for anything but science—will disappear at the same moment

In any event, what may happen here in the future will be governed by future discussion Will Imperial College, like the Massachusetts Institute of Technology, develop its department of humanities ? Experience in the United States has shown that attempts to force culture on those who are straightforwardly eager to secure a professional qualification and embark on practice are not very successful For my part, knowing my own limitations, I make no pretence of being a cultural physician, and I boast no desire to serve as the spoon that administers some unwelcome dose As for bridge-building, I would rather leave that to the civil engineers On the other hand, I believe history of science, and history of technology and philosophy, too, have a large role waiting for them in the more liberal scheme of British education that I hope will come, and at all levels, in schools, in undergraduate work, and in postgraduate study History, whether of science or of industry, seems to me like the history of politics, of society or of literature, part of our common culture Newton, Tolstoy, Renoir, Beethoven, Gauss, whatever one's profession or one's tastes, one should not greet any one of these names with a blank stare of vacuity Ideally, history of science at the university should find its natural and leading place in a general pattern of education that is bifocal rather than one-eyed, as one among a number of elective subjects for study I think the Americans have something there At the present time, however, it will be the chief task of the new Department in Imperial College to reinforce others in training those who, in various capacities, can promote this liberalization in education. Many here, as everyone knows, have long been striving towards the same end May I say that we look forward to close co-operation ? Wherever we can play a part in the life and activities of the College that is not directly devoted to pre-stressed concrete, catalytic crackers, or electron multipliers, I hope we may be able to do so But, like children, we would rather be heard as well as seen, for, in the immortal words of Archdeacon Spooner "It's beery work preaching to empty wenches"

SCIENTIFIC, TECHNOLOGICAL AND TECHNICAL MANPOWER*

By Sir WILLIS JACKSON, F.R.S.

Department of Electrical Engineering, Imperial College of Science and Technology, University of London

IN his recent presidential address ("Investment in Man") to the British Association for the Advancement of Science (*Nature*, 199, 877, 1963), Sir Eric Ashby, following Sir John Cockroft's preceding presidential address on "Investment in Science", said "The pre-requisites for investment in science are, first, scientists, and, second, a public opinion sufficiently well-informed and enlightened to give financial support to scientists Both these pre-requisites are acquired through education To invest in science you must first invest in man"

I wish to develop a variation or extension of this theme It is that, generally speaking, the impact of science on the life of the community, and in particular on its economy, is not a direct one but comes from the technological exploitation of its results Unless, therefore, we are strong in our technology, which requires that this need is recognized throughout the community, there is a danger that the economy may fail to provide for expanded scientific pursuit the increasingly expensive facilities and resources which this pursuit nowadays demands

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* Substance of the tenth Fawley Lecture delivered in the University of Southampton on November 21

A recent report by a committee of the Institute of Physics and the Physical Society entitled "Problems facing the University Physics Departments" details in an extremely convincing manner the needs of these departments if they are to meet their increasing teaching obligations satisfactorily and to remain in the forefront of international research in physics

I have no wish to argue against the claims put forward in this report in relation to what is good for physics Indeed, I am very sympathetically inclined towards them because, as an electrical engineer, I have good reason to be conscious of, and grateful for, the periodic stimulation of progress in my branch of technology which has come from fundamental work and discovery in physics Moreover, I am in no doubt that outstanding physicists, and those who show promise of becoming such, must be free to follow the directions which their scientific curiosity dictates without any necessary direct concern for the possible technological significance of the results of their work This freedom has been a well-safeguarded characteristic of university science in Britain, and I hope it will remain so It can be tampered with only at risk to the seed-bed from which important contributions to the future progress of technology will spring It follows also that these outstanding scientists must, so far as is practicable, be provided with the most up-to-date tools of investigation, and with adequate supporting assistance and technical staff, if they individually—and through them the country—are to remain in the forefront of scientific endeavour and achievement

But physics is only one branch of science Other branches could, and some already have, put forward similar claims for much expanded support, and I, as a comparative outsider, cannot but be impressed by the pertinency of such remarks as those of the Editor of the *Times Educational Supplement* who commented on the aforementioned report "The money that is just enough to make a significant difference to physical research would finance the whole of the national effort in biology" To which I am inclined to add—may not biology bear the same relationship to the next major technology as physics does to several present ones, and may not our neglect of the human sciences be the most serious unbalance of all ?

Acceptance of the unpredictability inherent in scientific pursuit, and of the necessity to maintain the freedom of enquiry which has been the traditional prerogative of basic science, affords no escape from such questions as Have we yet devised an effective means of making overall assessments of, and judgments on, the claims of the various branches of pure basic science ?*, Have we yet established satisfactory arrangements for the distribution of the available funds for this basic work ?, Are we anywhere near knowing on what criteria to decide how much of our national resources must be made available for its pursuit ?

It is not my intention here to seek answers to these questions They are matched, of course, by a number of related questions concerned with objective basic research* and applied research*, questions which are perhaps more difficult to resolve So far, decisions in these fields have been taken within the respective spheres of the establishment of the Government and public services and of industry, without significant co-ordination in the national sense It will not be easy to find, and many will no doubt argue that it is neither necessary nor desirable to find, a more effective alternative way of dealing with these problems

Nevertheless, the hoped-for devotion of increasing funds from central sources to research would seem to lead inevitably to a need for a clearer concept and formulation of national scientific policy, and for more co-ordinated means for its implementation

My justification for raising these issues is to make the point that the moneys available for devotion to research *for future purposes*, either from the central funds of Government or from the resources of individual industrial concerns, are dependent on the effectiveness with which the results of research *already completed* are translated into goods which can be sold internationally against increasingly severe competition

This raises a whole range of further questions concerning the factors which affect productivity and economic growth, now under consideration by the National Economic Development Council Not all these factors are, of course of a scientific or technical character, as these terms are normally understood—indeed, those which are most difficult to understand and resolve are certainly not But they seem to me to lead equally inevitably to the need for a clearer concept of scientific policy as an integral part of economic policy As put by C E Carter[1] "If we do not have an economic policy about the distribution of scientific resources, we are unlikely to have any policy at all A starting point for scientific policy is the examination of what we know about the pattern of exports ten to twenty years ahead At many points there will be a temptation to suppose that the problem of the distribution of scientific effort is too difficult, complex and vague to be solved Yet a few tentative moves towards rationality may be better than leaving the disposal of a valuable resource to random and undirected influences".

Since research can have military, social and political, as well as economic, consequences, scientific policy is becoming more and more an integral part of national, if not of international, policy in the widest sense In varying degrees the scientist, technologist, economist, sociologist, employer, Trade Union official, legislator and political leader all find themselves involved increasingly in the consideration of—if not in the taking of decisions on—the role which scientific research and technological development must occupy in national affairs, the resources which should be devoted to them, and how these resources should be allocated and balanced in the light of overall national policy

All this leads to the need for a clearer concept of manpower policy involving, for my present purposes, assessments and judgments on the likely future requirements of the community for the varied kinds of scientific, technological and technical manpower, and on the means by which these requirements are to be satisfied

I do not pretend that there is anything new or novel about the preceding Nor do I wish to appear unaware or inappreciative of the prolonged and careful attention which has been given to different aspects of the complex and difficult problems I have touched on by the responsible authorities, and by committees specially appointed by them for this purpose But it is surely right to say that we, and most, if not all, other countries, are still at a very early stage of our systematic consideration of the interrelationships between these various facets of national policy We do not yet know how to plan and implement deliberate changes in one sphere in appropriate scale and time relative to changes in others, so that the evolving needs of the overall situation—so far as we can reasonably hope to foresee them—can be most satisfactorily met Much hard thinking, study and experiment will be required before the largely empirical procedures which have been adopted so far can be much improved on Each country will no doubt evolve its own characteristic approach depending on its national traditions, attitudes, resources, forms of existing institutions, etc This diversity affords a fascinating starting point and opportunity for international co-operation in the resolution of problems which become increasingly matters of joint concern

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* The Zuckerman Committee on the Management and Control of Research and Development defined these categories of research as follows Pure basic, research carried out for the sole purpose of increasing scientific knowledge, Objective basic, research stimulated primarily by some practical need in a field of potential application, Applied, research differing from objective basic in which speed and the interests of the ultimate user come prominently into the picture

I must now seek to justify this extremely general, perhaps rather diffuse and impractical, introduction by relating to it some problems of scientific, technological and technical manpower which seem to me to give cause for concern The periodic reports of the Committee on Scientific Manpower published over the past seventeen years or so have made a very important contribution to clarifying the national situation in this respect by aggregating the anticipated future demands of industry, the Government and public services, education, etc , for scientists and technologists of various kinds, and by relating these estimates to the expected supply from the universities, colleges of technology, and other sources These reports have assisted the University Grants Committee and the Ministry of Education in the formulation of their plans for a considerable improvement and expansion in the facilities for scientific and technological education within the universities and the colleges of technology respectively, and done much to stimulate the marked change in the pattern of sixth-form work within the schools which has occurred during the post-war period The 1963 report[2] probably gives a better quantitative picture of the distribution of our existing scientists and technologists, and of the expected supply and anticipated demand over the next few years, than is available in any other country

It is evident, however, that we are a long way from knowing how to make an assessment of future *needs* for scientific, technological and technical manpower based on a forecast of the pattern of desirable national development and economic growth Taken literally, this may be asking for the impossible, and in any event such a pattern is only in the early stages of conception by the National Economic Development Council Certainly, as outlined particularly in its 1961 report, the Committee on Scientific Manpower has tried to make such projections, but it has necessarily had to base much of its assessments of likely *demand* on the opinions of individual employing organizations Some of these will not have been well situated, because of a dearth of suitably qualified and informed opinion within them, to judge their future need for scientific and technological manpower and to conceive how to make effective use of it The demand of these organizations is, therefore, unlikely to have reflected an adequate appreciation of the kinds of change which are now possible, and necessary, but which only qualified staff can formulate and carry to successful completion On the other hand, there are no doubt organizations which, though recognizing an expanding need for qualified manpower, may not have been able to foresee the financial possibility of initiating and sustaining desirable programmes of new development

In the second place, we do not yet know how, within a democratic community, to ensure a pattern of *supply* which is commensurate with that of anticipated demand—or need—at the time when this demand requires to be satisfied What we have sought to achieve is an adequate overall supply, subdivided into broad categories and characterized by sufficient flexibility and adaptability to meet unpredicted, or unpredictable, changes in the national situation Nevertheless, the 1963 report reveals a number of discrepancies which illustrate the kinds of problem for which we must surely seek more effective solutions

Thus the report states

"The information now available to us from the 1961 Census enquiry, together with the results of the 1962 Manpower Survey and the employers' estimates of requirements, suggests that we are still some way from a satisfactory balance of supply and demand, particularly as regards technologists

"The relation between supply and demand differs greatly from one discipline to another This has always been so, but in the conditions of extreme overall shortage which prevailed after the war, the first objective was to get near to an adequate total supply of scientific manpower As we approach the first objective, analysis by discipline becomes increasingly important "

In respect of some of these disciplines, the most notable discrepancy on the science side is in mathematics, where—"it seems likely we shall be short of nearly 1,500 mathematicians in 1965", while in physics—"the number of new physicists who will become available by 1965 would suffice to meet the requirements forecast by employers in the sectors covered by the Survey only if there were little or no growth in the employment of such men in other fields" On the technology side, although the number of students qualifying annually in electrical engineering has risen from 1,284 in 1960 to an estimated figure of 1,830 in 1964—"there will still be vacancies for about 4,000 in 1965, even when no allowance is made for the replacement of wastage or for possible growth in sectors not covered by the Survey" The position in mechanical engineering is particularly disturbing The number of students qualifying has fallen progressively over the same period, from 4,574 in 1960 to an estimated 3,385 in 1964 Although the report does not give a figure for the likely 1965 shortfall in this subject, the trend in output is clearly quite inconsistent with the central position which mechanical engineering occupies within technology as a whole

These figures provide one piece of evidence of the unattractiveness of technology relative to science to the scientifically minded products of our schools Much further evidence is given in the recently published booklet *Technology and the Sixth-Form Boy*[3], and it may be of interest to supplement this by the data of Tables 1 and 2 The former gives comparative figures for new entrants to the science and technology faculties of the universities over the period 1954–1962[4], and the latter the 'home' student applications per place in the several university faculties over the period 1960–1962[5]

Table 1 FULL-TIME STUDENTS ENTERING THE UNIVERSITIES OF GREAT BRITAIN FOR THE FIRST TIME

Academic year	Science	Technology	Total
1954–55	4,880	3,199	8,079
1955–56	5,400	3,341	8,741
1956–57	6,079	3,835	9,914
1957–58	6,644	4,269	10,913
1958–59	6,801	4,590	11,391
1959–60	7,043	4,491	11,534
1960–61	7,787	4,446	12,233
1961–62	8,519	5,064	13,583
Oct 1962 (provisional)	8,829	5,103	13 932

Table 2 HOME STUDENT APPLICATIONS PER PLACE IN VARIOUS UNIVERSITY FACULTIES

	1960	1961	1962
Arts and Social Science	6	7	8 5+
Science	7–	7 5+	8 5
Technology	4 5	4 5	5+
Medicine and Dentistry	6	8+	11 5
Overall	6	7	8+

The figures in Table 2 were calculated by Prof J T Allanson, from the annual returns of the Committee of Vice-Chancellors and Principals They embrace multiple applications and also students who fail to qualify for entry, and there may well be differences between faculties in these respects Nevertheless, the difference in respect of technology seems too large not to be significant

In respect of numbers, the technology situation is relieved somewhat by the gratifying growth in entrants to diploma in technology courses, as shown in Table 3 Nevertheless, even when the output from these courses, and from other, mainly part-time technical college, courses which satisfy the examination requirements of the professional institutions, is added to that from the universities, the position relative to science is disturbing This is revealed by the Scientific Manpower Committee data of Table 4

But there is a second discrepancy—that between the performance in the General Certificate of Education, Advanced Level, and related examinations of the entrants to full-time courses in science and technology In supplementation of other published evidence on this point[3]

Table 3 NEW ENTRANTS TO DIPLOMA IN TECHNOLOGY COURSES 1959–63

	1959	1960	1961	1962	1963
Engineering	1,108	1,199	1,211	1,517	1,718
Other technologies	450	570	558	843	979
	1,558	1,769	1,769	2,360	2,697

Table 4 ESTIMATED NUMBER OF STUDENTS QUALIFYING* ANNUALLY 1960–1964

	1960	1961	1962	1963	1964 forecast
Science	7,343	7,569	8,144	8,870	9,645
Technology (total)	9,783	9,916	9,907	9,800	9,910
(Mechanical engineering)	(4,574)	(4,443)	(3,939)	(3,635)	(3,385)

* By degree, diploma in technology and other examinations satisfying the requirements of the appropriate professional Institutions

Table 5 gives an analysis of the subject of undergraduate study chosen by the top thirty candidates in the Imperial College Entrance Scholarship examinations over the period 1954–1963 It will be seen that the ratio of engineers to scientists is about 1 to 3 5 and this has been getting worse, and of electrical and mechanical engineers to physicists 1 to 7 5 and 1 to 9 5, respectively Table 6 gives the mean percentage in the G C E "A" level examinations of all the undergraduate freshmen in physics and electrical engineering over the period 1956–1962

Table 5 ANALYSIS OF THE TOP 30 CANDIDATES IN THE IMPERIAL COLLEGE ENTRANCE SCHOLARSHIP EXAMINATIONS 1954–63

	Subject of later undergraduate study								
Yr	Maths	Physics	Chemistry	Elec Eng	Mech Eng	Civil Eng	Chem Eng	Aero Eng	Metallurgy
1963	6	14	4	–	2	–	2	–	2
1962	5	11	7	4	2	–	–	1	–
1961	9	14	2	1	–	1	–	3	–
1960	5	7	9	2	4	1	1	1	–
1959	8	8	6	2	2	1	3	–	–
1958	6	12	5	1	1	2	2	–	1
1957	3	14	6	1	–	1	4	1	–
1956	1	13	7	1	1	–	5	1	1
1955	5	9	8	2	–	–	4	1	–
1954	1	11	13	1	–	1	2	1	–
	49	113	67	15	12	7	23	9	4
		229				66			4

Table 6 MEAN PERCENTAGE IN THE GENERAL CERTIFICATE OF EDUCATION, ADVANCED LEVEL EXAMINATIONS OF THE UNDERGRADUATE FRESHMEN IN PHYSICS AND ELECTRICAL ENGINEERING AT IMPERIAL COLLEGE 1956–62

Session	Physics	Electrical engineering
1962–63	68 1	64 2
1961–62	68 5	63 5
1960–61	69 1	62 8
1959–60	68 8	63 0
1958–59	70 3	61 6
1957–58	69 1	65 9
1956–57	68 1	61 9
Overall average	68 8	63 3

I regard this second discrepancy as much the more serious, notwithstanding that I have no great confidence in examinations as a basis for predicting quality in later life Certainly I have no evidence of a close correlation between quality as judged by examination performance at the age of eighteen and quality as revealed later among practising technologists The successful pursuit of technology demands characteristics of mind and of personality which do not lend themselves to assessment by examination, and a quite different environment from that of school and university—and of the research laboratory—for their full development and refinement Nevertheless, if, as many people keep saying and as I believe, our national weakness, taken overall, lies not in our research but in our relative inability to translate its results into technological achievement, it seems to me that we cannot be content with a lower quality of entrant at the technological starting point, as judged by whatever criteria are generally applied at that stage of the educational process

What now are the essential differences between science and technology ? According to a recent definition[6], science is knowledge obtained by observation, by experiment and by subsequent deduction. Natural science is the investigation of the nature and properties of natural bodies and natural phenomena The object of the scientist is the determination of general laws and the deduction of principles from the effects which are observed Technology, on the other hand, is defined[7] as the science of the industrial arts, and the technologist is therefore one who applies scientific principles and imagination to the conception and creation of useful material products required by the community

J T Allanson[5] has drawn the distinction in the words "Just as the painter is judged by his paintings and not by his writings on æsthetics, so the engineer is finally judged by what he makes The making involves him in the social sciences and in relations with other human beings It is not possible for a good engineer to be a 'back-room boy', nor can he believe in that curious battle between the 'two cultures' invented by Sir Charles Snow He must make an engineering unity of the physical and social sciences by the kind of intellectual and moral effort supposedly characteristic of the man who reads 'Greats' With this he must integrate imagination and personal drive, for he is not a scholar but a doer, and the more visionary and far-reaching his ideas the greater must be his struggle to achieve them against the conservatism of the orthodox".

Technology spreads over applied research, development, design, manufacture, and operation, and the technologist may be found within any of these functional activities Scientists, on the other hand—until they also are more correctly described as technologists—are concentrated within the fields of basic and applied research, and of the initiating stages of development. The clear-cut area of demarcation is, therefore, design

In relation to mechanical engineering (and most design has strong mechanical associations) the recently published report of the Feilden Committee[8] defines design as—"the use of scientific principles, technical information and imagination in the definition of a mechanical structure, machine or system to perform pre-specified functions with the maximum economy and efficiency"

In the past, and indeed in the present, the majority of the country's designers have not been educated in degree or equivalent full-time courses, but through long practical experience and part-time courses, and often no courses at all The best of these men were, or are, outstanding in spite of the deficiencies of their educational preparation, and we have much reason to be deeply grateful to them for their inventiveness and their native engineering intuition and competence But this method of preparation will no longer suffice The origins of invention and the foundations of creative design are moving back more and more into science, and demand an educational discipline as prolonged and as searching as that required by the scientist But the discipline involves a different emphasis, for it is an art as well as a science, leads into a different environment with different objectives; and requires a preliminary training within this environment to provide contacts with the practices, the organization and the people necessary for the translation of design into production and operation It raises issues of economics and human relations from which the scientist is largely protected, and increasingly so as it moves beyond design into production, sales, operation and management Technology is not only an exercise in the application of science, but an even more difficult one in the motivation of people towards defined material objectives[9]

From now on, more and more of the equivalents of the men I have mentioned will remain at school until they are eighteen years of age, and will enter full-time university or college courses It is of great national importance that they shall not be lost to technology, for we need many more, not fewer, of them Not all design is concerned with major projects, of course, and even with these much of it is related to small components of the whole But this is no different from scientific research (except that, in the latter, study of the components leads more readily to publications of no great importance which fewer and fewer have time to read), and the sheer professional competence required is certainly of no lower order in one than in the other

I have heard it argued that they will not necessarily be lost to technology if their university education is on the science side, and indeed that a first degree in science is the best preparation for participation in the technology of the future It will be evident from what I have already said that I am in no doubt as to the necessity for strengthening the scientific foundations of technological education, but I have also expressed my conviction that this education involves different emphases than are to be found, or could reasonably or rightly be expected to be found, in pure science courses I am well aware of the substantial participation of physicists in the electronic branches of electrical engineering, but there is no evidence, and not much likelihood, of a corresponding help to mechanical engineering, for example, from this source It seems to me that the argument can be misleading both in respect of strengthening the country's technology at its weakest points, and of the best interests of the careers of the persons concerned—unless, that is, they are prepared to embark on appropriate technological study at the postgraduate stage At present most of those graduates of the university science schools who join industry enter its research departments In this sphere of industrial activity their contributions are invaluable, but, as a generalization, the environments in which research, and indeed development, are conducted, do not by themselves afford the kinds of basic training and experience which are essential to the successful pursuit of the design, production, operational and managerial aspects of technological activity.

Though at the recruitment stage the science graduate may not, of course, be interested in these activities, he may well become so later as his inclinations change, or should his creative abilities in research and his career prospects in it fail to mature It then becomes difficult, and the more so the older he gets, for him to move into other spheres of industrial or related employment, notwithstanding that these spheres may be much in need of men of his intellectual powers Indeed, an extremely sad feature of the research and development departments of industry and of Government departments, seen in both individual and national terms, is the number of men of considerable ability and promise who have become locked up in these environments from an early stage in their careers The immobility of qualified manpower between the interrelated spheres of scientific and technological life is an outstanding defect of our national situation Among those who pay the highest price individually, and are the greatest loss to the strengthening of our technology, are the able men who discover that they are not, as they had hoped to be, outstanding research scientists

I must not pretend that the employment situation for technologists is free from discouragements, knowledge of which has penetrated to levels which affect the choices of young people Those sectors of industry which are most in need of technological stimulation are too often unwilling to establish the conditions of employment for qualified manpower which are a prerequisite for it Other sectors, where this is not the case, are often said to cause frustration and discouragement to their qualified recruits during the necessary phase of preliminary training, to afford inadequate status and salary to their more senior technologists, and inadequate opportunity for those who wish to rise to the top levels of management I do not doubt that there is justification for these criticisms, though I have known them to be much distorted Where they are justified, however, they demand the most urgent attention and correction But it is not difficult to pin-point a number of other, quite different, reasons why we are failing to produce a public image of technology which is consistent with its national importance, and to attract a larger proportion of the most promising of our scientifically minded schoolboys to its pursuit

First, there is the widespread lack of acquaintance with technology among headmasters and their staff colleagues While this may not create, it is certainly not calculated to remove, the serious misconceptions present among schoolboys referred to in *Technology and the Sixth Form Boy*. In paraphrase, some of these are: that engineering studies are all right for boys who are good with their hands, but not for those who are good with their heads, that all the new ideas in engineering come from the scientist, that all the really difficult problems of engineering are solved by the scientist, and that the rest is just straight engineering In more recent comments, at a British Association meeting discussion on this Oxford University enquiry, W. D Hutchings remarked that the main single factor leading to the narrow and often naïve approach of many boys to their future careers is the intense and premature specialization to which they are subjected, causing the best boys to be channelled into the subjects in which they have been most successful at school, with the glamour and prestige of research as a prime incentive.

I find it quite understandable that the science departments of the universities do not discourage the latter trend But what I do not understand is the impression given by many staff members of these departments that they regard the process of translation of the results of scientific work into technological achievement as a markedly lower-grade activity than their own, and what I regret is their stimulation of the belief, which extends well beyond those students who are likely to be well suited to it, that research is the only valuable outcome of a scientifically based education

The situation is not improved by the otherwise quite excellent science programmes of sound and television broadcasting These not infrequently, but no doubt unconsciously, credit scientists with much that is essentially achievement in technology, while other programmes do little to correct the widespread misunderstanding that engineering and a pair of overalls are synonymous—though at a very important, but non-professional, level they undoubtedly are

The university engineering schools have themselves done a dis-service to their cause by differentiating too early between the several branches of engineering, and fostering the concept of narrowness and rigidity in their undergraduate courses. In fact, there are now strong movements, notably within the plans of the new universities, towards more general courses in engineering science This involves, however, an increasing participation in postgraduate courses of study in more specialized fields, and a willingness on the part of employers to sponsor the return of selected graduates for this purpose

The professional engineering institutions have also contributed by retarding the emergence in the public, and indeed in the Governmental mind, of a clear concept of technology and of the position which the profession of engineering occupies within it No doubt each of the many such institutions has a distinctive service to perform in catering for the technical interests of a different branch of the profession, but only since the formation about a year ago of the Engineering Institutions' Joint Council, representative of thirteen specialist institutions, has there been a deliberate approach to the formulation of an overall common policy on such questions as education and training and professional recognition It is one of my hopes that the corporate members of all of them will soon come to be known as 'chartered engineers', and that steps will be taken to ensure that the meaning and significance of this common title is clearly and widely understood

Finally, the Civil Service is also not free from responsibility in so far as the majority of the professional engineers whom it employs form part of the so-called works group of professional classes, a term which suggests that their status is lower—as their career prospects undoubtedly are—relative to the members of the Scientific Civil Service

This complex situation will not be easily resolved However, the considerable attention which the subject has received in reports, speeches and the Press during recent months, and to which the Duke of Edinburgh has made several notable contributions[10], is gratifying evidence of a growing recognition of the need to do so. The next step must be a determination by each of the sectors of the community which I have mentioned to make its appropriate contribution towards this resolution

It seems to me that the most crucial people for this purpose are the scientists, and particularly the outstanding scientists, and, if for no other reason, in the long-term interests of science I have not only in mind the contributions, of which many examples can be cited, which new tools of investigation resulting from technological progress can make to the further progress of science, but also the relationship which must exist between the scale on which new scientific work can be supported and the growth of the national economy, and the dependence of the latter on the economic consequences of the technological exploitation of what is already known in science I would, therefore, ask the outstanding scientists, to whom and through whom the opportunity to pursue new ideas is of outstanding importance, to show a greater readiness to recognize publicly that the speedy translation of the results of research through development, design and manufacture into efficient, reliable and economic pieces of equipment is an activity which can make intellectual demands no less severe, if different from their own; and that these activities justify a public prestige commensurate with the one which their own activities enjoy It is probable that such assertions as these will be really believed by schoolmasters, parents and the best boys only if they are made by outstanding scientists—and one must hope first that the outstanding scientists themselves really believe them

I have already mentioned a number of aspects of the employment situation for technologists which require urgent attention, but there is another which leads me into another category of manpower My experiences and observations of recent years have left me in no doubt that some, if not much, of the work being carried out by professionally qualified people could, with appropriate replanning and the fuller use of modern techniques, perfectly well be performed by persons of lower academic qualifications The process of devolution is, of course, a continuous ,one in any progressive situation It is a process which tends to increase, not decrease, the need for highly qualified people, through stepping up the standard of the overall activity. But it demands for its progression the availability of others, themselves well educated and trained, though to a different level or levels, on whom the work can be devolved

The effects and the demands of technical change in industry and elsewhere are emphasizing the growing significance of the category of manpower lying between the technologist and the craftsman and operative—a category which, in engineering, is coming to be described collectively as that of the technician engineer Regrettably, in our concern for our shortage of scientists and technologists, we have been inclined to overlook somewhat the importance of their supporting contribution and their needs in education and training, so much so that our greater present shortage may well be at this level So far the members of this category have come mainly through promotion from the ranks of craftsmen and operatives, or are men who, having aspired to professional status, have failed to achieve it All too few of the former have pursued their technical studies to a standard adequate for the evolving requirements of their new activities and responsibilities, or have received any specific training in preparation for them This will no longer suffice—if, indeed, it has sufficed so far—to deal with the rapidly increasing technical complexity of engineering equipment and systems, and of the problems involved in their manufacture and operation This does not mean that all the functions performed by the technician engineer call for high qualification in the academic sense, for they vary considerably in their technical demands What it does mean is that more specific steps must be taken in both education and training to ensure adequate preparation for the duties to be performed

The Ministry of Education made an extremely important contribution towards this end in its formulation of a new pattern of technical education in a White Paper issued in 1961 This pattern is now taking shape in technical college courses, with the assistance of the City and Guilds of London Institute, but much remains to be done by many employers in respect of their willingness to afford day-release facilities to their young recruits and to provide associated training of adequate standard within their own concerns

About a year ago the Committee on Scientific Manpower initiated exploratory steps in preparation for a national statistical survey on technicians These are taking the form of sample enquiries involving a small number of firms in selected industries Their purpose is to ascertain the relationships which exist within them between technicians—if so-called—and other categories of personnel employed; the kinds of duties with which the technician is identified, the nature of the responsibilities which these duties involve, and the status attaching to them, the evolving changes in the character of technician employment, the type of preparation in education and training which the present technicians have received, and the modifications to these which are thought to be necessary for future purposes The objectives to be aimed at must be, of course, not merely to state the present position but, so far as practicable, to assess qualitatively and quantitatively the aggregate needs that lie ahead so that the earliest possible preparation can be made for meeting them.

This is a difficult and long-term exercise, but it is obvious that the assessment is integral with the one for scientific and technological manpower—indeed, that neither can be made reliably without the other The pity is that we are only at the beginning of an attempt to interrelate them

In conclusion, I wish to make two points, both of which have no doubt been made many times in different ways

The first is that those young people who choose, or are persuaded, to prepare themselves for careers in science and technology must, as an integral part of their education, have their minds opened to the sociological implications and consequences of scientific and technological progress, and of their special responsibilities towards them, and no less important, that those who choose one or other section of the faculties of arts must be helped to extend their knowledge and understanding of the history, the methods, the achievements and the aims of science and technology well beyond what has been thought necessary in the past

The problem of the arts student is probably more difficult than that of the scientist and technologist, for while the latter can draw on a vast accumulation of literature in the arts, there is as yet little literature in science and technology that is well suited to his purpose Yet, notwithstanding this difficulty, the time has surely passed when the humanist—or so-called humanist—can continue to remain as ignorant as many now are of the scientific and technological origins of the developments which are changing the pattern of life, both nationally and internationally

The second point is a related one—it is a plea for a more serious attempt to establish what are variously called the human, social or behavioural sciences (if it is yet permissible to refer to them as sciences) in a more rational relationship to the physical and biological ones The scale of effort in the physical sciences and in technology is increasing rapidly The innovations which will result will bring further material benefit, and indeed this is greatly needed in many places and by many people But, as in the past, we are likely to be found woefully incapable of resolving the human problems which will also flow from them I would not know how to set about achieving a better understanding of the factors which govern even the rational modes of human response and behaviour The most I can do is to hope that more good people will devote themselves to the search for it—not in expectation of early benefits, but because longer-term ones will not come otherwise

I have tried to portray the need for, and some of the difficulties that beset, the formulation and implementation of a scientific, technological and technical manpower policy This is, of course, only one aspect of manpower policy within the context of national policy as a whole, and here we are far from having reached a clear concept of the kind of country, and society, we would wish to see emerge with the aid of science and technology

My main theme has been the strengthening of technology. This is only one of the means to the achievement of the wider aims that should inspire us But it is an indispensable means, since I am convinced that only through the material benefits that flow from successful technology shall we be able to afford to do the things which most need doing

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[1] Carter, C E , "The Distribution of Scientific Effort", *Minerva* (1963)

[2] *Scientific and Technological Manpower in Great Britain*, 1962 (H M S O , Cond 2146, Oct 1963) (*Nature*, 200, 297, 1963, also p 1133 of this issue)

[3] Oxford University Department of Education (1963)

[4] Supplied by the Office of the University Grants Committee

[5] Allanson, J T , *Education*, 24 (May 24, 1963)

[6] Dame Kathleen Lonsdale

[7] *Concise Oxford Dictionary*

[8] *Engineering Design*, Department of Scientific and Industrial Research (1963)

[9] See Sir Christopher Hinton's Presidential Address on "Design and Research" to the British Electrical Power Convention, June 1963, for a stimulating treatment of this subject

[10] Graham Clark Lecture, *Inst Civil Eng Proc* ,19 (July 1961), First Handley Page Lecture, College of Aeronautics, Cranfield (May 1963)

NEWS and VIEWS

Chemistry at the University of East Anglia: Prof. N. Sheppard

Dr N Sheppard has been appointed professor of chemistry in the School of Chemical Sciences in the University of East Anglia, Norwich Dr Sheppard went to Hymers College, Hull, and was an undergraduate at St Catharine's College, Cambridge, from which he graduated in 1943 During the Second World War he worked with Dr (now Sir) Gordon Sutherland in the Department of Colloid Science, Cambridge, on the infra-red spectra of rubbers. He obtained a Ph D degree in 1947 and then spent a year as visiting assistant professor at the Pennsylvania State University studying Raman spectroscopy with Prof D H Rank On returning to the United Kingdom, he rejoined the spectroscopy group in the Department of Colloid Science, Cambridge, as a Ramsay Memorial Fellow and later as an 1851 Senior Exhibitioner In 1956 he was appointed assistant director of research in spectroscopy in the University Chemical Laboratory, Cambridge, and a Fellow of Trinity College His research interests have been concerned with the application of spectroscopic methods to the investigation of molecular structure and molecular dynamics His group has applied infra-red spectroscopy to the examination of problems in molecular conformation and internal rotation, hydrogen bonding, and physically and chemically adsorbed molecules In nuclear magnetic resonance spectroscopy the group has principally studied the steric dependence of the (H,H) coupling constant in proton spectra of hydrocarbon groupings—particularly those of simple and symmetrical molecules obtained with the help of spectra from ^{13}CH groups Structural investigations of organic natural-product and inorganic co-ordination compounds have been made in collaboration with colleagues at Cambridge and elsewhere Dr Sheppard has taught physical chemistry at all levels and given undergraduate and postgraduate lecture courses on spectroscopy

Mechanical Engineering at the Manchester College of Science and Technology: Prof J K Royle

Dr J K Royle has been appointed to a second chair in mechanical engineering in the Faculty of Applied Science of the University of Sheffield as from April 1, 1964. Dr Royle, after graduating at the College of Technology, University of Manchester, in 1944, became a scientific officer at the Royal Aircraft Establishment, where, among other things, he worked on power plant, aerodynamics and supersonic flow. In 1949, Dr Royle was appointed assistant lecturer in mechanical engineering in the College of Technology, Manchester, lecturer in 1953, and senior lecturer in 1961 He was an associate professor of mechanical engineering at the Massachusetts Institute of Technology for the session 1960–61. Dr Royle's interests have been in the teaching of fluid mechanics and in research into hydraulic servo-mechanisms. He is the author of a considerable number of papers, for one of which he was awarded the Bernard Hall Prize of the Institution of Mechanical Engineers in 1959 He has acted as consultant to many firms and organizations and he holds a number of patents relating to machine tools, to which many of his developments in hydraulic servos have been applied

The National Research Council (Canada) Steacie Memorial Fellowship: Prof. N. Bartlett

The National Research Council of Canada has announced in Ottawa that Prof Neil Bartlett, associate professor of chemistry in the University of British Columbia, has been awarded the first E W R Steacie Memorial Fellowship by the National Research Council (Canada) This senior research award, established by the Council in March 1963, perpetuates the name of Dr E W R Steacie, president of the Council during 1952–62, and one of Canada's most distinguished scientists (*Nature*, 196, 110, 1962) The purpose of the fellowship is to give outstanding and promising young staff members of Canadian universities the opportunity to spend two or three years in uninterrupted research During his tenure of the award, Prof Bartlett will be relieved of all teaching and administrative duties He will receive his normal university salary, paid in equal shares by the University of British Columbia and by the National Research Council Prof Bartlett, who is thirty-one, became well known when, in October 1962, he prepared the first true compound of the rare gas xenon By successfully combining xenon with another gas to form a stable chemical compound, a reaction previously regarded as impossible, Prof Bartlett not only overthrew a number of existing theories on chemical bonding, but also opened up a whole new field of scientific investigation As a result of his work with xenon, the chemistry of the rare gases is now being examined in laboratories all over the world Prof Bartlett is a native of Newcastle upon Tyne, and holds the degrees of B Sc and Ph D of the University of Durham.

The European–American Nuclear Data Committee

Dr E Bretscher, head of the Nuclear Physics Division of the Atomic Energy Research Establishment, Harwell, was elected chairman for two years of the European–American Nuclear Data Committee at the Committee's sixth meeting held in Athens Dr Bretscher will succeed Dr J Spaepen, head of Euratom's Central Bureau for Nuclear Measurements at Geel in Belgium The secretaries of the Committee are Mr R Batchelor, Atomic Weapons Research Establishment, Aldermaston, and Prof H Goldstein, Columbia University, New York The European–American Nuclear Data Committee was set up in October 1959 by the Steering Committee of the European Nuclear Energy Agency in agreement with Euratom, the United States and Canada The Committee consists of fifteen experts from nuclear establishments in member countries of the Organization for Economic Co-operation and Development The work of the Committee includes periodical reviews of activities in the field of neutron cross-sections, and collation, comparison and diffusion of the results of this work It also establishes lists of priority requests for nuclear data measurements, so as to help in directing and co-ordinating research activities in the participating laboratories and to ensure co-ordination in the use of scarce materials needed for such measurements The Committee was responsible for the recommendations which led to the formation of the European–American Committee on Reactor Physics and the European Nuclear Energy Agency's Group of Specialists on Neutron Data Compilation The Committee has also sponsored various symposia

Building up Education in Britain

In a written answer in the House of Commons on December 5, the Minister of Education, Sir Edward Boyle, stated that the two most important recommendations of the Newsom Report on secondary education addressed to him were those relating to raising the school age and to the school building programme He had recently announced a greatly increased level of school building for 1965–66 and 1966–67, and recognized the importance of continuing the improvement of educational provision in secondary schools on which Britain was already engaged He had already promised to make a statement during the Session about raising the school-leaving age Some work was already in hand relating to other recommendations of the Report He was supporting from his research vote an investigation by the Liverpool Institute of Education of the relationship between environment and educational progress and by the London Institute of Education of the relationship between verbal intelligence, learning ability and social class Consideration of how best to follow up other recommendations was continued, and he would be ready to consider supporting from his research vote other proposals for research and development formulated in this way In reply to an earlier question, Sir Edward Boyle had stated that the increasing recognition throughout the building industry of the need for scientific management techniques, and the inclusion of building management in the examinations of the Institute of Builders, had increased the demand for specialist teachers of building management His Department would shortly be discussing this and other problems with the Institute, and the discussions would cover the question of text-books

British Space Research

In a written answer in the House of Commons on December 5, the Minister of Aviation, Mr J Amery, stated that his Department had been carrying out a programme of research into the technology required for the civil and military exploitation of space, and this programme was at present being reviewed The design study of a communications satellite had been completed and its implications were being investigated Twenty separate systems, including fifteen types of satellite, were considered in a co-operative investigation with the Post Office and industry, and discussions with Europe for British participation in a satellite communications system would be followed by talks with the United States and other countries Britain, he said, was keeping in close touch with the Commonwealth and continued to participate in the negotiations for establishing the European Space Research Organization, the Preparatory Commission of which had placed contracts for two preliminary satellite design studies with the Ministry of Aviation The first *Blue Streak* would be launched from Woomera, probably in April 1964, and under the Minister for Science's space research programme, the second Anglo-American scientific satellite awaited launching in the United States His Department had placed a contract for a third satellite in the programme which would be the first British-made satellite and should be ready for launching by the United States in about three years. Development of a stabilized version of the *Skylark* sounding rocket had reached an advanced stage

Telstar and its Problems

A RECENTLY-PUBLISHED booklet, edited by R. M Foster, jun , *Satellite Communications Physics*, is designed primarily as an aid to school science education (Pp 88 By Members of the Staff of Bell Telephone Laboratories New York Bell Telephone Laboratories, 1963) Written by scientists who have participated in the *Telstar* project, the booklet gives a general review of communications by satellite, followed by six interesting individual 'case histories' on specific questions raised by *Telstar* The six topics are the method of calculating orbits; the choice of colour and surface finish to maintain the required temperatures, the use of optical observations to determine the direction of the spin axis, the protection of solar cells from radiation damage; the effects of time delays of up to 1 2 sec in telephone conversations via a synchronous satellite, and, finally, the problem of repairing a satellite in orbit

Research and Design in Engineering

In a lecture entitled "Research and Design in Engineering", originally delivered at the National Engineering Laboratory in October 1962, and which has only been published this autumn, Mr H Clausen renews the plea for a better balance between the science and the art of engineering which he had advanced in earlier lectures on engineering design as the background and basis of contemporary life and on the borderland between science and engineering. Quoting a remark of Sir Henry Tizard that science may make many things possible but does not necessarily make them happen, Mr Clausen suggests that Britain's educational and industrial weaknesses still call for more attention at the point where two disciplines, fields of activity, or techniques overlap The problem is largely one of communication of ideas and media of expression He suggests that the gap between research and the application of its results is due first to the disappearance of the older relation between master and apprentice and then to the failure, in the educational, engineering and industrial fields, to develop some system to replace it effectively Probably arising out of the latter is the failure of Britain's older educational system to realize that national leadership cannot be effective without a full appreciation of the way the country earns, or might be able to earn, its living a mastery of the industrial arts, on which everything depends, and which can no longer be taken for granted Probably, in consequence, the control of much industry has drifted into the hands of men who are mainly interested in finance and profits and have pushed the creative aspects of industry, on which everything depends, so far down in the hierarchy that they are no longer effective, or attractive to men of talent, quality or ambition Finally, he insists that Britain's existence as an independent nation depends on maintaining a proper balance between science and the technologies

Industrial Psychology and Take-over Bids

A STUDY by Mrs W Raphael and Mr M W Zimmerman of the reactions of some 5,500 employees in a group of electrical equipment companies indicates that unrest and anxiety among employees whose firm has been taken over by another could be reduced if they were given more information as soon as possible after the change occurs (National Institute of Industrial Psychology. Report No 15 *After the Take Over—A Study of Reactions of Employees in a Group of Companies* Pp iii+35 London National Institute of Industrial Psychology, 1963) Two surveys were made one shortly after the take-over, and a similar survey about a year later At the time of the first survey, people were anxious about their jobs and initially excited rather than despondent about the future, and willing to co-operate rather than tending to resist all change. The Institute comments that even if few changes are intended, it is better to explain the nature of those that are intended than to say there will be few Employees will be reassured more by knowledge of the direction that affairs will take than by general expressions of benevolence The results of the second survey are treated with more reserve. and a particular distinction is suggested between the purposes of take-overs On the whole, views about the effects on companies and on products and customers remained favourable Ignorance of the procedure by which a take-over is conducted was widespread a circular which explained this procedure and its reasons could help to allay the suspicions to which misunderstandings lead

British Aid to Developing Countries

A FURTHER pamphlet in the series of independent factual surveys of British aid being issued by the Overseas Development Institute, Ltd , reviews the main features of aid to developing countries, completing the picture given in the White Paper issued in September (*British Aid—1· Survey and Comment.* Pp 63 London Overseas Development Institute, Ltd , 1963 3*s* 6*d*) Objectives, organization, financial assistance, technical assistance and, finally, education and training, are discussed in successive chapters, and in the last three chapters the factual survey is followed by critical comments As regards objectives, attention is directed to the need for more public explanation and discussion of the more specific aims of aid if policy is to be deliberate, consistent and publicly supported On organization, establishment of a Ministry for Overseas Development with a senior Minister at its head is suggested This Ministry would take over from the external affairs departments the responsibility for policy-making and allocation of aid An Agency or service department, it considers, should be established to handle the technical aspects of aid, but policy would remain in the hands of the present departments—the Agency acting as adviser—while a senior Minister should be appointed to act as general overseer of aid and its spokesman The pamphlet is critical of some aspects of technical assistance, particularly emphasizing the need for an overseas career service and suggesting also a home-based technical advisory service Accordingly, it suggests that recruitment could be improved by making contracts significantly longer, and more attractive, and by more determined efforts at making an overseas tour of duty an accepted part of a successful career On education, it questions whether the 7 per cent of Government aid devoted to educational assistance represents the right order of magnitude It strongly urges the need for more educational research, particularly in determining the priorities of need in the developing countries and the relative efficiency, and actual costing, of various methods of educational assistance Apart from its value as a concise factual summary, the pamphlet is to be commended for its constructive criticism which, except perhaps in the matter of organization, is sound as well as informed

Conservation of Nature and Landscape

AT the second meeting of the Committee of Experts for the conservation of Nature and landscape, held in Strasbourg during November 12–15, Mr J. P Harroy (Belgium) was elected chairman, and Mr W B Yapp (Britain) and Mr Z Bayer (Turkey) were elected vice-chairmen. The agenda included soil conservation and unwise use of land; preparation of a map of potential natural vegetation in European countries, water conservation; pesticides, sea pollution, criteria to be adopted for the selection of biotopes to be preserved in Europe and list of these biotopes, plant and animal species threatened by extinction, a European label for protected objects; protected areas in Europe, education and information; national legislation on Nature conservation The Committee of Experts asked the Committee of Ministers to recommend that member States (1) take care in pursuing their forestry policies and conifer-planting projects to respect the principles of soil and water conservation and to protect fish and game, (2) ensure the protection of unusual biotopes and the preservation of the character and harmony of the landscape whenever these are threatened by such projects, (3) take preventive measures to conserve aquatic life by reserving a strip of land on either side of water-courses where the natural vegetation must be conserved, (4) hasten the ratification of amendments to the 1954 International Convention for the prevention of Pollution of the Sea by Oil, and the provision of facilities for the reception of oil residues in ports The Committee of Ministers was also asked to repeat its recommendation to Governments to hasten the ratification and full application of the 1950 International Convention for the protection of birds The next session of the Committee is scheduled for July 21, 1964

Systematics

THE Institute for the Comparative Study of History, Philosophy and the Sciences was founded in 1946 to conduct research and other scientific work relating to the problem of human perfectability, or the factors that determine progress or retrogression in individuals and communities Coombe Springs, the headquarters of the Institute, has been a laboratory for studying ideas and methods directed to progress Some of the results of this experimental work have been published, and the desire to bring this work before a wider audience has led to the decision to launch a journal, *Systematics*, which is intended to become the principal medium for the publications of research work conducted by the Institute itself (1, No 1; June 1963 The Journal of the Institute for the Comparative Study of History, Philosophy and the Sciences Pp 1–103 Kingston upon Thames Coombe Springs Press, 1963 Annual subscription 42*s* non-members, 30*s* members Separate issues 11*s* 6d non-members; 8*s* 6*d* members) The title has been chosen as expressing the fundamental notion of integration without rejection, which has been the guiding principle in the work at Coombe Springs For papers to be accepted for publication in the journal they must bear on the systematic hypothesis and show sufficient originality in content or treatment to contribute significantly to the better understanding of human development they may deal with any branch of history, philosophy and science, including psychology Two numbers of the new journal have now been published That for June 1963 includes, besides J G Bennett's paper on general systematics, A. M. Hodgson's short paper on the education of technologists, describing a project of the Integral Science Education Research Group. The September 1963 issue includes a paper, "Systematics of a Manufacturing Process", based on a study by the late C E King, chief engineer of Vauxhall Motors, Ltd , and one by J G Bennett, "Geophysics and Human History", discussing some new light on Plato's Atlantis and the Exodus

Piston Gauge Pressure Measurements

THE dead-weight piston gauge, also called the 'gauge-tester', 'dead-weight tester', 'pressure balance' and 'piston manometer', can be used to measure pressure in terms of the fundamental units of force and area. The piston is inserted into a close-fitting cylinder, and weights loaded on one end of the piston are supported by fluid pressure applied to the other end. The construction varies as to the method of loading, the methods of rotating or oscillating the piston to reduce friction, and the design of the piston and cylinder. Errors in the measurement of pressure with the gauge arise from failure to take into account the parameters of the instrument or environment, or from uncertainty as to their measured values. In National Bureau of Standards Monograph 65, J. L. Cross discusses the variables which affect the pressure measurement and presents a general equation and simplified procedures for the calculation of the pressure (*Reduction of Data for Piston Gage Pressure Measurements*. By J. L. Cross. Pp. iii+9. Washington, D.C.: Government Printing Office, 1963. 15 cents.) Uncertainty of the mass of the loading weights and of measurements of the effective area of the piston and cylinder is an obvious cause of error. Less easily recognized sources of error are air buoyancy on the weights, fluid buoyancy on the piston, the value of local gravity, the force on the piston because of the surface tension of the fluid, thermal expansion and elastic deformation of the piston and cylinder, and the fluid heads involved. In the various appendixes to the monograph, working equations, examples of calculations and guides for compiling correction tables are given.

Farm Motorization, Consumption and Prices of Motor Fuels

Development of Farm Motorization and Consumption and Prices of Motor Fuels in Member Countries is the title of a publication recently issued by the Organization for Economic Co-operation and Development (Pp. 96. Paris: Organization for Economic Co-operation and Development, 1963.) It is essentially statistical in outlook although relieved by pertinent comments on developments of interest to all concerned with European trends in replacement of manpower by machines in agriculture. The report is divided into three sections: (*A*) development of motorization from pre-war until 1960; (*B*) consumption of motor fuels used in agriculture from 1952 until 1960; (*C*) development of prices of motor fuels from 1950 until 1960. In the summary under (*A*), the main conclusions reached are that the number of farm tractors has steadily increased commensurate with the equally steady decline in available active agricultural (human) and draught animal populations. In section (*B*), dealing with consumption of motor fuels in European agriculture during 1952–60, "... it can be stated that the increase by 61 per cent was less noticeable than the rise in the number of tractors by 161 per cent for the same period. Consequently, the annual average consumption per tractor declined for the European O.E.C.D. area as a whole from 2,100 kg in 1952 to 1,600 kg in 1960 and that per horse-power from 82 kg to 66 kg accordingly". It is thought that the future trend will be slowed down because farmers will make a wider and more efficient use of their modern tractors annually compared with past years. As regards prices for motor fuels used in agriculture in countries covered by this report, the conclusion is reached that farmers' expenses for this vital factor in their economy are increasing annually, reaching in 1960 the somewhat staggering total of about 491 million U.S. dollars, naïvely described as "... rather an important cost factor of agricultural production".

Mineral Dust in Industry

THE proceedings of a symposium, *Mineral Dust in Industry*, which was held in the laboratories of the British Ceramic Research Association, Stoke-on-Trent, during December 19–21, 1961, have recently been published (Pp. v+153. London: H.M.S.O., 1963. 22*s*. 6*d*. net.) The symposium was sponsored by the Department of Scientific and Industrial Research which is showing increasing interest in industrial health, which is not unreasonable because of the close relationship between health, the human working environment and the engineering, physics and chemistry of the processes and products which are being handled. In the symposium are papers on pneumoconiosis (dust disease of the lung) in the pottery and foundry industries. The latest magic word 'parameter' comes up in a description of the biological significance of dust parameters but, happily, there is no excess usage of the word 'integrate'. The sampling and control of dust and technicalities of instrumentation are dealt with, as also are ventilation of dusty shops, the design of protective clothing for the workpeople, and the amount of disability which they may suffer if they breathe in dust for long enough. The title of the booklet is a little wide for its subject range, in view of the large areas of the problem unmentioned. The contents possess a medical bias rather than one of fundamental physics and chemistry, and the emphasis is a little on potteries. It is interesting to note that in the study of dusts the physics is well ahead of the chemistry, and little appears to be known, as yet, about what happens on the surface of a particle of dust when it is inside a biological cell. The booklet is well charted and illustrated and will be useful for anyone with either a medical or technical interest in mineral dust.

Nutrient Requirements of Livestock: Poultry

THE first of the Agricultural Research Council's pamphlets on *The Nutrient Requirements of Farm Livestock* has recently been published (No. 1. *Poultry—Summary of Recommendations*. Pp. v+50. London: Agricultural Research Council. Obtainable from H.M.S.O., 1963. 2*s*. 6*d*.) It covers the needs of "all those tame birds reared for their flesh, eggs or feathers, and provides some information about the pheasant". The intention is that the *Summary* shall be brought up to date periodically, and that Technical Reviews will detail the mass of material now abridged. Although special attention has been paid to conditions in the United Kingdom, and therefore to British experiments, the recommendations differ little from those of the U.S. National Research Council since, indeed, American studies predominate overwhelmingly in this field. Feed compounders and farmers will not, therefore, gain an immediate advantage from this statement. However, since each section provides a precise and careful examination of present-day knowledge, it exposes the deficiencies of available information and should stimulate work in these areas. Subsequent issues should profit from this; so, too, should research workers, teachers and students, who will also be grateful for the comprehensive sectional bibliographies. To-day, the poultry industry is changing rapidly both with respect to the breeding of the birds it exploits and in its husbandry techniques. It can be anticipated that its most costly item, feeding stuffs, will be improved concurrently, and that this pamphlet will be a standard reference point in this development.

Folklore in French Canada

Bulletin No. 182 of the National Museum of Canada describes the work of recording folk traditions in the Islands of Saint-Pierre and Miquelon (*Saint-Pierre et Miquelon: Une Mission Folklorique aux îles*. Pp. 192. Par Carmen Roy. Ottawa: Imprimeur de la Reine et Contrôleur de la Papeterie, 1963. 3 dollars.) A description of the area, its history and economy, and its people, introduces the major portion of the Bulletin, which considers the traditions under various headings, including place-names, customs and beliefs, stories and songs. Full transcriptions are given where possible The overall work is an outstanding contribution to folklore studies, and it is hoped to extend this to other areas which also form a part of the Acadian group of French Canada

Bulletin No 188 of the National Museum of Canada describes the moulds of early historic times in Quebec (*Les Moules du Québec* Pp vii+141 (75 plates) Par Robert-Lionel Seguin Ottawa Imprimeur de la Reine, 1963 2 50 dollars) This is the first in a series of *Bulletins d'Histoire* The moulds are classified on the basis of function of their products, and represent the household equipment of a time when manufactured products were otherwise not available The most common are moulds for the shaping of food, maple sugar, butter and pastry Some of these wooden moulds are skilfully carved to produce animal and other figures Moulds for religious purposes, for the making of altar breads, are also included The itinerant tinkers' moulds include sets for religious figures and domestic objects such as spoons, goblets and buttons The report is well illustrated and documented Typical household sets might well have been grouped or listed together Further *Bulletins* in this series should continue to provide more insight into the way of life of seventeenth to nineteenth century Quebec

Archæology in South Africa

THE *South African Archæological Bulletin* for June (17, No 66, 1963), which has recently been published, contains a number of interesting articles Dr J T Robinson describes at some length the Sterkfontein stratigraphy and the significance of the extension site, and later, in part 2, R J Mason describes the tools found, both parts are well illustrated So much has been said about this site, where almost the earliest works of man have been found, that it is admirable that in this number of the *Bulletin* a more or less complete account of the artefacts of these Australopithecines, as well as of where they lived, is now easily available In a supplement to the *Bulletin*, R J Mason pays tribute to the work of the late C Van Riet Lowe and lists his principal publications during the period 1922–57 There is also a report on the Research Unit of the University of the Witwatersrand, to which the Archæological Survey has been transferred

The American Society for the Study of Sterility

THE following three awards will be presented at the annual meeting of the American Society for the Study of Sterility to be held at Bal Harbour, Florida, during May 15–17, 1964 *The Ortho Medal* and a sum of 1,000 dollars will be awarded to a recipient selected by the Awards Committee of the Society on the basis of his outstanding contributions in fertility and sterility or related subjects in recent years. The money is to be used by the recipient for expenses in visiting medical and research centres of his choice *The Rubin Award*, consisting of a certificate of merit and the sum of 250 dollars, will be awarded to the author(s) whose paper is deemed by the Awards Committee to be the most significant contribution among those appearing in the Society's journal, *Fertility and Sterility*, during the year 1963 *The Carl G Hartman Grant-in-aid* of 500 dollars will be awarded to the most meritorious research project in fertility and sterility or related subjects, as chosen by the Awards Committee Applications for the grant-in-aid should be sent to Dr Michael Newton, American Society for the Study of Sterility, 2500 North State Street, Jackson 6, Mississippi, before March 15, 1964, from whom further information can also be obtained

The Night Sky in January

NEW moon occurs on Jan 14d 20h 44m U T and full moon on Jan 28d 23h 23m The following conjunctions with the Moon occur Jan 17d 01h Saturn 2° N , Jan 17d 17h, Venus 3° N , Jan 20d 19h, Jupiter 4° N Venus is in conjunction with Saturn on Jan 9d 22h, Venus being 0 6° S There is a partial eclipse of the Sun on January 14, invisible at Greenwich Mercury is a morning star, but is not well placed for observation Venus is an evening star, setting at 18h 30m, 19h 15m and 20h 5m on Jan 1, 15 and 31, respectively Its stellar magnitude is −3 4, its distance decreases during the month from 128 to 111 million miles, and the visible portion of the apparent disk from 0 862 to 0 786 Mars is too close to the Sun for observation Jupiter sets at 0h 20m, 23h 30m and 22h 40m at the beginning, middle and end of the month, respectively Its stellar magnitude is −1 9 and its distance from the Earth on January 15 is 470 million miles Saturn sets about two hours after the Sun, and conditions for observation are now unfavourable The Quadrantid meteors are active on January 3–4, the radiant being near R A 15h 28m, Dec +50° The Earth is at perihelion on January 2, its distance from the Sun being 91 3 million miles

Announcements

A SYMPOSIUM on "Major Weather Hazard Affecting British Agriculture" will be held in the Department of Geography and Anthropology, University of Wales, on March 18 Further information can be obtained from Mr J A Taylor, Department of Geography and Anthropology, Alexandra Road, Aberystwyth, Cardiganshire Wales

THE eighteenth annual symposium on fundamental cancer research entitled "Cellular Radiation Biology", organized under the auspices of the M D Anderson Hospital and Tumor Institute, University of Texas, will be held in Houston during March 2–4 Further information can be obtained from the M D Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas

THE third European regional conference on "Electron Microscopy", organized by the National Committee for Electron Microscopy of the Czechoslovak Academy of Science under the auspices of the International Federation of Societies for Electron Microscopy, will be held in Prague during August 26–September 3 Further information can be obtained from the Organizing Committee, Albertov 4, Prague 2

A TWO-DAY course on "Expanded Plastics" will be held at the Bradford Institute of Technology during February 6–7 The programme will include chemical aspects of expanded polymers; the physical and mechanical properties of expanded polymers, developments in urethane foams, developments in expanded polystyrene, expanded polyvinyl chloride, expanded ebonite Further information can be obtained from R P Sheldon, Department of Chemical Technology, Bradford Institute of Technology, Bradford 7

A CONFERENCE on "Low and Medium Energy Nuclear Physics", arranged by the Institute of Physics and the Physical Society, will be held in the University of Sussex during September 9–11 Topics under consideration will include nuclear structure, electromagnetic and weak interactions, two nucleon potential and Brueckner theory, nuclear reactions Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S W 1

A JOINT computer conference on "The Impact of the Users' Needs on the Design of Data Processing Systems", arranged by the British Computer Society, the British Institution of Radio Engineers and the Institution of Electrical Engineers under the ægis of the British Conference on Automation and Computation, will be held in Edinburgh during March 31–April 3 Further information can be obtained from the Computer Conference Secretariat, Institution of Electrical Engineers, Savoy Place, London, W C 2

POINT DEFECTS IN NON-METALLIC SOLIDS

DIAMONDS, graphite, oxides and phosphides, zeolites and alumino-silicates all came up for discussion at a conference at Leamington Spa organized by the British Ceramic Society through its Basic Science Committee during October 14–16, 1963. The wisdom of making the programme so wide as regards materials examined, and so well defined in limiting the discussions to the properties of point defects, was exemplified by the liveliness of the discussions and the encouraging overlap of the technical terms now current in the field. Despite the wide selection of materials the majority of the audience was interested for the majority of the time. From the point of view of a physicist, one of the most encouraging features was the growth of the recognition that the basic properties of any pure material need to be isolated from those deriving from the defects it contains. This recognition has spurred on the production of purer materials for investigation. Of course, the example of semi-conductors and the immediate pressure of laser and maser requirements has given a real edge to these considerations, and the preparation of large, highly pure crystals of many halides and a few oxides is now possible.

There are two kinds of purity to be discussed for these ionic substances; chemical contamination by extraneous material must be avoided, but also, perhaps less obviously, the proper proportions of the main constituents to produce a stoichiometric crystal must be maintained. It was with two papers on this topic that the conference opened. It was shown that careful density and lattice parameter work can give information on the numbers of vacant lattice sites, and that extra diffraction lines may betray complex ordering behaviour of the defects. The advantages of neutron diffraction over X-rays were brought out clearly by investigations of oxygen defects in compounds between UO_2 and U_4O_9. Another paper in this section showed how the electron microscope can identify small clusters of defects as interstitial atom groups rather than vacancy aggregates.

The difficulties of positive identification of individual defect centres in II–VI compounds were outlined, but the conference quickly settled down on the second day to detailed discussion of recent experimental information on alkali halides, diamond, magnesia and rutile. Most of these papers sought to set up models to account for the electron spin resonance or optical properties. In diamond, magnesia and calcium fluoride, many of the optical bands have now been shown to have sharp, zero-phonon, lines at the high-energy edge of the band. It is often of good intensity and, in one case at least, was split by application of stress to the diamond. There seems no doubt that such lines will repay more detailed investigation than has been possible on the broad bands seen hitherto.

Coloration in rutile under irradiation has been investigated at Brookhaven Laboratories, but virtually no specific effects in the optical spectrum can be identified. A general small rise in absorption is all that has been seen in room temperature irradiation experiments on crystals of carefully controlled stoichiometry. Photoconduction peaks were, however, produced by neutron irradiation.

Impurity coloration due to cupric ion in a number of solvent crystals—ZnO, MgO and ThO_2—was interpreted as demonstrating marked differences in the ion's electric levels in crystal fields of different symmetries. The effects of tetragonal Jahn-Teller distortions from cubic symmetry were identified and values of the crystal field splitting parameter tabulated. The explanation of the susceptibility of the cupric oxide crystal itself seems to demand some significant antiferromagnetic coupling of pairs of spins even in the paramagnetic state.

The next section of the meeting was concerned with atomic mobility in solids. It was interesting to have some discussion at last of the role of anion vacancies and interstitials in simple ionic crystals, and some experimental evidence that they could not be permanently ignored was presented. The analysis of diffusion mechanism has always been fruitful in identifying the most mobile of the possible atomic defects, and it is most impressive now to hear of the delicacy and accuracy of the experiments which allow the relative rates of motion of different isotopes to be followed. The measurements on ^{22}Na and ^{24}Na in sodium chloride have shown again that the most exacting test is still satisfied by the idea that anion vacancy diffusion is dominant. That the fluctuation in the kinetic energy to carry the system to the 'activated complex' state resides mainly on the jumping atom is established by the closeness with which the basic rates are proportional to the square root of the mass of the jumping atom. The discussion brought to light some interesting measurements from the Imperial College of Science and Technology on the charges absorbed on dislocations, which were interpreted as a measurement of the different partial supersaturation of positive and negative ion vacancies in sodium chloride, and hence of their energies of formation. Despite some reservations as to the rigour of the argument, this seemed a promising new departure. Another short contribution from the same group showed how the temperature range, where diffusion rates are intrinsic, can be extended in the alkali halides by adding oxide or sulphate ions to depress the number of non-thermal free positive-ion vacancies. The observed absence of a reduction in diffusion coefficient below the intrinsic line is hard to understand. This section closed with an address dealing with the significance of the non-equilibrium thermodynamic formulation of atomic mobility, which showed that, despite (or because of ?) its generality, it was easy to fall into error by failing to define carefully all the variables and their interdependences. For example, vacancy flux must be introduced with care since it can be deduced from atomic fluxes for some boundary conditions and not for others. It was concluded that it was worth while to develop some familiarity with the formation by translating the results of some atomic models into the language of irreversible thermodynamics, before trying to use the technique in unfamiliar situations.

Prof. Barrer gave a succinct guide to the features controlling ionic diffusion in various zeolite-type structures. In some of these, wide continuous channels exist and diffusion barriers are essentially electrostatic in nature, but in others, narrow points separate atomic cages, and repulsive forces come into play. The connexions between surface chemistry, surface diffusion, and bulk diffusion became obvious, and the practical importance of interdisciplinary conferences of this kind was again emphasized in the minds of those present.

The intricate question of how ionic is a salt was raised by a paper on the E.M.F. of the diffusion cell. It was shown that the transport of charge during diffusion of, say, aluminium atoms through alumina is not necessarily precisely that corresponding to the motion of Al^{+++} ions. The actual value depends on the precise mechanisms of compensation of the charges of the defects responsible for diffusion.

The final section related to the mechanical properties of ionic solids, as affected by point defect. The introductory lecture by Prof. Pratt showed clearly that two main categories of effects could be considered, the direct effects of defects or their aggregates, and the indirect effect of the interaction of the defects with pre-existing dislocation or dislocations formed by mechanical deformation. It appears that in some circumstances a main effect may be the indirect pinning of dislocations by their electrostatic or stress interaction with re-orientable dipole-type defects. A short paper on sodium chloride with controlled concentration of divalent cations supported this view. The nature of the relaxation of dipoles formed by trapped defects was discussed in group theoretical terms in the next paper, and it was shown that except for very short times a single relaxation time appropriate to nearest neighbour orientations of the defect is reasonably accurate.

The effects of irradiation on the plastic properties of sodium chloride showed a rise in yield stress, a tendency to smaller slip-step heights and to simultaneous slip on equivalent planes with suppression of kinking. None of the changes was related to *F*-band colour, but *V* band and interstitial density were not checked. The effects observed in the Youngs modulus of polycrystalline graphite after low-temperature electron irradiation appeared to be due to the pinning of dislocations, but no detailed mechanism could be proposed.

Electron microscope examinations have been made on a number of deformed and defective crystals. The development of oxygen deficiency in titanium dioxide helps the annealing out of stacking faults by a mechanism which may well involve the dissolution of titanium interstitial atoms, after condensation of oxygen vacancies has eliminated planes of oxygen. The titanium interstitials are in fact identified by colour studies. Other dislocation reactions have also been observed. A separate group reported an increase in optical absorption associated with an increase in titanium interstitial content after high-temperature plastic deformation of an already oxygen-deficient titanium dioxide crystal, and also attributed this to either stacking fault elimination or to dislocation climb. In view of the lack of structure in the optical absorption investigations, several comments were made to the effect that an increase in titanium interstitial content could not yet be accepted as proved. Electron microscope examinations were also reported on plastically deformed TiO, and annealing investigations pointed to an activation energy of some 5.5 eV for atomic mobility.

To sum up, this was a good conference to be at. It brought together groups of people who meet too seldom, and showed again the unity of the physical sciences. It was the right size—about 120 people—and the programme was not so packed that it was impossible to find a moment to talk to most of those one meant to. The wisdom of holding the conference during university term, when many people found it hard to be away for three days, and when it was not possible to use the lecture theatres of a university, may be queried. A proper lecture room is really quite important, though not nearly so important as the programme, content and participants. I am sure the success of this meeting will encourage the British Ceramic Society to hold more meetings of similar kind to bring these neglected substances under regular scientific review.

W. M. Lomer

MECHANISMS OF ADSORPTION FROM SOLUTION

An informal discussion of the Faraday Society under the above title was held in the Department of Mechanical Engineering, Imperial College of Science and Technology, during September 19–20, by kind permission of Prof. O. A. Saunders. The meeting was attended by 144 people, 81 of whom came from industrial organizations and 63 from academic institutions. There were 16 overseas visitors.

Sir Eric Rideal opened the meeting and, as the first item, Dr. J. J. Kipling (University of Hull) gave a review of the present position in this subject. After outlining the main historical periods, and the progress which was made in each, Dr. Kipling discussed at some length the problems encountered when, instead of an adsorbing vapour, we have to deal with a two (or more) component liquid system. The basic equation relating changes in the surface and bulk mole fractions of the components was given, and its relation to the Gibbs surface excess and the individual adsorption isotherms for the components was described. The use of adsorption from solution for measurement of surface area was examined critically, and, in particular, the difficulty of selecting an appropriate cross-sectional area for the adsorbate molecule was stressed. The difficulty of satisfactorily taking into account localized site adsorption was also discussed. Finally, Dr. Kipling mentioned the question of chemisorption of molecules from solution and foresaw the problem well known in vapour adsorption of distinguishing between chemically and physically adsorbed molecules.

The first paper was given by Prof. D. H. Everett (University of Bristol) and was entitled "Thermodynamic Models of Adsorption from Solution". The adsorption of the components of a binary liquid mixture on to a uniform surface was described in statistical mechanical terms and a new definition of the activity coefficients of the components in the adsorbed layers was proposed. Prof. Everett showed that the "isotherm of concentration change" may be markedly dependent on the nature of the solution concerned, for example, whether it is a regular solution, or contains molecules of different size. The use of adsorption from solution for the determination of specific surface areas was mentioned and it was suggested that useful results could be obtained from measurements of adsorption from mixtures of molecules of approximately the same size (for example, *cyclo*hexane + benzene).

This was followed by a paper on the "Thickness and Structure of Layers of High Polymers adsorbed from Solutions to Solid Surfaces", by F. Rowland, R. Bulas and Prof. F. R. Eirich (Polytechnic Institute, Brooklyn, New York). The paper was given by Prof. Eirich, who explained the difficulties and uncertainties of investigating the nature of polymer adsorption and then presented results obtained principally by observing the change in flow of liquids through sintered glass disks of known pore size distributions and surface areas. From an analysis of the hydrodynamics of the system, the change in effective radius of the capillaries resulting from polymer adsorption was estimated. Several polymers in different non-aqueous solvents have been examined and it was shown that the film thicknesses were of the same order of magnitude as the diameters of the free coils in solution. A detailed discussion was given of the nature of the adsorption isotherms, of their reversibility and of the influence of good and bad solvents.

A contribution to the investigation of the adsorption of ionized polymers was made by J. B. Craig and Dr. P. Meares (University of Aberdeen) under the title "The Adsorption of Polyacrylates at a Charged Mercury–Salt Solution Interface". Experimental data had been obtained from electrocapillarity investigations, and from derived thermodynamic relations, the surface excess concentrations of polyanions, counter-ions and co-ions were estimated. The plot of surface excess of polyanion against increasing bulk concentration showed a maximum followed by a decrease to an almost constant value. A detailed model was proposed to account for this and related phenomena which was based on configurational changes of the polyanions normal and parallel to the surface. From the calculated co-ion surface excesses an estimate was made of the thickness of the adsorbed polyion film; this appeared, in some cases, to be greater than one monolayer.

On the following morning, under the chairmanship of Dr J. A. Kitchener, Dr C. H. Giles (University of Glasgow) presented a paper on "The Association of Adsorbed Organic Solutes". The adsorption of a number of organic molecules, many of them dyes, on to a variety of adsorbents was described and compared with the surface area indicated by *p*-nitrophenol adsorption. The differences relative to *p*-nitrophenol were expressed as 'coverage factors', and it was shown that these factors bore a simple relation to the molecular weight of the adsorbate. The 'coverage factors' were suggested to arise from the adsorption of the organic molecules as an aggregate or micelle. Some direct evidence for such a phenomenon was presented.

"Adsorption of Cyanine Dyes at Silver Halide Surfaces" was the title of a paper by Dr J. F. Padday (Kodak, Ltd.). The surface areas of specimens of silver chloride, bromide and iodide were determined from argon and benzene vapour adsorption. It was deduced that under conditions of close packing of the cyanine dyes at the crystal surfaces the area per molecule of a given dye was different for the various halides. From this evidence and from measurements of spectral reflectance the manner in which the substrate affected the state of the dye was discussed.

The chairman for the final session was Prof. F. C. Tompkins. In the first part Dr I. T. Smith (Paint Research Station, Teddington) gave a paper on "An Infrared Spectroscopic Study of the Adsorption of Carboxylic Acids on Titanium Dioxide Pigments". After adsorption equilibrium had been established in each case, for a variety of carboxylic acids in benzene in contact with several different samples of titanium dioxide, the oxide was made into disks and examined by absorption spectroscopy. The results suggested that the acids were bound to the oxides, either through a carboxylate ion, or by means of hydrogen bonds. Both types of bonding could usually be found for a given pigment. It was found that on washing the oxide with its adsorbed molecules in pure solvent the hydrogen bonded molecules, but not perceptibly the ions, were removed. Immersion in the original acid solution restored the specimen to its original state.

The meeting ended with an extended discussion on the adsorption of polar molecules on to metal oxides. Dr J. J. Kipling and Dr E. H. M. Wright mentioned that stearic acid molecules adsorbed from benzene on to silica lay flat on the surface, but on alumina and titanium dioxide were approximately vertical. They also showed that for stearic acid on titanium dioxide and alumina the adsorption was close to that expected for a localized adsorption on to lattice points. Dr D. A. Haydon and P. T. Dawson (University of Cambridge) described the effectively irreversible adsorption at 20° C of formic acid from the vapour phase and stearic acid from benzene on three different dry activated rutiles. The possible reasons for a slightly lower adsorption, in all cases, of stearic acid relative to formic were discussed. Both stearic and formic acids gave fractional coverages relative to nitrogen of approximately 0.5. Dr G. D. Parfitt and I. J. Wiltshire (University of Nottingham) discussed the adsorption of a homologous series of aliphatic alcohols from xylene on to a pure rutile. The amount of alcohol adsorbed showed a strong dependence on chain-length, being a minimum at octanol. Prof. Everett commented that such behaviour might be expected from his theoretical treatment of adsorption. The important part played by adsorbed water on the rutile surface in the adsorption process was also stressed.

During the meeting it was announced that it was proposed to hold a further meeting during September 17–18, 1964, in the Chemistry Department, University of Bristol, on the subject, "The Electrical Double Layer in Surface and Colloid Chemistry".

D. A. HAYDON
R. H. OTTEWILL

METABOLISM AND PHYSIOLOGICAL SIGNIFICANCE OF LIPIDS

LIPID biochemistry is one of the scientific topics which have become prominent during the past fifteen years, due both to major technical advances easing their study and to fundamental observations showing their importance. Thus only part of the field could be surveyed at the advanced study conference on the metabolism and physiological significance of lipids, held at Cambridge during September 16–21, 1963, and supported by the Scientific Affairs Division of NATO, although about 150 participants (from institutions in sixteen countries, of which six do not belong to NATO) heard 38 formal discourses given at nine day-time sessions, besides two informal evening symposia, and were admirably provided by the organizers, Drs R. M. C. Dawson and D. N. Rhodes (Great Britain), with time and electronic encouragement for free discussion.

The first major topic was lipid biosynthesis. Prof. S. J. Wakil (United States) began by describing how animals form long-chain fatty acids. First, a reductive synthesis of saturated acids from malonyl and acetyl coenzyme *A* gives mainly palmitate; it, or longer-chain acids, can then be oxidatively desaturated. Secondly, in the most recently examined process, acetyl coenzyme *A* provides two carbon atoms for the reductive chain-lengthening of saturated or unsaturated acids; thus palmitoleate gives *cis*-vaccenate, now known to be an important component of 'oleate'.

According to Prof. K. Bloch (United States), some organisms form unsaturated acids anaerobically by the dehydration of hydroxy acids, but most do so aerobically. Only green plants can make linoleate, which is then further dehydrogenated via α-linolenate; or, in animals, via γ-linolenate. The route used and the type of compound lipid produced by green euglenids depend on whether they are functioning as plants or animals. Photosynthetic bacteria do not produce polyunsaturated fatty acids, which may therefore be specifically concerned in plants' oxygen-yielding type of photosynthesis.

Prof. B. Shapiro (Israel) said that synthesis of triglycerides by the labile enzyme systems of liver preparations *in vitro* is greatly assisted by lecithin or by serum proteins, and closely dependent on the concentration of α-glycerophosphate, which varies enormously under different physiological conditions *in vivo* and may therefore control the process.

Dr G. Popják (Great Britain) then showed how deuterium-labelled mevalonate and tritium-labelled pyridine nucleotides of known absolute configuration have been used to establish the precise stereochemical specificity of the reactions whereby mevalonate is converted via its pyrophosphate and isopentenyl, geranyl and farnesyl pyrophosphates into squalene, the precursor of steroids.

Prof G V Marinetti (United States) had examined glycerol metabolism in rat liver homogenates, important products were dihydroxyacetone, phospholipids and glycerides. Adenosine triphosphate stimulated lipid biosynthesis, with high concentrations, the only lipid formed was phosphatidic acid

According to Dr R O Brady (United States), the first specific precursor of animal glycolipids is palmitaldehyde its acquisition from serine of one nitrogen and two carbon atoms and subsequent dehydrogenation yields sphingosine, which then reacts with uridine diphosphate galactose to form psychosine, reaction of an acyl coenzyme *A* with the amino group to form the amide then completes the biosynthesis of a cerebroside Higher glycolipids probably result from the addition of further sugars, etc , to the cerebrosides

Dr J Asselineau (France) then explained how the very large and complex molecules of the higher fatty acids of mycobacteria are formed by comparatively simple mechanisms, comprising methylations, such as that of oleate to give tuberculostearate, the condensation of propionate residues with each other and with higher acids yielding the phthienoic and mycocerosic acids, and finally, the condensation of long-chain α-hydroxy acids to give the branched-chain hydroxylated mycolic acids of nearly 90 carbon atoms

Two plant fatty-acid-forming systems described by Prof P K Stumpf (United States) resembled animal mechanisms examined by Prof Wakil, one synthesized saturated acids from malonyl coenzyme *A*, while the other lengthened the carbon chain of assorted acids, ricinoleate being one product Thirdly, the safflower seed made linoleate from acetyl rather than malonyl coenzyme *A*, a single gene controlled the synthesis of linoleate instead of oleate

Discussion of the further metabolism of lipids began with an examination of the enzymatic hydrolysis of fats Dr P Desnuelle (France) has developed a simple and efficient means of purifying pancreatic lipase, it requires finely emulsified water-insoluble α-glycerides as substrates, and the liberated fatty acids prevent long-chain β-monoglycerides also produced from isomerizing to α-monoglycerides, which would be poor substrates for mucosal fat re-synthesis Any short-chain β-monoglycerides, however, isomerize and are destroyed

Prof L L M van Deenen (Holland) went on to describe how the unambiguous synthetic work of his laboratory has firmly established the specificities of the phospholipases, which can now safely be used to elucidate the structures of natural lipids Thus it has been shown unequivocally that the plasmalogens and the batyl or chimyl phosphatides are α′-glyceryl ethers, not β-glyceryl compounds as first thought, again, all phospholipases attack phosphatidylglycerol C, yielding a diglyceride and D-α-glycerophosphate

The substrate specificities of several phospholipases have been shown by Dr R M C Dawson (Great Britain) and his colleagues to be matters not only of chemical structures but also of micellar surface charges, which can be measured as electrophoretic mobilities and regulated with suitable counter-ions This can even be demonstrated with the specific phosphatases attacking the polyphosphoinositides, which are virtually water-soluble

The unusual metabolism of these very polar compounds in animal tissues was then discussed by Dr J N Hawthorne (Great Britain) They probably arise from the phosphorylation of monophosphoinositide, which is in turn formed by a unique reaction between cytidine diphosphate diglyceride and free inositol A possible function of the inositol phosphatides may be the transport of divalent cations across mitochondrial membranes

The gaps in our knowledge of the metabolism of plasmalogens were then exposed by Dr E F Hartree (Great Britain), the fatty acid of choline plasmalogen is a major source of energy for mammalian spermatozoa, but little is known about the enzymatic rupture and synthesis of the α′-1-alkenyl ether bond It seems to be formed from a long-chain acyl-coenzyme *A*, yet such compounds can be reduced enzymatically giving free aldehydes which are not incorporated into plasmalogens

At an informal and lively symposium that evening, many examples were given of the great but mysterious physiological importance of the structures of certain fatty acids, which is only partly explicable by their physical properties but also depends on the particular lipids in which they occur.

The conference next examined the digestion and absorption of fat– Prof B Borgstrom (Sweden) explained how triglycerides are emulsified by bile salts and digested by pancreatic lipase to give free fatty acids and β-monoglycerides, this mixture is readily absorbed, helped by the re-synthesis of fat in the mucosa In the absence of bile salts lipase itself catalyses triglyceride synthesis

Dr G H Hubscher (Great Britain) then outlined two other and probably more important processes of mucosal fat re-synthesis, both requiring coenzyme *A* for the acylation of α,β-diglycerides, which arise either directly from β-monoglycerides or by enzymatic dephosphorylation of phosphatidic acids produced by acylation of L-α-glycerophosphate The phosphatic synthesis needs two new co-factors, one thermolabile, the other probably related to carnitine

Quantitative ideas of fat absorption and transport *in vivo* have, according to Dr A M Dawson (Great Britain), been seriously distorted by technical difficulties of measurement It now appears both that free glycerol can be an important source of glycerophosphate for mucosal triglyceride re-synthesis, and that up to a third of the long-chain acids of ingested fat pass into the portal vein in the free state

Drs W M F Leat and T Gillman (Great Britain) then dealt with the absorption, transport and clearance of fat by ruminants, they absorb neutral fat poorly and cannot tolerate it as an intravenous emulsion, but readily absorb free fatty acids which are then kept at a steady level in the plasma by hormonal regulation.

The conference then turned to its second major theme, the physiological significance of lipids in the general economy of animals and bacteria, beginning with a session devoted to large-scale events in living animals

Dr D S Robinson (Great Britain) began by suggesting that only a small amount of fat is supplied to tissues by mammalian blood as free fatty acids, since a large proportion of any available are rapidly converted by the liver into plasma lipoprotein triglycerides, the latter are readily used by the tissues after hydrolysis by lipoprotein lipase, which is probably in the capillary endothelium and is controlled both by the tissues' needs and by the supply of fat

Even in ruminants, said Dr E F Annison (Great Britain), isotope dilution experiments have shown that glucose is the main substrate for respiration *in vivo*, long-chain fatty acids are used anabolically, as in other animals, although acetate is oxidized to some extent

Dr R G Macfarlane (Great Britain) summarized the confused history of theories connecting lipids with blood clotting Two stages in this very complex process still appear to require lipids, even after the elimination of misleading phenomena such as non-specific 'contact activation' and the massive thromboses produced by free fatty acids The lipids' micellar surface charges seem to be more important than their chemical structures

Ruminants have peculiar depot fats because, said Dr A G Garton (Great Britain), all the fatty acids which they absorb arise either by microbial alteration of the dietary lipids or by digestion of the rumen micro-organisms themselves The former process yields short-chain acids from glycerol and galactose, and partially hydrogenated and isomerized long-chain acids, branched-chain acids are derived from bacterial protoplast membranes.

The ruminant's milk fatty acids, on the other hand, have, according to Prof G Peeters (Belgium), been shown by recent experiments *in vivo*, and with perfused isolated udders, to be mostly synthesized from blood acetate, with a small contribution from propionate, long-chain acids, however, can be obtained from the plasma's scarce triglycerides, but will only exchange with its free fatty acids, without net uptake

The next session was concerned with the locations and properties of lipids in the surfaces and internal parts of cells Prof W Bartley (Great Britain) described the lipid compositions of the intracellular organelles of rat liver and brain The fatty acids are characteristic of the lipids rather than of the organelles, except that 'essential' fatty acids are unusually abundant in mitochondrial cardiolipids, which may be functionally important, being the only mitochondrial lipids remaining in oxidatively phosphorylating 'digitonin particles'.

Possible structures of animal cell membranes were then discussed, Drs J A Lucy and J T Dingle (Great Britain) demonstrated that certain lipid mixtures could give electron micrographs showing hexagonal arrays of uniform spherical micelles, on the other hand, Drs. A D Bangham and R Horne (Great Britain) had obtained pictures confirming the more usual idea of a lamellar structure

Dr. M G Macfarlane (Great Britain) then surveyed the lipids of bacterial protoplast membranes, which contain some unique compounds such as the phosphatidyl glyceryl amino-acids and diacyl glyceryl mannose, besides resembling mitochondria both functionally and in being rich in acidic phosphatides Their fatty acids are mostly saturated, but a large proportion have highly branched chains, and hence show physical resemblances to the polyunsaturated acids essential for higher animals

Drs L E and M R Hokin (United States) then described how secretory activity and the turnover of phosphatidic acid labelled with phosphorus-32 in avian pancreas or salt gland are reversibly stimulated by pancreozymin or acetylcholine *in vitro*, when the hormone is removed, the label goes to phosphatidylinositol This may represent the activation and inactivation of a sodium carrier by the conversion of phosphatidylinositol to phosphatidic acid and vice versa

The second evening symposium was devoted to a popular and stimulating discussion of recent progress in the separation and analysis of lipids by chromatographic methods, with perhaps the greatest interest being shown in thin-layer chromatography

Some pathological and endocrinological aspects of lipid metabolism were then examined Dr C R C Heard and Prof B S Platt (Great Britain) reported some deleterious effects of low-protein diets on young animals With the worst diets, even a trace of casein was very beneficial, whereas extra carbohydrate led to obesity, œdema, fatty livers and low glucose tolerance, besides severe regression of the thyroid and, suggestively, of the β-cells of the islets of Langerhans

Dr. K R Rees (Great Britain) then showed how another defect in protein metabolism may be the direct cause of the fatty livers induced by, for example, poisoning with carbon tetrachloride; the poisoned liver converts plasma free fatty acids into triglycerides as usual, but can no longer return them to the plasma in low-density lipoproteins Certain detergents, however, will move this fat into the bloodstream, thus preventing fatty livers

Prof W Fromkel (United States) had found that, *in vitro*, adipose tissue's utilization of glucose for respiration and fat synthesis was greatly and immediately stimulated by insulin, the effect persisting somewhat after the hormone's destruction, while thyroid slices treated with thyroid stimulating hormone oxidized more, not less, glucose when fatty acids were added, and incorporated them into kephalins rather than triglycerides these effects resembled events *in vivo*

Dr G B Ansell (Great Britain) reviewed present knowledge of the effects of electricity and drugs on lipid metabolism in the nervous system, and described some experiments with chlorpromazine It was known to increase the turnover of phosphatidic acid and phosphatidylinositol in brain slices, but decreased that of lecithin, probably by inhibiting the formation of cytidine diphosphate choline

Leucocytes will ingest starch particles by phagocytosis, or, when stimulated by deoxycholate, inulin solutions by pinocytosis Prof M L Karnovsky (United States) reported experiments in which these phenomena, presumably requiring the formation of much new cell membrane and hence expected to stimulate phospholipid metabolism, were indeed accompanied by six-fold increases in the turnover of phosphatidic acid, with smaller increases for other acidic phospholipids

The last afternoon session was devoted to the nervous system Prof R Rossiter (Canada) gave an account of the ability of brain to synthesize phospholipids *in vitro* Preparations from young animals show the usual 'Kennedy pathway', as well as a base-exchange reaction interconverting phosphatidylethanolamine and phosphatidylserine, a decarboxylation of the latter, and an especially rapid synthesis of plasmalogens Phosphatidylinositol is rapidly formed from cytidine diphosphate diglyceride; di- and tri-phosphoinositide are even more actively metabolized.

On the other hand, Dr A N. Davison (Great Britain) and his colleagues have confirmed that the myelin sheathing the larger nerve fibres of the central nervous system does not metabolize in adults, it has a negligible enzyme content, and isotopically labelled cholesterol, cerebrosides, sulphatides, sphingomyelin, ethanolamine plasmalogen, phosphatidyl serine and proteolipid proteins, once laid down in young animals, persist throughout life

Prof R. H. S. Thompson (Great Britain) went on to outline the evidence for a possible explanation of the disappearance of myelin, normally so stable, from the lesions of disseminated sclerosis An observed deficiency of essential fatty acids could cause mitochondrial failure in the metabolically active oligodendroglia, and hence cell lysis with the release of phospholipases, known to occur in brain and to form the myelinolytic lysolecithin, which also lyses cells to release more phospholipase; this autocatalytic process will stop only when the systems removing lysolecithin regain control

Prof L Svennerholm (Sweden) then outlined the complex interrelations between the gangliosides of brain neurones, in connexion with Tay-Sachs disease. The infant victims' brains accumulate excessive quantities of an unusually simple ganglioside, due to a deficiency either of the enzymes normally destroying it or of those which would normally use it for the synthesis of higher gangliosides by attaching further galactose, *N*-acetylgalactosamine and *N*-acetyl-neuraminic acid residues to it

Prof F R N. Gurd (United States) opened the final meeting by demonstrating, from the structure of myoglobin, the specific bonding of 'lipids', such as the non-polar groups of hæm, with the globin's non-polar amino-acid residues, which are collected together away from the protein's polar regions

Dr D. E Green (United States) went on to describe analogous properties of mitochondria, careful extraction with aqueous acetone leaves their protein skeletons intact but abolishes their enzymatic activities, which can then be restored by treatment with aqueous emulsions of the extracted lipids Lecithin will reactivate the dehydrogenases, but both coenzyme *Q* and acidic phosphatides—preferably cardiolipins—are needed for oxidative phosphorylation, which seems to involve stoichiometric reactions between the cytochromes and groups of the lipids' polar 'heads', the hydrophobic 'tails' being attached to the non-polar regions of the protein skeleton.

Taken together with observations by other speakers, including Prof K Bloch, Prof W Bartley and Dr M G Macfarlane, Prof Gurd's and Dr Green's findings suggest one possible aspect of the 'essential' nature of particular fatty acids These acids are prominent in the lipids of the main energy-yielding apparatus of the bacterial, plant or animal cell, perhaps the non-polar regions of certain vital sections of the protein skeleton of this apparatus require specific structures in the hydrophobic 'tails' of the lipids which must be present for full enzymatic activity to be displayed

G. H Sloane-Stanley

CHARACTERIZATION AND USES OF HUMAN DIPLOID CELL STRAINS

A SYMPOSIUM on "The Characterization and Uses of Human Diploid Cell Strains (HDCS)", sponsored by the Permanent Section on Microbiological Standardization of the International Association of Microbiological Societies, was held in Opatija, Yugoslavia, during September 24–26 Ninety-six delegates from 18 countries attended The sessions covered the following topics (1) The management of human diploid cell strains (2) Biochemical, cytogenetic and immunological studies on human diploid cells (3) Virus spectrum of human diploid cells (4) Production of human virus vaccines in human diploid cell strains (5) Results of clinical trials on the use of vaccines prepared in human diploid cell strains

Many laboratories receiving the human diploid cell strains reported their success in propagating the cells and attributed most of their early failures in handling these strains to variations in media constituents The cells showed the classic normal human diploid karyotype until approximately the 40th cell generation

It was reported that, at least for some loci, cultured diploid cells carry and express the genes of their donor The human diploid cell strains retain the donor's chromosomal complement, they also maintain biochemical and immunological properties of the donor cells In a sense these observations are complementary, for they indicate at two structural levels an apparent genetic resemblance between the cultured cells and the somatic tissues of the host

The numerous attempts to isolate latent viruses from these cells have been unsuccessful Dangers from extraneous oncogenic viruses, inherent in the use of primary explants of animal tissue, are largely circumvented in the opinion of many workers when human diploid cell strains are used for vaccine production

Investigations on the virus spectrum of human diploid cell strains indicated their susceptibility to many viruses These strains were thought to be particularly valuable in work with the rhinoviruses which cannot generally be detected in other cell substrates Since different human diploid cell strains vary in their sensitivity to rhinoviruses, it was felt important to use the most sensitive strains

Both live attenuated and killed vaccines for parenteral inoculation or oral administration have been made in human diploid cell strains from poliovirus, rhinovirus, adenovirus, varicella, measles, vaccinia and rabies Following adaptation to human diploid cell strains, all viruses investigated were said to give yields equal to those in other cell systems Investigations with poliovirus showed that they retained their genetic markers when propagated in human diploid cell strains

It was urged that all laboratories using human diploid cell strains for vaccine production limit themselves to a few standardized cell strains, since the use of a well-characterized tissue culture system is as logical and necessary as the use of a well-characterized virus to be propagated in this system

Clinical investigations on a number of virus vaccines are in progress It was reported that an oral poliomyelitis vaccine produced in human diploid cell strains has already been successfully tested in a large-scale field trial and that no untoward reactions have been noted in 7,000 subjects who have received vaccine during the past two years It was also reported that such vaccines proliferated in the gastro-intestinal tract and elicited an antibody response

On the basis of the investigations suggesting the feasibility of large-scale production of virus vaccines in human diploid cells strains, a sub-committee of the conference drafted "Minimum Requirements for Human Diploid Cell Strains to be Used in Vaccine Preparation" These requirements were presented to the symposium participants and adopted at a plenary session

Leonard Hayflick
Frank T Perkins
Robert E Stevenson

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1963*, includes the Minister's statement, the report of the Council, the longer report of the Secretary, to which are attached brief reports from the various Divisions, the Incorporated Research Associations, and on granted-aid research in the Cawthron Institute, Lincoln College, Massey University College of Manawatu, and the Industrial Development Department, University of Canterbury The fifth annual report of the Ross Dependency Research Committee is appended, to which, as to the Divisional reports, a list of publications is attached The Council in its report reiterates the view that a much more comprehensive review of research is needed than was given by the Royal Commission on State Services, but supports the proposal to replace the present Council, which is advisory only to the Department of Scientific and Industrial Research, with a National Research Council responsible for advising the Government on all aspects of research in Government Departments The new Council should be advised by a number of expert committees, but the Department of Scientific and Industrial Research must retain many of the existing expert committees Research and development expenditure in New Zealand in 1961–62 was still only 0 3 per cent of the gross national product or £1 9 per head of the population, the Department's gross expenditure of £2 19 million being 0 163 per cent of the gross national product Staff at March 31, 1963, numbered 1,229, including 455 scientific officers, compared with 1,170 and 442, respectively, the previous year An increase of 60 in the staff has been approved for 1963–64, as well as re-equipment of the Dominion Physical Laboratory over the next three years at an estimated cost of £120,000

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* New Zealand Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1963 Pp 87 (H 34) (Wellington Government Printer, 1963) 3s

The Council's report also notes the steady improvement in the educational qualifications of the Department's scientific officers, and the present staff now includes 17 with a D Sc degree and 99 with a Ph D The senior research fellowship programme continues to be most successful, and an increase in the number of awards from two to five is recommended Grants to research associations in 1962–63 totalled £169,720, to universities and agricultural colleges £37,070, and to the Antarctic Division £126,607 The highest items in the total expenditure of £2,389,000 are £237,000 on the Dominion Laboratory and £238,000 on the Dominion Physical Laboratory The only other items exceeding £100,000 are £132,000 to the Geological Survey, £154,000 to the Geophysics Division, £114,000 to the Grasslands Division, £105,000 to the Plant Diseases Division, and £137,000 to the Soil Bureau, but £90,000 went to the Crop Research Division

The Secretary's report stresses the value of geochemical prospecting for mineral resources in New Zealand and gives some details of the iron-sand investigations in North Island Field trials are demonstrating the excellent performance, under farm conditions throughout New Zealand, of the new hybrid ryegrass developed by the Grasslands Division over the past fifteen years The Plant Chemistry Division has been seeking to find a culture suitable for bacteria of lucerne, clover and trefoil Two enzyme systems which hydrolyse or decompose sucrose are now being investigated in some New Zealand soils, particularly under introduced pasture With the present-day retreat of the Franz Josef Glacier, the opportunity has been taken to examine the microbiology of a series of soils in process of formation from unweathered glacial detritus Besides investigations on the rate of chemical breakdown, residue and metabolism of demeton methyl, phosphamidon, guthion and dimethoate (Rogor) used to control aphids, a programme of research has been initiated with the view of integrating biological and chemical methods of controlling pests

An Australian method has been applied successfully in the ripening of peaches for canning to prevent loss from brown rot, and field trials have established the considerable scope for mechanical soil fumigation in New Zealand Immediate relief from the chironomid midge in oxidative ponds has been obtained by the judicious use of insecticides, and there seems every prospect of a permanent solution of the problem Reference is also made to the work of the Botany Division in connexion with the control of noxious weeds and on the incompatibility of plants A review has been prepared of the features of the Antarctic ionosphere during calm and disturbed conditions, and the effect of γ-rays and X-rays on certain sugars has been examined with an electron spin resonance spectrometer Five narrow-angle Geiger tube telescopes have been installed at the Institute of Nuclear Sciences to investigate the daily variations in the intensity of cosmic rays coming from outer space, while industrial research has shown that high-grade rayon pulp can be made from *Pinus radiata*, which can be used to manufacture dissolving pulps A simple and effective instrument has been developed for comparing reflexions from opposite surfaces of sheet glass and thus give a measure of its 'waviness' or distorting properties.

TALENT FOR TO-MORROW

A BOW Group pamphlet, *Talent for Tomorrow**, by R Smith and R Eddison, arguing that any policy of reform of higher education must be based both on a thorough understanding of the way in which the present system works and on some attempt to estimate what will be required of it in the future, discusses the problem in the light of higher education as a determining factor in the character and effectiveness of primary and secondary education It is in the universities and colleges that teachers are taught the fundamentals of their profession Such institutions act not only as a vital factor in our economic potential but also as a means of providing a haven for older men to think as well as for younger men to learn Educational policy is not simply a matter for Cabinet discussion and a few crucial decisions at the top it depends on many decisions at many levels, and, while arguing for closer integration of the political machinery at the centre, the pamphlet also insists on the need for considerable devolution in policy making

Surveying in the first of the four chapters of the pamphlet the national need, the Bow Group maintains that the major problem in the next twenty years, given continuing economic growth, will be that of educating men and women to a high enough level, and, allowing some 7–10 per cent places for overseas students, as at present, it estimates that in 1980 some 630,000 places will be required in full-time higher education, including 320,000 in universities These figures compare with 560,000 and 346,000 in the Robbins Report (*Nature*, 200, 925, 1963), and would provide an annual entry of about 180 000 home students, including 72,000 to universities, and about 16,000 overseas students

The present pattern of opportunity is considered in the second chapter, which leans very definitely towards 10,000 as a reasonable maximum size for a university, and stresses the need for careful preparation before institutions are given the right to confer degrees It suggests that three of the colleges of advanced technology should be selected for promotion, providing, with the Manchester College of Science and Technology, the Imperial College of Science and Technology, and the new University of Strathclyde, six technological universities which could lead the way in trying out new ideas in technological education The remaining colleges of advanced technology would keep their present status, but no immediate change in the status of the teacher training colleges is proposed By 1980 the technological universities would have some 70,000 students, and there would be 170,000 in the regional colleges of technology and commerce and 140,000 in the professional and teacher training colleges In the 1970's the opportunity to found another 6–10 universities would be welcomed as well as more professional colleges and colleges of technology Emphasis is laid on the danger of drawing staff from secondary schools in this expansion and on the importance of promoting greater mobility of employment between higher education and other professions, including civil servants and industrial management Lower staff ratios in the universities seem inevitable but could be offset by employing more administrative, clerical and technical staff, by providing better library facilities and by a more imaginative approach to technical teaching aids, especially television The final chapter considers the financial expenditure involved as a national investment, urging that Britain must revise her scale of priorities where education, and especially higher education, is concerned, and be prepared to spend 1 5 per cent of her national income on higher education. At this rate there would be no difficulty in meeting the cost of the expansion plans advocated in the pamphlet

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**Talent for Tomorrow* (A Bow Group pamphlet) Pp 68 (London Bow Publications, Ltd , 1963) 4s

STRUCTURE OF LIQUID SILICATE SLAGS

THE investigation of steel-making slags goes back probably to the 1880's when Fouqué and Lévy experimented with synthetic minerals made from fusion of pure oxides at controlled temperatures. Later, Doelter, Vogt and Tammann carried the work a stage further in their investigations of silicate phase-diagrams. The work of Rankin, Wright and Bowen at the Geophysical Laboratory in Washington, on binary and ternary silicate phase-diagrams, is well known and contributed much to our knowledge of composition and structure of metallurgical slags. It was otherwise, however, with liquid silicates, the 'magma' from which minerals in solid slags crystallized out. This complex problem appears to have been investigated by Vogt in 1902, but his conclusion that the crystalline minerals found microscopically in a slag represented those fundamental to the mother-liquor was later disproved by Schenck in the 1930's. Schenck's thesis was that knowledge of the crystallized compounds found in slags was of little value as a guide to their behaviour in the liquid state. The concentration of oxides of lime, iron and silica in a slag gives no clear indication of its chemical properties because of the ramified chemical changes occurring when they react to form a solution. Later work by Sauerwald and Neuendorff in 1925 demonstrated the ionic nature of liquid silicates by causing deposition of metallic iron from an iron-silicate melt by electrolysis.

In a stimulating article by J. B. Henderson of the Department of Physical Chemistry, Central Research Laboratories, the Broken Hill Proprietary Co., Ltd., Australia (*Technical Bulletin*, 7, No. 2; July 1963), the present-day theories of the constitution of liquid silicate slags are reviewed and their limitations clearly defined. Although brief, this article gives an indication of the technique now being applied in contemporary studies in the elucidation of the structure of the more complex silicate slags. Henderson comments: "Until recently little interest has been evinced in these liquids. There are two reasons for this: in the first place, it has been exceedingly difficult to study these melts under well-controlled conditions in the laboratory, owing to the lack of good refractories and the tiresome techniques involved. In the second, these liquids are themselves so complex that theoretical prediction of their structure has so far proved impossible".

Such knowledge as we have to-day of liquid silicates is essentially founded on the X-ray results of researches by Zachariasen, Bragg and Biscoe and Warren (see F. D. Richardson, "Oxide Slags—A Survey of Our Present Knowledge", *The Physical Chemistry of Steel-Making*, 55, John Wiley and Sons, and Technology Press, New York, 1958). In his article Mr. Henderson reviews some of the modern ideas of liquid silicate structure and shows how such models are derived by reference to "measurements of self-diffusion coefficients". He contrasts by schematic representation (after Richardson) the silicate tetrahedron which is fundamental and the molten silica pattern in which the structure is distorted. The addition of metal oxides, such as lime or magnesia, to the molten silica produces profound structural changes which are indicative of the electrochemical forces at work.

The complexity of this problem of the constitution of liquid silicates is here clearly demonstrated. Present-day theories may help to explain 'qualitatively' the nature of binary melts, but 'quantitatively' they cannot be described nor can the structure of melts of more complicated compositions be predicted. These demand individual investigation and many different types of measurement are needed. The approaches to such research now in train in the Broken Hill Proprietary Company's Laboratories include X-ray, neutron and electron diffraction, absorption spectroscopy, electrolysis, ultra-sonic absorption, density, cryoscopy, refractive index and vapour pressure.

H. B. MILNER

FIBRE OPTICAL PROPERTIES OF ULEXITE

By MRS. E. J. WEICHEL-MOORE and DR. R. J. POTTER

Thomas J. Watson Research Center, Yorktown Heights, New York

THE optical fibre bundle, now a commercially available optical component, requires very high precision in manufacture. It is essential that the optical fibres be assembled in a carefully controlled array such that the position of each fibre with respect to the others is maintained. In many cases, there are hundreds of millions of individual fibres in a given bundle. The manufacture, insulation and assembly of optical fibres are major technological problems which have been overcome by a number of manufacturers.

The early published work on fibre optics by Baird[1], Hansell[2], and Lamm[3] described devices for transmitting images with bundles of bare glass fibres.

In the early 1950's, fibre optics was essentially rediscovered by A. C. S. van Heel[4,5], H. H. Hopkins[6] and Brian O'Brien[7]. Their work is important in the development of the technology. They realized the need for optical insulation, that is, low index cladding between the fibre cores in a close-packed structure.

Since that time, much has been published on the properties of clad optical fibres and the applications of clad fibre bundles. It is significant, however, to note that virtually no important application of fibre optics without cladding has survived. Actually, the technique of assembling and drawing glass fibre bundles was developed by glassmakers in Palestine[8] in the first century B.C. They assembled fibres of coloured glass in the form of a portrait, fused the fibres together, and drew the resulting rod to reduce the portrait in size. The rods were then cut into cross-sections, polished, and pierced to be used as beads. We thank Dr. Dietrich von Bothmer, curator of the Greek and Roman Department of the Metropolitan Museum of Art in New York City, for his co-operation in affording several of these ancient beads for microscopic examination; they were found to be remarkably well preserved. The glasses of which the beads were made are mostly opaque in the bead thickness of approximately $\frac{1}{8}$ in. × $\frac{1}{4}$ in. The fibre structure was eliminated in the

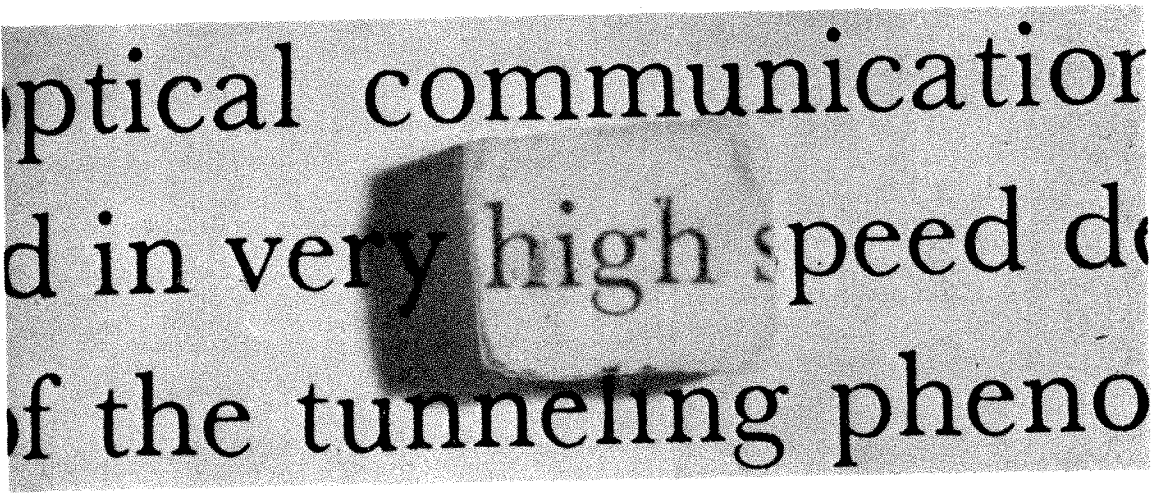

Fig. 1. Image transfer of ulexite fibre bundle

process of fusion so that the final product is not fibre optics, although the fabrication process is presumably essentially the same as the modern method.

With all due respect to the ancient and modern technologies, the purpose of this article is to describe a material known as 'ulexite', found in Nature, which has precisely the same optical properties as a man-made optical fibre bundle. Nature has found another way of solving the cladding problem and has been equally successful in solving the orientation problem. Ulexite fibre bundles are capable of good resolution and exhibit little 'cross-talk'.

Ulexite is a fibrous mineral with the chemical composition, $NaCaB_5O_9{\cdot}8H_2O$ (ref. 9). Ordinarily, the fibres mat together in an aggregate resembling a cotton ball. However, a variety of ulexite is found at Boron, California, in which the fibres are oriented in parallel bundles. Much of the material is pearly and translucent, but some is transparent and of optical quality, yielding samples of 'natural fibre optics'. A small bundle of ulexite, approximately 45 mm in thickness, has been cut and polished perpendicular to the fibre axes; it is able to transmit an image with remarkable clarity, as shown in Fig. 1.

The existence of this variety of ulexite and its fibre optical properties have been known for several years[10], but the phenomenon has not been described in detail. The apparent lack of a low refractive index cladding on the fibres might cause one to believe that the numerical aperture and resolution would be low and subject to the same problems which existed in the early fibre bundles. In fact, however, ulexite has a respectable numerical aperture of $\sim 0{\cdot}3$ and a resolution in excess of 10 lines/mm. In essence, the fibres are cladded because of the random optical orientations of nearest neighbours.

The mineral is triclinic[9], elongated on the *c* axis. Fig. 2 is a photomicrograph of a ulexite fibre bundle taken between crossed polarizers at a magnification of fifty. The adjoining 0·001-in. scale was taken at the same magnification. As the photograph shows, there is tremendous variation in the size and shape of the fibres. Optically, the crystals are biaxial positive[11]. The orientation of the fibres around the *c* axis is completely random, as shown by the variation in extinction between crossed polarizers. X-ray photographs have been taken by Takeda and Donnay[12] of a sliver of the fibre bundle. Their rotation photographs, taken about the fibre axis, show that the fibres are perfectly parallel along the *c* direction. It would be interesting to ascertain the growth conditions which led to such perfect orientation.

According to Larsen and Berman[13], later confirmed by Murdoch[11], the refractive indices of ulexite are $\alpha = 1{\cdot}491$, $\beta = 1{\cdot}504$, $\gamma = 1{\cdot}520$. The intermediate index, β, shows some variation in orientation with respect to the *c*, or fibre, direction. When $y \wedge c = 0^\circ$, β coincides with the fibre axis and the limiting refractive indices in the fibre cross-section are α and γ.

Since the orientation of the fibres is random about the fibre axis, it is possible for the γ direction of a given fibre to be adjacent to the α or α′ direction of a neighbouring fibre; that is, a fibre boundary separates a high- and low-index material. The maximum possible index difference is $\gamma - \alpha = 0{\cdot}029$. Using this value in the expression for the numerical aperture of a perfect, circular, non-birefringent fibre, we calculate a meridional numerical aperture of 0·30. This corresponds to an angular aperture in air of 17·5°.

Actually, the numerical aperture will vary from fibre to fibre depending on the mutual orientations of the high and low refractive index directions at the fibre boundaries. Moreover, the numerical aperture will vary with direction in a single cross-section, since the high index direction, γ,

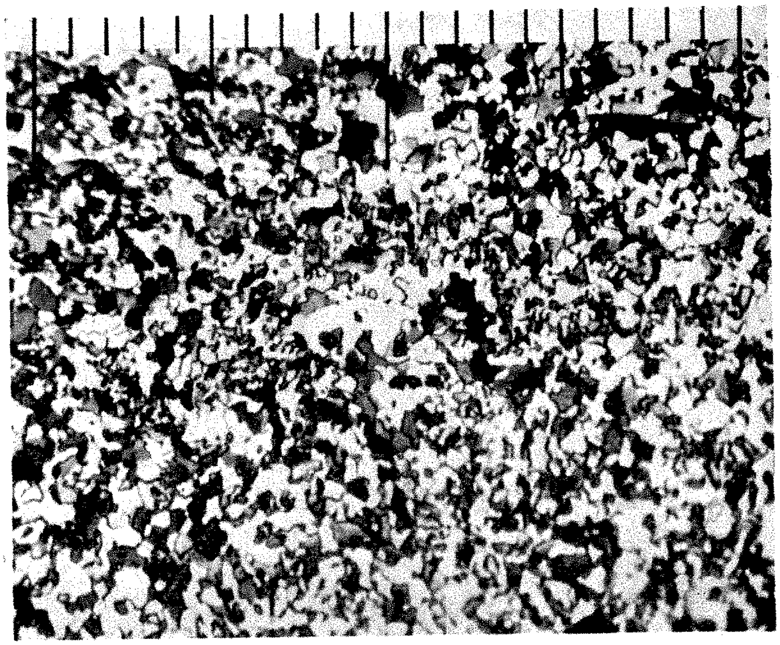

Fig. 2. Fibre cross-section of ulexite, crossed polarizers (× 50; 0·001 in. scale)

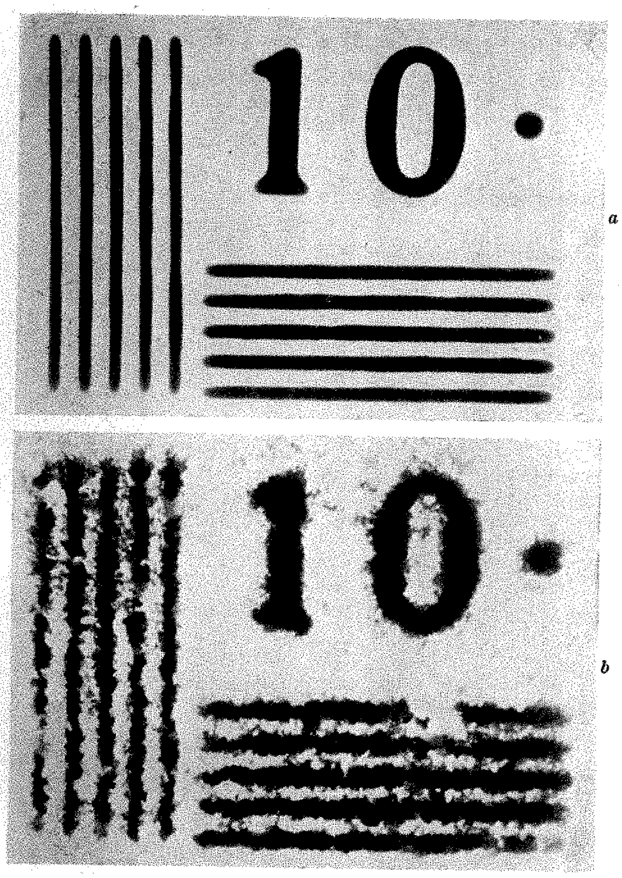

Fig. 3. *a*, National Bureau of Standards, 10 lines/mm (× *c*. 15); *b*, image transfer of National Bureau of Standards, 10 lines/mm by ulexite fibre bundle (× *c*. 15)

can be clad by a lower α or α′ direction, but the α direction cannot be clad by a neighbouring fibre, since there is no lower index available. The numerical aperture of the bundle is a statistical average of the numerical apertures of the individual fibres.

The rays associated with the unclad α direction will always leak into the neighbouring fibre and will thus be unavailable for image transmission in the parent fibre. Rays in higher index directions which are not appropriately clad by a neighbouring fibre will be similarly lost. This can be clearly seen by illuminating the fibre bundle with linearly polarized light and rotating either the polarizer or the sample. The transmission of each fibre varies with respect to its orientation relative to the polarizer, that is, with index direction.

In unpolarized light, the overall transmission of each fibre and the cross-talk between neighbours depend on the number of rays which are appropriately reflected at the fibre boundaries. Because of the random core to cladding index difference between the limits 0–0·029, there will be variations both in the transmission of individual fibres and in the cross-talk between neighbours. These effects are shown in Fig. 3*a*.

The angular aperture of the ulexite bundle shown in Fig. 1 was measured on an Eichner goniometer. As in commercial fibre optics, when one observes an image on the end of the bundle, the transmitted image begins to blur as one exceeds the meridional numerical aperture. The numerical aperture of ulexite was measured by observing the angle at which the transmitted image began to blur. A subjective judgment had to be made; but the measurements were reproducible to ± 1°. The technique was verified using a glass fibre bundle of known numerical aperture and experimentally determining the angular aperture. In this case, of course, the angular aperture was as predicted. The experimental measurement of 18° ± 1° for ulexite compares remarkably well with the theoretical meridional angular aperture of 17·5°. Since the fibres are highly irregular in shape, skew rays probably play an important part in image transmission and influence the average numerical aperture. Because of the skew rays[14], the effective numerical aperture of a circular fibre bundle has been shown to be larger than that predicted by the meridional approximation.

A photomicrograph of National Bureau of Standards resolution chart with 10 lines/mm is shown in Fig. 3*a*. The photograph was taken at 15 times magnification. In Fig. 3*b*, the chart was photographed through the ulexite fibre bundle which was approximately 45 mm thick; the chart is just resolved. Variation in transmission of the different fibres is evident from the photomicrograph, and, as one would expect, some of the fibres do not transmit at all because they are, in fact, not fibre cores but, rather, fibre cladding.

Although one cannot establish a 'fibre diameter', as one would in a commercial fibre bundle, the resolving power of ulexite is measurable, and the numerical aperture is both explicable in terms of optical properties of the material and capable of experimental verification. The discovery and study of ulexite show that it is possible to find in Nature a device almost as good as a man-made fibre bundle.

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RELATIONSHIP BETWEEN REFRACTIVE INDEX AND CONCENTRATION OF SOLUTIONS

By F. A. GLOVER and J. D. S. GOULDEN

National Institute for Research in Dairying, Shinfield, Reading

IT is frequently pointed out in light-scattering and other studies that the refractive index of a solution increases linearly with concentration, only when concentration is expressed in terms of weight of solute per unit volume of solution, that is, w/v units[1,2]. In many analytical applications of refractive index measurements, concentrations are normally expressed in terms of weight of solute per unit weight of solution, that is, w/w units, and conversion into w/v units requires a knowledge of the density of each sample. No theoretical justification for the choice of w/v units rather than w/w units of concentration appears to have been published although these relationships can readily be deduced.

Consider a beam of radiation traversing a unit cube of solution, as shown in Fig. 1. The volumes occupied by the solvent and solute molecules, after any interactions during dissolution have occurred, are V_1 and V_2 respectively.

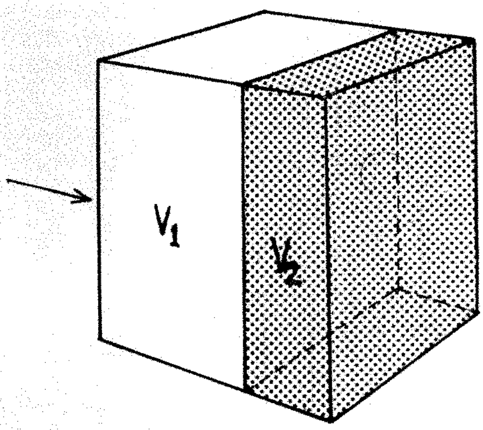

Fig. 1

If at any given temperature the velocities of light in vacuum, solvent, solute and solution are u_0, u_1, u_2 and u_3 respectively, the refractive indices of the solvent, solute and solution are given by

$$n_1 = \frac{u_0}{u_1}, \quad n_2 = \frac{u_0}{u_2}, \quad n_3 = \frac{u_0}{u_3} \qquad (1)$$

In the unit cube the optical path lengths in the solvent and in the solute are numerically equal to V_1 and V_2.

Time taken for light to pass through the solution = time through solvent + time through solute

$$= \frac{V_1}{u_1} + \frac{V_2}{u_2}$$

Velocity of light in the solution is therefore:

$$u_3 = \frac{V_1 + V_2}{V_1/u_1 + V_2/u_2}$$

Hence:

$$n_3 = \frac{u_0(V_1/u_1 + V_2/u_2)}{V_1 + V_2}$$

Using (1) to eliminate u_0, u_1 and u_2:

$$n_3 = \frac{n_1 V_1 + n_2 V_2}{V_1 + V_2}$$

or:

$$n_3 = \frac{V_2(n_2 - n_1)}{V_1 + V_2} + n_1 \qquad (2)$$

If the w/v solute concentration is c_v and the density of the solute in the dissolved state is ρ_2 at the same temperature at which the refractive indices are measured:

$$c_v = \frac{\rho_2 V_2}{V_1 + V_2}$$

Substitution in equation (2) gives:

$$n_3 = \frac{c_v}{\rho_2}(n_2 - n_1) + n$$

or:

$$\Delta n = n_3 - n_1 = \frac{c_v}{\rho_2}(n_2 - n_1) \qquad (3)$$

In the case of w/w concentration units, the density of the solvent ρ_1 must be introduced, so that:

$$c_w = \frac{V_2\rho_2}{V_1\rho_1 + V_2\rho_2}$$

and

$$V_1 = \frac{V_2\rho_2(1 - c_w)}{c_w\rho_1}$$

Substitution for V_1 in (2) and rearranging gives:

$$n_3 = c_w\left(\frac{\rho_1}{\rho_2}\right)(n_2 - n_1)\left[\frac{1}{1 - c_w(1 - \rho_1/\rho_2)}\right] + n_1$$

or

$$\Delta n = c_w\left(\frac{\rho_1}{\rho_2}\right)(n_2 - n_1)\left[1 + c_w\left(1 - \frac{\rho_1}{\rho_2}\right) + c_w^2\left(1 - \frac{\rho_1}{\rho_2}\right)^2\right] \qquad (4)$$

Thus equation (3) predicts a linear relationship of slope $(n_2 - n_1)/\rho_2$ for refractive index increment against concentration in w/v units, whereas equation (4) shows that a curvilinear relationship must hold when w/w units are used.

As shown in Fig. 2, the published data for sucrose[3] confirm these calculations and similar results have also been obtained by Goulden[4] with condensed milk.

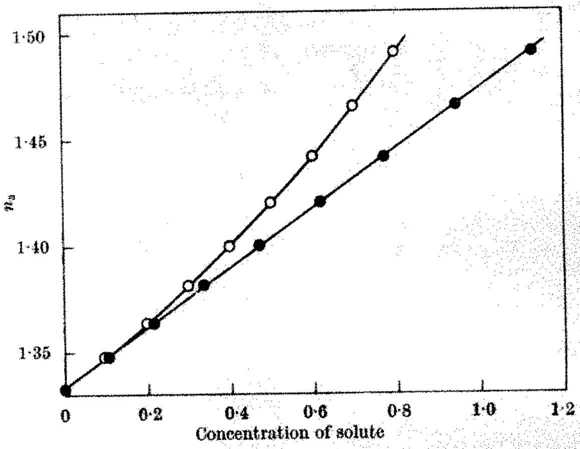

Fig. 2. Refractive index of sucrose solutions n_3) against solute concentration in g/g –○–○–○–, g/ml. –●–●–●–. Wave-length = 0·589 μm; temperature, 20° C

Both equations (3) and (4) contain the density (ρ_2) and the refractive index (n_2) of the solute in solution as distinct from their values in the liquid or solid state, so that neither n_2 nor ρ_2 can be independently evaluated from the variation of refractive index with concentration. In cases such as some protein solutions where linear relationships between density and c_v have been established[5], ρ_2 can be independently evaluated from density measurements and used in conjunction with equation (3) to evaluate n_2. For solutions of strong electrolytes and other cases where interactions with the solute molecules lead to changes in either n_2 or ρ_2 with concentration, deviations will occur from the linear relationship of equation (3).

We thank Dr. R. A. Gibbons for preliminary discussion which led to the mathematical analysis.

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A RAPID METHOD FOR DETERMINING THE CORROSIVITY OF THE ATMOSPHERE AT ANY LOCATION

By D. P. DOYLE and HUGH P. GODARD

Aluminium Laboratories, Ltd., Kingston, Ontario

THE use of Sample's 'wire-on-bolt' test to measure galvanic corrosion of metals in the atmosphere has been described[1-3]. This article indicates how the test can be adapted to provide a numerical assessment of corrosivity of the atmosphere in a period as short as three months, as compared with several years required in conventional atmospheric exposure tests.

Two instances where numerical assessment of corrosivity of the atmosphere would be very valuable are when metallic structures are planned for locations where the corrosivity of the atmosphere to the metal involved is unknown, but suspected to be severe, and when metals must be stock-piled in similar circumstances. In the first case, the availability of corrosivity data at an early date permits proper choice of alloy and thickness of sections or sheet, adoption of suitable protective measures if needed, and settlement on design which will ensure as long a life as desired for the structure. In the second instance, the stock-piled metal may be given suitable protective treatments to combat corrosivity of the degree indicated by the test.

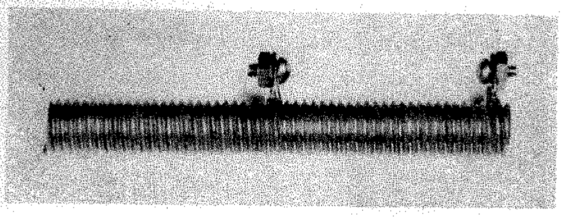

Fig. 1. 'Wire-on-bolt' test specimen

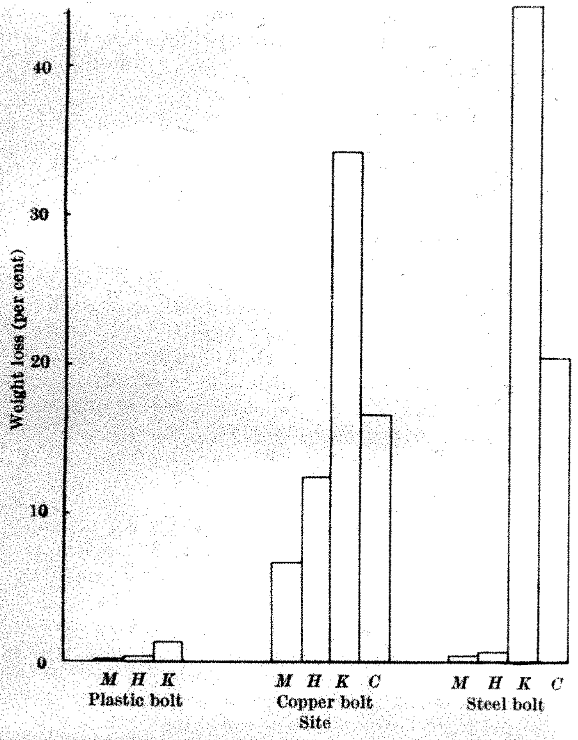

Fig. 2. Weight loss of 0·020-in. diam. 1*S* alloy wire from 'wire-on-bolt' samples exposed for three months at four different sites
M, Montreal; *H*, Halifax—industrial atmosphere. *K*, Kure Beach, N.C.; *C*, Chebucto Head, N.S.—marine atmosphere

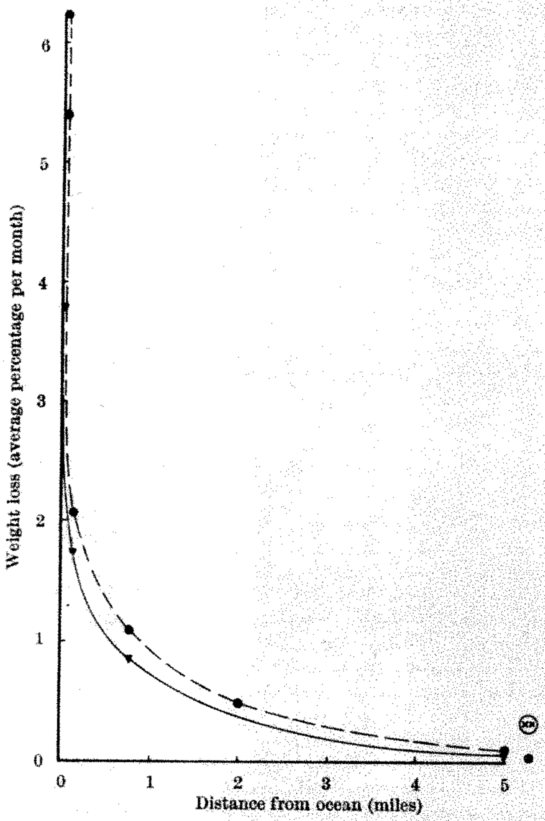

Fig. 3. Graph showing the drop in corrosivity with distance from the ocean at a coastal area of North Carolina, measured by the amount of attack on 2*S* alloy 0·035 in. diam. Aluminium wire wound on mild steel and copper bolts
●, Copper bolt used; ▼, mild steel bolt used; ×, Kingston, Ontario, value—steel bolt (no marine influence); × ×, Montreal, Quebec, value—steel bolt (industrial atmosphere)

In using the test for aluminium, a short length (about 36 in.) of thin (0·035 in. diam.) wire, wound tightly in the threads of a bolt (0·5 in. diam.) or a more cathodic material, is very sensitive to changes in the corrosivity of the atmosphere. The extent of corrosion is assessed by exposing the test piece for three months in the atmosphere being rated, and determining the loss in weight of the aluminium wire. The bolt may be steel, copper or other material the influence of which on the corrosion rate of aluminium is known.

By exposing similar specimens in areas where the performance of aluminium is known and comparing the results the corrosivity of the new location can be judged.

A typical 'wire-on-bolt' test specimen is shown in Fig. 1. The ends of the wire are bolted to 'spade' lugs driven into a snugly fitting hole in the bolt and the connexions are protected from the weather by paint.

Prior to assembly, the aluminium wire is weighed to 0·1 mg. After exposure, the wire is unfastened from the bolt, the corrosion products removed, and the wire re-weighed. The corrosion products are removed by a

30-min immersion of the wire in a hot solution of chromic and phosphoric acids (A.S.T.M.–*B*137–45). A correction is applied for the very small loss of weight during cleaning; this is determined on a similar length of new wire which is treated at the same time.

Comparison of two industrial and two marine atmospheres. Fig. 2 shows the results of an experiment carried out at two industrial and at two marine atmospheres, which demonstrates the relative corrosivity of these atmospheres towards one another, as well as the influence of the bolt material used. It is seen that: (*a*) A copper bolt promoted a high rate of attack on the aluminium wire, but did not alter the relative order of attack, at the four sites, from that which was found for a plastic bolt. Because it is more sensitive, the aluminium–copper couple is preferred. (*b*) A steel bolt promoted a high rate of attack on the aluminium wire, when exposed to a marine atmosphere, and was comparable to copper, but did not do so in an industrial atmosphere. For this reason the aluminium–steel couple may be used to distinguish between marine and industrial atmospheres.

Corrosivity of a marine atmosphere versus distance from shore. Fig. 3 shows the results of an experiment carried out to determine to what distance from the coast the marine corrosive influence is present in the atmosphere. It can be seen that the relative corrosivity drops off very rapidly with distance from the ocean-front. At five miles distance the corrosivity is as low as that of the mild semi-rural inland atmosphere at Kingston, Ontario.

While the examples given here relate to aluminium, other metal pairs could be used for investigating the corrosivity of an atmosphere to any particular metal in wire form which can be paired to a more cathodic metal in bolt form.

Once sufficient data have been accumulated with the 'wire-on-bolt' test it will be possible to dispense with the controls recommended for simultaneous exposure in areas of known performance.

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FALSE POSITIVE DIRECT COOMBS TESTS FOLLOWING TOTAL-BODY X-IRRADIATION OF MICE

By OSCAR GARFEIN, NANCY TREACY* and JOHN G. GORMAN*

Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, and Blood Bank, Presbyterian Hospital, New York

NOSSAL *et al.*[1–3] have shown that acquired immunological tolerance can be destroyed by sub-lethal whole-body X-irradiation. If the immunological mechanisms subserving acquired tolerance and natural tolerance are similar, then natural tolerance to auto-antigens may also be destroyed or altered to some extent by treating normal animals with X-ray[4]. To test this hypothesis we treated *A/JAX* and *CBA/JAX* male and female mice with sub-lethal whole body X-irradiation delivered in various dose schedules, and examined serial blood samples from these animals with a direct Coombs test, looking for an auto-immunization to red blood-cell antigens as an indication of breakdown of natural tolerance.

Five test groups were irradiated according to the method described here to examine the effect that fractionation in delivery of a 500-r. dose of X-ray might have on the development of auto-immunity. Group 1*X* received the total dose of X-ray in one exposure; group 2*X* received half of the total dose at one time and received the remaining 250 r. (or 200 r. in the case of the *A/JAX* males) one week later; group 4*X* received one-quarter of the total dose, four times, at weekly intervals; and group 8*X* received one-eighth of the total dose on eight separate occasions, with three and four days alternating between doses. Group 1*X-SP* received the total dose of X-ray in one exposure and, 7 h after this, received approximately fifty thousand isologous spleen cells intravenously. Following the beginning of a course of X-irradiation, serial direct Coombs tests were performed on all mice at 4–6 day intervals.

Each test group, with the exception of one which received isologous spleen cells after its single dose of X-ray, was composed of 2 *A/JAX* males, 2 *A/JAX* females, 2 *CBA/JAX* males, and 2 *CBA/JAX* females. The group given spleen cells after X-irradiation had the same relative composition as the other groups but was composed of sixteen mice. All *CBA/JAX* males and females, and all *A/JAX* females in all test groups received a total of 500 r. whole-body X-irradiation. The *A/JAX* males in all test groups received a total of 400 r. whole-body X-irradiation.

Mice were irradiated in 'Lucite' boxes in groups of 2 (except 500-r. and 400-r. single dose groups which were irradiated 6 per box). The conditions of X-irradiation were as follows: 184 kVp.: 30 m.amp; target—subject distance (from focus to centre of mouse) 72 cm from each of two tubes (cross-fire technique); dose rate 50 r./min; filtration: 0·28 mm copper, 0·5 mm aluminium; half-value layer = 0·6 mm copper.

Five groups of mice identical in composition to the test groups were used for controls. The sole difference in the handling of the control mice was that, once placed in the 'Lucite' boxes used for holding test animals during X-irradiation, they did not receive X-ray but were kept in these boxes for times equivalent to those required for X-irradiating the test animals.

The direct Coombs tests were performed as follows: The mice were tail bled into normal saline and the cells washed 4 times in normal saline. One drop of a 5 per cent suspension of those cells was then added to one drop of rabbit anti-mouse globulin diluted 1 : 32 with normal saline, incubated at room temperature for 30 min and the tubes centrifuged for 1 min before reading. All results were read both macroscopically and microscopically for agglutination.

With each batch of tests the following controls were done and were required to be satisfactory. Saline controls, substituting one drop of normal saline for the rabbit anti-mouse globulin serum, were run simultaneously on every blood sample. To provide an anti-mouse globulin reagent control a Coombs test was performed on known Coombs positive cells and known Coombs negative cells with each batch of cell samples tested. Coombs positive cells were prepared in the following manner: one volume of a 50 per cent suspension of red cells, obtained from a single non-experimental *A/JAX* or *CBA/JAX* mouse, was washed 4 times with normal saline and coated by incubating for 60 min at 37° C with 2 volumes of a 1 : 4

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* Present address: Immuno-Hematology Research Unit, Lenox Hill Hospital, 100 East 77th Street, New York 21.

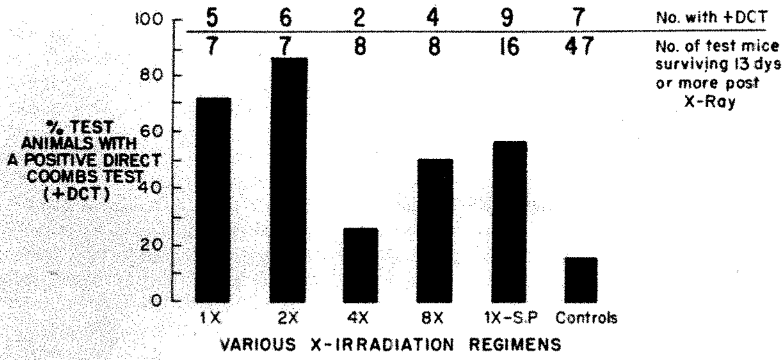

Fig. 1

saline diluted iso-immune mouse serum. This iso-immune serum, used to render both *A/JAX* and *CBA/JAX* red cells Coombs positive, was obtained from *DBA/JAX* mice immunized with *C57/JAX* spleen cells. Coombs negative cells were prepared in the same way except that they were incubated with normal mouse serum. Before use, both coated cells and negative cells were washed 3 times with normal saline and a 5 per cent suspension made. Mouse cells coated in this way gave a strongly positive direct Coombs reaction and cells incubated with normal mouse serum were uniformly negative. Saline controls as described here were also run on these positive and negative control cells. A further negative anti-mouse globulin control consisted of incubating 1 drop of a 5 per cent suspension of 4 times washed *A/JAX* unsensitized cells with 1 drop of the rabbit anti-mouse globulin serum.

The results of the experiment are shown in Fig. 1. 71 per cent of the animals that received a single dose of X-ray developed a positive direct Coombs test. 86 per cent of the animals receiving two equal doses of X-ray became positive, while 25, 50 and 56 per cent of the animals in the remaining three groups developed a positive direct Coombs test. These Coombs tests were transient in most cases, becoming negative spontaneously within seven days. 15 per cent of non-irradiated mice showed a positive direct Coombs test at various times.

In Fig. 2 are indicated the periods in time after the beginning of X-irradiation when the test and control mice that did become positive first showed a positive Coombs test. There were two periods when most of the animals became positive. The first period occurred 14–21 days after the beginning of all X-irradiation procedures. Most of the animals in this group had received single or dual doses of X-ray. The second period occurred at about the 39th day. The bulk of the animals which first showed a positive Coombs test at this time were those animals which had received multiple doses of X-ray. The spleen-protected mice behaved on the whole like the other mice receiving a single dose of X-ray, but tended, as a group, to develop a positive test several days earlier than the mice not receiving spleen cells.

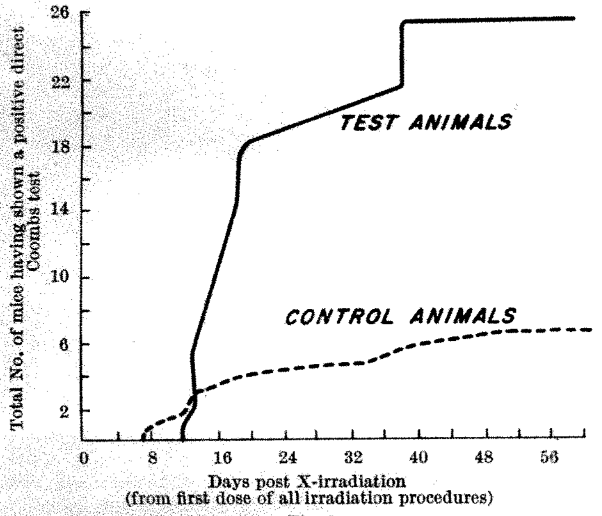

Fig. 2

These results, with special attention to the non-irradiated mice which showed a positive Coombs test, were discussed with Dr. Robert Schwartz of the New England Center Hospital of Boston, Mass. Noting that Muirhead *et al.*[5] have reported positive direct Coombs tests in dogs following phenylhydrazine administration and that Nelken[6] has shown that false positives could occur with human blood samples with high reticulocyte counts, he suggested that this possible source of error in our experiments should be excluded. Such false positive direct Coombs tests associated with reticulocytosis are thought to be due to an anti-iron binding globulin (anti-transferrin) antibody in the Coombs serum reacting with iron binding globulin which is normally present on the surface of reticulocytes[7].

On the basis of this information we repeated, on a much smaller scale, our original experiment and found that of 10 *A/JAX* mice that were given a single dose of 500 r., 8 of 9 that survived 13 days or more developed a 30–50 per cent reticulocytosis and showed a strong positive direct Coombs test when tested with our original Coombs serum. In 5 mice given isologous spleen cells after irradiation, reticulocytosis and false positive Coombs tests were seen as early as 5 days after X-ray. When simultaneous blood samples from these mice were tested with the same Coombs serum absorbed with reticulocytes, negative results in all cases were obtained. We also noted that when Coombs serum unabsorbed with reticulocytes was used, the addition of 1 drop of guinea pig serum (intended as a source of complement) to the test mixture inhibited the false positive agglutination reaction but did not inhibit the positive reaction with our positive control cells coated with iso-antibody. This inhibition is apparently due to the presence of iron binding globulin in guinea pig serum sufficiently similar antigenically to mouse iron binding globulin to inhibit rabbit anti-mouse iron binding globulin antibodies.

To prepare reticulocytes used for absorbing the Coombs serum healthy *A/JAX* mice were given intravenously 0·04 mg phenylhydrazine hydrochloride per g body-weight as a 1 per cent solution in normal saline daily for three days[5]. Three days after the last dose, reticulocyte counts ranged from 30 to 35 per cent and all mice were Coombs positive with unabsorbed Coombs reagent, but were negative when guinea pig serum was added to the reaction and negative with reticulocyte absorbed Coombs serum. In other words, phenylhydrazine produces false positive Coombs tests due to reticulocytosis regularly in mice. Reticulocytes used for absorbing the Coombs reagent were obtained by killing these mice on the fourth day after the last dose of phenylhydrazine.

On checking our non-experimental *A/JAX* mice we found that reticulocyte counts ranged regularly from 10 to 15 per cent. It is probable that occasional reticulocyte levels ranging even higher than this accounted for the few positive Coombs tests seen in our non-irradiated control animals. These levels are considerably higher than earlier published values[8] which still stand as typical of levels being observed at present in reticulocyte counts on *A/JAX* mice at Bar Harbor[9].

It is clear that the positive direct Coombs tests reported here in mice after whole-body irradiation do not indicate the breakdown of natural tolerance to red-cell auto-antigens but rather just reflect the existence of post-irradiation reticulocytosis. In the light of the true nature of these positive Coombs tests it is easy to explain the temporal relationships of the onset of positive tests to the beginning of irradiation. Whole-body X-ray doses of the size that were administered suppress erythropoiesis for 7–10 days[10], after which a brisk reticulocytosis would be expected which might reach its peak in the period of 14–21 days, the period in which we did note most of our positive results. Re-population with unirradiated isologous donor erythroid cells would account for the earlier reticulocytosis and positive Coombs tests seen in mice given spleen cells.

The question of whether natural auto-tolerance is impaired by X-ray, however, remains open for further experimental investigation. It should be noted that in Nossal's experiments[2,3] breakdown of the tolerance mechanism was seen in situations of partial tolerance, where at least a small 'forbidden' auto-immune clone is already established and might quickly grow once free from inhibition by a tolerance mechanism which has been put out of action by irradiation. In cases of complete tolerance as in natural tolerance to red cells, any such emerging 'forbidden' clone would have to be initiated as a new primary response and multiply from a few stem cells[4]. Since the ability to initiate an immune response is severely affected by total-body irradiation[11] and the tolerance mechanism is probably able to repair itself fairly soon after 500-r. X-irradiation[4], it is not too surprising that our test system might fail to detect a breakdown of the tolerance mechanism.

We have demonstrated once again, this time in mice, that high percentages of reticulocytes can cause false positive direct Coombs tests. Coombs reagents by their nature often contain antibodies against serum proteins other than immunoglobulins. If one such non-immune protein happens to be attached to the surface of red cells a false positive Coombs test may be recorded. It is essential that investigators be alert to the fact that positive direct Coombs tests do not always indicate that red cells are coated by immunoglobulins.

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We thank Dr. Philip Levine and Mr. William Pollack of Ortho Pharmaceutical Co., Raritan, N.J., for the preparation of the rabbit anti-mouse Coombs serum used. We also thank Dr. Douglas Gilmour, Department of Agriculture, University of Cambridge, for a gift of the mouse iso-antibodies used to coat positive control cells and for supervising the absorption of the Coombs serum, and also Dr. Harald H. Rossi and Miss Marlis Wohlfromm, Department of Radiological Research, University of Columbia, for providing irradiation facilities and technical supervision.

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TRANSFORMATION OF HÆMOPOIETIC CELLS OF BONE MARROW ALLOGRAFTS INTO LYMPHOCYTES IN X-IRRADIATED DOGS

By I. L. CHERTKOV, M. N. NOVIKOVA, L. S. ROGACHEVA, L. L. SHEPSHELEVICH,
A. S. MAXYMENKO and M. O. RAUSHENBACH

Central Order of Lenin Institute of Hæmatology and Blood Transfusion, Moscow, U.S.S.R.

GOOD therapeutic effects in LD_{95} X-irradiated dogs (600 r.) following transplantation of allogenic bone marrow have already been observed[1]; more than 70 per cent of dogs survived. Examination of the bone marrow action mechanism showed that the graft cells proliferate in the recipient for 2–3 weeks. At this time there was an enlargement in the peripheral blood of the donor's leucocyte count (female in male) and donor's erythrocyte count (by the differential agglutination method with anti-*A* dog's test-serum). After 15–20 days the marrow graft was rejected and simultaneously restoration of the recipient's hæmopoiesis began. Immunological complications were not observed.

In the experiment described here, the dogs X-irradiated with 1,000 r. received bone marrow allograft. The bone marrow was injected intravenously, $5–8 \times 10^9$ nuclear cells on the day after radiation. Bone marrow was removed from the sternum and ribs and suspended in aliquots of '*TC*-199'. The marrow was passed through two layers of gauze. Staining with erythrosine demonstrated that more than 98 per cent of the cells were viable. This was also suggested by the ability of the marrow cells to synthesize DNA (by incorporation of tritiated thymidine).

In these conditions bone marrow transplantation failed in its therapeutic effect; the dogs died 11–15 days after radiation. At this level of irradiation, the white blood cell count invariably fell during 6–7 days. After 2 days there was a considerable fall of all the elements in the bone marrow; only mature myeloid cells could be observed. However, by the fifth to sixth day after irradiation the stem and immature cells appeared, and the number of mature cells fell (Fig. 1). Restoration of the recipient's hæmopoiesis at this time was impossible; moreover, in bone marrow female donor's leucocytes among mature neutrophils were identified, and in peripheral blood by the sixth to eighth day 2–3·5 per cent of the donor's erythrocytes appeared. These results indicated that graft cells had re-populated and differentiated in the normal way. However, after the seventh to eighth day in bone marrow peculiar 'hyperbasophilic' cells were revealed and normal hæmopoiesis stopped. In bone marrow and peripheral blood it was possible to observe hæmocytoblasts transforming into lymphocytes through several transitional forms. The first of them was a hæmocytoblast with basophilic cytoplasm (Fig. 2), and a young laciniate often plicate nucleus. The next form was like a lymphoblast (Fig. 3, left), the nucleus was more mature, chromatin more solid, the nucleoli were distinct. There was a perinuclear zone in these cells. Lymphoblast differentiated into prolymphocyte (Fig. 3, right). The nucleus was round or kidney-shaped; the chromatin more compact. Sometimes remains of nucleoli could be seen, the perinuclear zone more pronounced, basophily usually marginal. That type of cell converted into lymphocyte, at first hyperbasophilic and then normal.

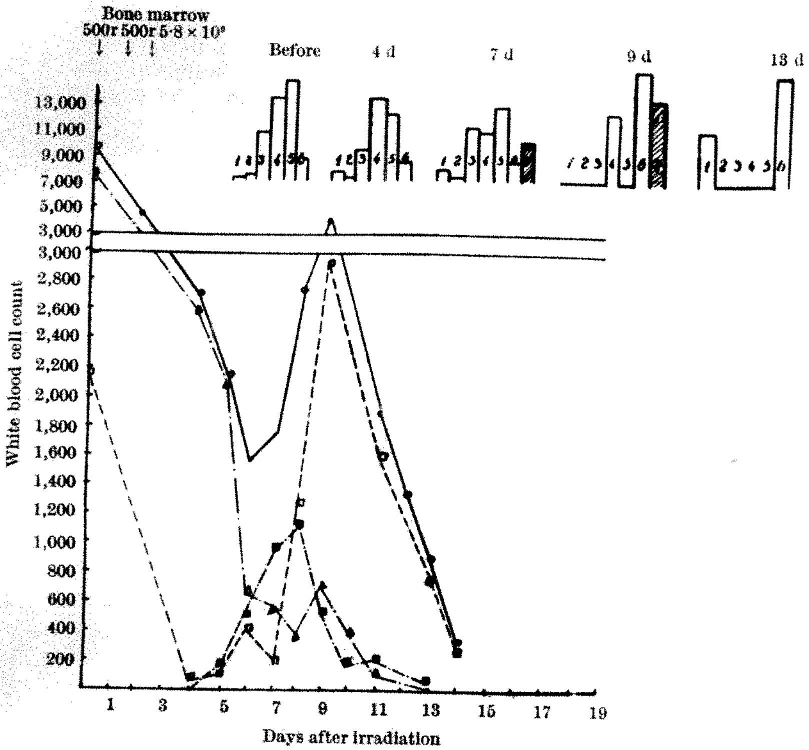

Fig. 1. White blood cell counts and myelograms in a dog irradiated with 1,000 r. for two days. On the day after radiation the dog received allogenic marrow. 1, Reticular cells; 2, stem cells; 3, immature cells of myeloid row; 4, mature cells of myeloid row; 5, erythropoietic row; 6, lymphocytes; 7, hyperbasophilic cells. ○, leucocytes; △, neutrophils; □, lymphocytes; ■, hyperbasophilic cells

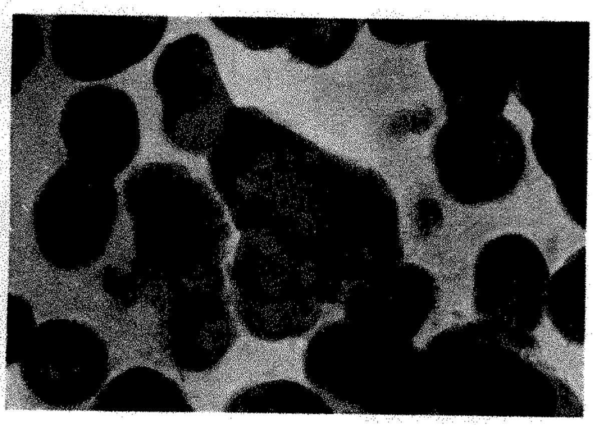

Fig. 2. Hyperbasophilic 'haemocytoblast' from peripheral blood

Consequently, hæmopoiesis was of the lymphoid type. The picture of the peripheral blood reflected this transformation of hæmopoiesis. On the eighth to ninth days the leucocyte count rose greatly (Fig. 1). The rate of proliferation was very high; for 1–2 days the white blood cell count rose 3–4 times (from 400–1,500 to 1,700–4,200 cells/mm^3); moreover, the neutrophil number did not enlarge practically. The effect was caused mainly by hyperbasophilic forms, the number of which was 60–80 per cent from the general leucocyte count on the eighth day. The next day the number of hyperbasophilic cells reduced sharply: simultaneously the lymphocyte count rose. On the following days hyperbasophilic cells disappeared, white blood cells, represented almost only by lymphocytes gradually fell. In bone marrow reticular hyperplasy developed, only reticular cells and lymphocytes were observed (Fig. 1).

Autopsy disclosed massive hæmorrhage in lungs, gastrointestinal tract, and aplasy in lymphoid nodes and bone marrow. Hence supra-lethally X-irradiated dogs had 'takes' of bone marrow allograft which differentiated normally during 3–4 days and then myeloid hæmopoiesis of graft stopped, and hyperbasophilic cells from hæmocytoblast formed. They intensively proliferated for 1–2 days (on the smears figures of amitotic and mitotic division in hyperbasophilic cells were observed); afterwards proliferation stopped and cells transformed into lymphocytes. On the whole this phenomenon is close to that observed in lymph nodes after antigenic stimulation[2]. This suggests that following the strong antigenic stimulation bone marrow allograft transforms into immunologically competent cells which are able to induce the reaction 'graft versus host'. This assumption is confirmed by the fact that transformation does not begin for 3–4 days, this period being necessary for immunization of graft cells. On the other hand, immunological genesis of this graft transformation is confirmed by the similarity of the hyperbasophilic cells described here and of those known as immunologically competent in transplantation immunity[3]. Immunologically inverted genesis of allogenic bone marrow cell is supported by our experiments on screening of dogs' hind-legs during irradiation. No hyperbasophilic cells were seen in the stage of active proliferation of autological bone marrow; hæmopoiesis was normal and the dogs survived.

Special investigations confirmed that hyperbasophilic cells had been formed from the graft's hæmocytoblasts but not from any occasional immunologically competent cells available in the transplant. Intraperitoneal transplantation of 5–15 × 10^9 lymphoid cells derived from donor's spleen and lymph nodes to supralethally X-irradiated dogs was accompanied only by an inconsiderable number of hyperbasophilic cells. These results suggest that hyperbasophilic cells and their derivatives (lymphocytes) are morphological bases of secondary disease and any other reaction of transplantation immunity. Some clinical investigations[4,5] suggest that hæmopoiesis transformation of the type described may occur in bone marrow transplantation in irradiated human beings.

In reactions of 'host versus graft' type, in particular in skin grafts, hyperbasophilic cells are probably formed from stem cells of lymph nodes[3]. It is suggested that some transplantation immunity reactions occur also in disease

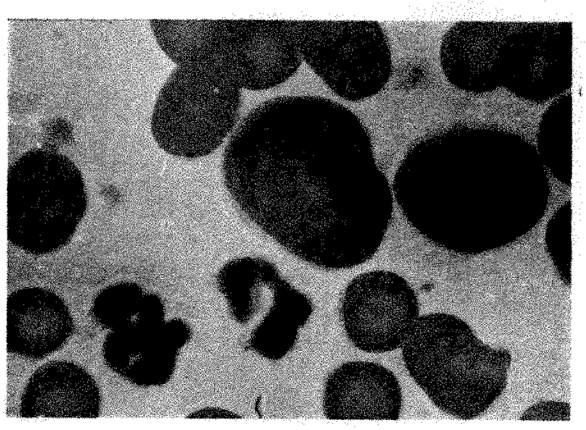

Fig. 3. Hyperbasophilic 'lymphoblast' (left) and 'prolymphocyte' (right) from peripheral blood

accompanied by intensive disintegration of tissues. So we observed the appearance of hyperbasophilic cells in blood of X-irradiated or burnt dogs without any tissue transplantation during the regenerative period.

Transformation of hæmopoietic cells into immunologically competent cells participating in reaction of transplantation immunity is reported here for the first time. Many authors[5–10] consider that the only morphological basis for secondary disease is immunologically competent cells of bone marrow or blood. Our results indicate that secondary disease can be induced by the immunologically competent cells derived from the stem cells of bone marrow, but not by the available immunologically competent cells of bone marrow or blood. In other words, the morphological basis both of protective action of bone marrow transplantation and of immunological complications is identical; that is, stem hæmopoietic cells. Hence it is doubtful whether reducing the number of available immunologically competent cells in the transplant can prevent secondary disease. It is necessary for prophylaxis of secondary disease first of all to prevent transformation of the graft after lymphoid type. The allogenic bone marrow transplantation in supra-lethally irradiated dogs is an excellent model for investigating this problem. The possibility of transformation of hæmopoietic cells into immunologically competent cells following a strong antigenic stimulation suggests that similar processes can also occur in unirradiated hosts. Perhaps this immunological mechanism is significant in pathogenesis of some forms of leukæmia. It is very important to ascertain why in some cases transformation of hæmopoiesis developed very early, and in other cases secondary disease was revealed considerably later if at all[11]. Perhaps the solution of these questions will render possible treatment of the secondary disease, and using of the latter, in leukæmia therapy.

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INHIBITORY ASPECTS OF BILE SECRETION

By Dr. TOM HARGREAVES and Prof. GRANT H. LATHE

Departments of Chemical Pathology, St. George's Hospital Medical School, London, S.W.1, and School of Medicine, Leeds

A KNOWLEDGE of the mechanism of bile secretion is probably as important for understanding various types of jaundice, as the mechanism of renal function is the key to understanding kidney disease. Unfortunately, our knowledge of bile secretion is limited due to the inaccessibility of the biliary tract to direct observation. Nevertheless, in recent years some important advances have been made: particularly important is the suggestion that a distinction should be made between the formation of bile and the secretion of substances into it.

Some substances, for example, bilirubin and bile salts, are secreted in bile at a higher concentration than in plasma; others, for example, sodium, potassium and glucose, occur at approximately plasma concentrations. There is also a third class of substances present in bile in lesser amounts than in plasma[1]. Many foreign substances are concentrated in bile; some, like bromsulphthalein, serve as useful agents in assessing liver function, others are used for X-ray examination of bile channels. We propose to use the term 'cholephil' for all endogenous and exogenous substances which are concentrated in the bile.

It appears probable that bile formation involves two simultaneous processes, the formation of a fluid similar to deproteinized plasma and the secretion of cholephilic substances into this fluid. This is suggested by experiments in which the two functions can be dissociated; bile salts increase the flow of bile without increasing the total yield of administered cholephilic substances, for example, bromsulphthalein[2]. Bile production does not depend on the hydrostatic pressure of blood perfusing the liver[3] and must, therefore, depend on a chemical mechanism to provide the energy for bile production. It has been shown that the flow of bile after administration of sodium taurocholate is directly proportional to the taurocholate secretion rate and the electrolyte composition was similar at all rates of secretion[4]. Since the concentrations of the major inorganic anions, chloride and bicarbonate, change very little over the entire range of bile salt secretion rates, the output of these anions must also be directly proportional to the bile salt secretion rate. These observations lend support to the view expressed by Sperber[5] that organic anion secretion (normally bile salt secretion) is actually the primary event in bile formation. It is possible that the movement of inorganic electrolytes and water into the bile may proceed passively and independently along gradients of concentration and electrical potential created by active bile salt secretion into the canalicular lumen, or may be coupled in some manner with active bile salt transport[6]. This suggests that the secretion of high concentrations of other cholephils may enhance bile secretion, as shown for chloral[7]. Cyanide produces a dissociation of the reverse type, reduced bile flow with maintenance of bilirubin and bromsulphthalein secretion[8]. A third type of dissociation, reduction of secretion of cholephilic substances with normal or enhanced bile secretion, can be produced experimentally by administration of phloridzin[8]. Jenner and Smyth[9] found phloridzin, or a product of it, was excreted in the bile at a concentration of up to 0·9 per cent (20 m.equiv./l.).

A number of organic anions, for example, bile pigments, several phthalein dyes, indocyanine green and the bile salts are excreted into the bile in such high concentrations that it is generally assumed that active transport mechanisms must be involved. There is a limiting rate of bromsulphthalein secretion: this mechanism can be saturated by increasing the plasma concentration. Similar saturation phenomena have been described for sodium dehydrocholate, bilirubin and fluorescein[10].

Various reports have suggested that cholephilic substances compete with one another at several stages in their transport from plasma to bile. Dragstedt and Mills[11] examined the rate of clearance of intravenously administered bromsulphthalein; simultaneous administration of bilirubin reduced the rate of clearance by 20–50 per cent. Mendeloff, Kramer, Ingelfinger and Bradley[12] found a reduced clearance of bromsulphthalein from the plasma when dehydrocholic acid was injected and a similar but smaller effect with rose bengal Wheeler, Cranston and Meltzer[13], using dogs, demonstrated that during the constant injection of bromsulphthalein, indocyanine green increased the plasma bromsulphthalein concentration without any reduction of bromsulphthalein excretion Both these results were open to the criticism that bromsulphthalein was being displaced from extra-hepatic tissues, but Andrews and Richards[14] showed that in dogs conjugated bile salts led to a release of bromsulphthalein into the hepatic vein Hunton, Bollman and Hoffman[15] have shown that in unanæsthetized dogs the clearance of bromsulphthalein is reduced by indocyanine green, rose bengal, bilirubin and sodium dehydrocholate In rats bromsulphthalein and indocyanine green each affected the clearance of the other

Rate of clearance cannot be equated with rate of secretion, as the former may be affected by hepatic blood flow and the latter by storage of administered material in the liver It seems possible that for a brief period secretion into the bile might be reduced without clearance being altered, and vice versa Direct measurements of rates of secretion have suggested that competition occurs though usually it has been impossible to say whether this was a primary effect on secretion or secondary to competition for uptake

Investigation of liver uptake has been made by direct observation of the fluorescent substance uranine[16], bilirubin reduced the rate of uranine uptake and its subsequent biliary secretion Fauvert[17] suggested that competition for hepatic uptake exists between bilirubin and bromsulphthalein, the latter being selectively removed Bradley[18] investigated the uptake of bromsulphthalein, as distinct from its secretion, by following the clearance during continuous intravenous administration at increasing rates *In vitro* investigations have shown that biligrafin inhibits the uptake of bromsulphthalein by liver slices[19]

It has been postulated that anionic substances are excreted by a different pathway from cationic substances[20] A number of quaternary ammonium compounds inhibited the biliary excretion of each other, while others were without effect Anions, for example, bromsulphthalein and glycocholate, did not compete with the cations for biliary excretion

In considering the mechanism of competition for a common pathway of secretion into the bile, account must be taken of chemical alterations of the secreted substance Thus bilirubin is secreted in the bile as a diglucuronide, since animals in which the conjugating mechanism is defective cannot excrete bilirubin but can excrete injected bilirubin glucuronide[21], conjugation is essential for secretion of bilirubin In contrast to bilirubin, bromsulphthalein can be excreted unchanged, but most of the injected pigment appears in the bile as a conjugate of glutathione[13] In a third category are those cholephils like indocyanine green[13] and rose bengal[18] which are secreted into the bile without chemical alteration

Other examples of cholephils competing for excretion in the bile are the retention of bilirubin[22] and bromsulphthalein[23] following bunamiodyl administration, and bilirubin retention in the new-born following novobiocin administration[24]

We examined the reduction of bile secretion of one cholephilic substance by another as it seemed possible that this investigation might help to define the biochemical nature of the secretory mechanism We are now reporting the inhibitory effect of a number of cholephilic substances on *in vitro* glucuronide synthesizing systems, and on the *in vivo* secretion of two cholephilic substances, bilirubin glucuronide and indocyanine green

The rate of bilirubin glucuronide formation was determined in rat liver slices, and rat and rabbit liver homogenates, by the method of Lathe and Walker[25] *o*-Aminophenol conjugation was measured by the method of Stephenson and Dutton[26] In the homogenate experiments uridine diphosphate glucuronic acid, Sigma Grade (Sigma Chemical Co), was added in a final concentration of 0 3 m mole Conjugated bilirubin in liver slices was determined according to Hargreaves[27] Glucose-6-phosphatase was estimated by the method of de Duve, Pressman, Gianetto, Wattiaux and Appelmans[28], the hexobarbitone metabolizing enzyme was determined by the method of Cooper and Brodie[29]

The secretion of substances in the bile was investigated in male Wistar rats, anæsthetized with veterinary 'Nembutal' (Abbott), in whom the bile duct was cannulated[30] Bile was collected over 10-min intervals Administered substances were given intravenously Conjugated bilirubin was given as diluted human bile collected post-operatively from a patient with a T-tube in the common bile-duct Norethandrolone was administered in propylene glycol which was found to have no effect on dye or pigment excretion Conjugated bilirubin was determined by the method of Lathe and Walker[25] Indocyanine green was estimated by diluting the bile 1 to 10 or 1 to 100 with water and reading against an appropriate blank at 805 mμ in a spectrophotometer (Unicam *SP* 600)

In vitro *effect of cholephils on formation of glucuronides* Increasing concentrations of cholephilic substances were added to rabbit liver homogenates in order to determine the approximate concentration at which 50 per cent inhibition of bilirubin and of *o*-aminophenol conjugation occurred

Table 1 CONCENTRATIONS REQUIRED TO PRODUCE 50 PER CENT INHIBITION OF MICROSOMAL ENZYMES IN RABBIT LIVER HOMOGENATE

Inhibitor $10^4 \times$ conc (M)	Glucuronyl transferase		Glucose-6-phosphatase
	o-Aminophenol	Bilirubin	
Novobiocin	1	2	50
Bunamiodyl	3	2	>100
Norethandrolone	>100	8	>100
Methicillin	20	30	>100
Bromsulphthalein	< 0 1	2	6
Indocyanine green	3	5	90
Rose bengal	0 6	2	40
Fluorescein	8	—	5
Chloral	50	>100	70
Phenolphthalein	0 2	0 3	40
Phenolphthalein glucuronide	4	0 6	>100

The results of the experiments of this type have been summarized in Table 1 All but methicillin, chloral and norethandrolone inhibited glycuronyl transferase at concentrations of less than 1 m mole As the enzyme conjugating substances with glucuronic acid occurs in the microsomes, two other microsomal enzymes were tested The results with glucose-6-phosphatase are given in Table 1 The enzyme metabolizing hexobarbitone was examined in the presence of four cholephilic substances The rate of hexobarbitone metabolism was inhibited 41 per cent by 0 1 m mole bunamiodyl, 27 per cent by 0 1 m mole indocyanine green and 33 per cent by 1 m mole sodium deoxycholate, 10 m mole methicillin had a negligible effect on this enzyme

In vivo *effect of cholephils on secretion into the bile* Figs 1 and 2 show the rise and fall of bilirubin glucuronide and indocyanine green respectively in rat bile following intravenous injection, alone and with the simultaneous injection of novobiocin The results of 27 experiments of this type are summarized in Table 2 (indocyanine green) and Table 3 (conjugated bilirubin) All cholephilic substances reduced the secretion of conjugated bilirubin and indocyanine green

In experiments designed to eliminate competition for uptake, the injection of one cholephilic substance (bunamiodyl, novobiocin or norethandrolone) was delayed for 20 min after the administration of the bilirubin glucuronide or indocyanine green in order to allow the liver to take up the latter without competition Each of the substances inhibited the excretion of indocyanine green and bilirubin glucuronide

Table 2 INHIBITION OF BILIARY SECRETION OF INDOCYANINE GREEN (0 13 μMOLE) IN THE RAT

Inhibitor	Dose (μmole)	Relative depression of secretion (%)
Novobiocin	1 3	47
	13	92
Novobiocin (Gunn rat)	13	72
Bunamiodyl	1 3	33
	13	93
Norethandrolone	6 5	74
	65	84
Methicillin	1 3	48
	13	95
Chloral	1 3	0
	130	77
Phenolphthalein	1 3	46
	13	93
Phenolphthalein glucuronide	1 3	59
	13	74

Table 3 INHIBITION OF BILIARY SECRETION OF CONJUGATED BILIRUBIN IN THE RAT

Inhibitor	Dose (μmole)	Conjugated bilirubin (μmole)	Relative depression of secretion (%)
Indocyanine green	0 13	0 3	29
Novobiocin	3 0	0 6	53
	30	0 6	65
Bunamiodyl	40	0 4	66
Methicillin	6	0 6	27
	60	0 6	66
Chloral	13	0 4	0
	130	0 4	43
Phenolphthalein	3	0 3	10
	30	0 3	72
Phenolphthalein glucuronide	3	0 3	13
	30	0 3	33

Secretion by liver slices The accumulation of bilirubin glucuronide and *o*-aminophenol glucuronide in a medium in which liver slices were incubated was the basis of the standard procedures for demonstrating conjugation by slices The addition of novobiocin, bunamiodyl or norethandrolone reduced the yield of glucuronides in the medium In order to define the secretory process conjugated bilirubin was estimated in both slices and medium (Table 4). At certain concentrations, 0 01 m mole novobiocin, 1 m mole bunamiodyl, and 1 m mole norethandrolone it was noted that there was a reduction in the glucuronide passing into the medium during the 2 h of incubation although substantial amounts of glucuronide were formed, as demonstrated by the amount remaining in the slices Under these conditions it was concluded that the secretory process was inhibited more than the uptake of bilirubin, or its conjugation. Novobiocin reduces the total amount of bilirubin in the slice, indicating that it also inhibits the uptake of bilirubin

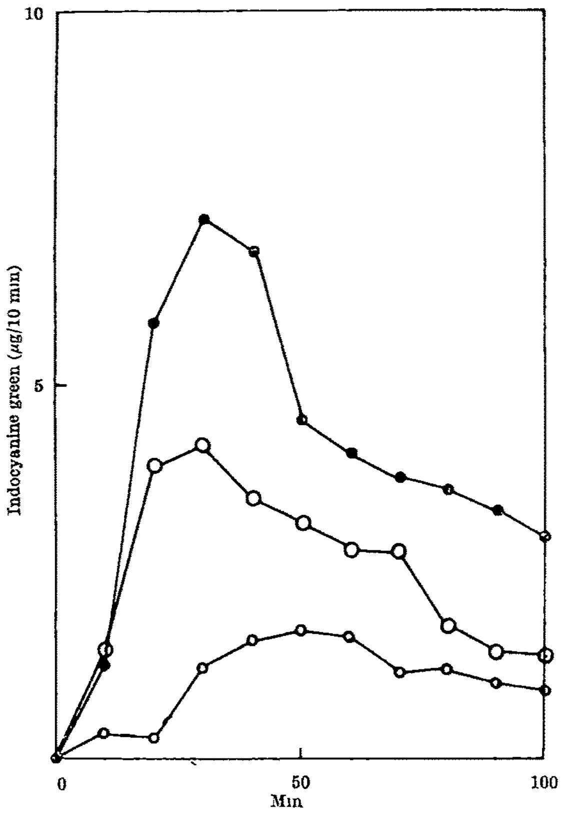

Fig 1 Effect of novobiocin on the concentration of indocyanine green in bile ●, control animal injected with 0 13 μmole indocyanine green Test animals injected with 0 13 μmole indocyanine green together with 1 3 μmoles (○) and 13 μmoles novobiocin (○)

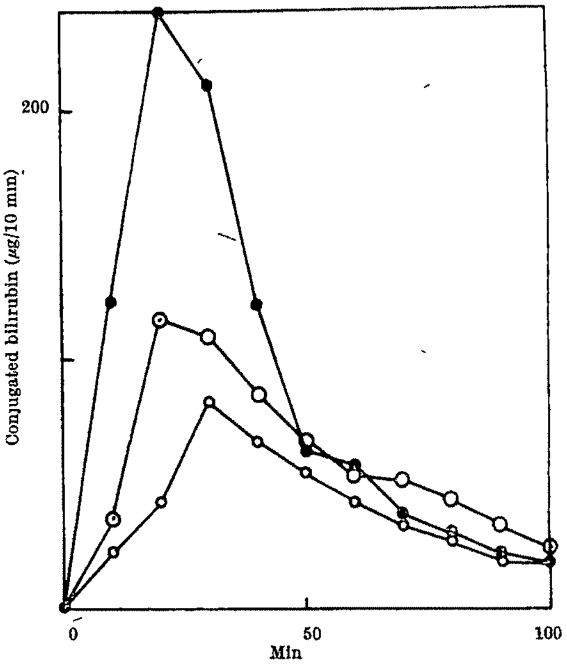

Fig 2 Effect of novobiocin on the concentration of conjugated bilirubin in bile ●, control animal injected with 0 6 μmole conjugated bilirubin Test animals injected with 0 6 μmole conjugated bilirubin together with 3 μmoles (○) and 30 μmoles (○) of novobiocin

Table 4 RATE OF CONJUGATION AND ACCUMULATION OF BILIRUBIN IN LIVER SLICES IN THE PRESENCE OF CHOLEPHILS

Conc (mM)	bilirubin in slice (μg/g) Conjugated	Unconjugated	Rate of conjugation (μg/g/h)
		Novobiocin	
0	67	22	38
0 01	55	17	24
0 1	34	15	10
1 0	25	16	0
10	13	16	0
		Bunamiodyl	
0	67	22	38
0 01	54	32	37
0 1	57	29	30
1 0	40	39	0
10	21	29	0
		Norethandrolone	
0	67	22	38
0 01	57	16	28
0 1	63	17	25
1 0	64	10	24
10	41	15	17

In order to test the effect on the secretory mechanism which excluded the conjugating process, rat liver slices were incubated in the same medium used for examining conjugation of bilirubin, but bilirubin glucuronide was added in place of unconjugated bilirubin The effect of adding novobiocin, bunamiodyl and norethandrolone to such a system is given in Table 5 Each of the substances caused the slices to accumulate it

Table 5 INHIBITORS ON THE ACCUMULATION OF CONJUGATED BILIRUBIN IN LIVER SLICES

Time (min)	Control	Conjugated bilirubin (μg/g slice) Novobiocin	Bunamiodyl	Norethandrolone
0	16	16	16	16
30	40	46	45	78
60	54	71	68	88
90	56	88	90	101
120	64	105	112	124

Most of the cholephilic substances examined have two properties in common they inhibit the *in vitro* formation of glucuronides in liver homogenates (Table 1), and the *in vivo* biliary excretion of both bilirubin glucuronide and indocyanine green, methicillin, chloral and norethandrolone appear to be inhibitors of glucuronide formation at higher concentrations than other cholephilic substances Fluorescein and phenol phthalein are excreted as glucuronides in bile, chloral is excreted as the glucuronide of trichlorethanol The mode of excretion of novobiocin, bunamiodyl, norethandrolone and methicillin is not known Billing[31] did not find an increase in glucuronic acid in the bile when novobiocin was administered to rats. All cholephils tested inhibited the biliary excretion of bilirubin glucuronide and indocyanine green *in vivo* Eight of eleven cholephilic substances inhibited glucuronide formation at a concentration of less than 1 m mole, including four cholephils which are not secreted as glucuronides

The *in vivo* investigations are open to the criticism that they may be affected by factors other than the biliary concentrating capacity Secretion into the bile could be retarded by a decreased hepatic circulation as a result of the administration of cholephilic substances, this seems improbable since in each experiment the control study was made following the injection of one cholephilic substance. Circulatory factors do not play a part in the slice experiments (Tables 4 and 5) in which the secretory phenomenon was demonstrated We conclude that circulatory factors are unlikely to be very important Secretion into the bile can also be retarded by reduced uptake of indocyanine green or bilirubin by the liver, due to direct competition Novobiocin reduces the secretion of bilirubin glucuronide from the liver slice, but it also reduces the total amount of bilirubin in the slice (Tables 4 and 5) These results suggest that novobiocin affects the uptake of bilirubin as well as the secretion of bilirubin glucuronide In the case of bunamiodyl and norethandrolone the experiments shown in Tables 4 and 5 indicate that secretion into the medium is reduced more than uptake into the liver slices This suggests that the secreting mechanism is more sensitive to bunamiodyl and norethandrolone than either the mechanisms of uptake or of glucuronic acid conjugation Novobiocin has an effect on the uptake of bilirubin and on the secretory mechanism In order to demonstrate that all three substances affect the secretory mechanism the injection of the inhibitory cholephil was delayed in the *in vivo* investigations until the liver had taken up a large part of the bilirubin glucuronide or indocyanine green, this did not prevent the inhibitory effect

There are three possibilities to explain why cholephilic substances inhibit glucuronide formation by homogenates and the secretion of cholephils into bile First, glucuronide formation may play a part in the concentrating mechanism It seems improbable that the formation of glucuronides of all cholephilic substances is essential for concentration in the bile Indocyanine green was chosen for detailed investigation, together with bilirubin glucuronide, because the former is excreted chemically unaltered, rose bengal and bromsulphthalein can also be excreted unchanged. Bromsulphthalein is excreted normally in patients with familial non-hæmolytic hyperbilirubinæmia lacking the ability to conjugating bilirubin, and in the parallel condition in the jaundiced Gunn rat[32] We have also shown (Table 2) that indocyanine green is excreted by the jaundiced Gunn rat These observations do not exclude participation of glucuronyl transferase in the concentrating mechanism It is probable that there is more than one enzyme Gunn rats are able to form N-glucuronides[33], they can form glucuronides of N-acetyl o-aminophenol and menthol as can patients with familial non-hæmolytic hyperbilirubinæmia, albeit at a reduced rate[32] These observations suggest that a number of glucuronyl transferase enzymes occur Glucuronyl transferases have a second substrate, uridine diphosphate glucuronic acid Inhibition by cholephils could equally well occur at this enzyme site, should this be true it would suggest that uridine diphosphate glucuronic acid was involved in some way in the concentrating mechanism of bile secretion

Secondly, cholephilic substances may have a chemical property which makes them inhibitors of glucuronyl transferase This is unlikely because of the wide variety of chemical types represented (Table 1) unless the same chemical property which makes these substances cholephilic confers the capacity to inhibit glucuronyl transferase This is another way of suggesting that there is a chemical similarity between glucuronyl transferase and some key participant in the concentrating mechanism.

The third possibility is that there is a common pathway in the two processes of conjugation and secretion of cholephilic substances Glucuronide formation requires the enzyme glucuronyl transferase and the specific donor substance uridine diphosphate glucuronic acid. In slice experiments uridine diphosphate glucuronic acid is constantly generated; in broken cell experiments we added an amount of uridine diphosphate glucuronic acid which was nearly optimal for the reaction However, one may not assume that under these conditions the only factor is the kinetics of the enzyme, for glucuronyl transferase is not in solution, but part of the microsomal fraction In the intact cell it is attached to, and possibly embedded in, the phospholipid-rich, endoplasmic membranes In microsomal preparations cholephils may retard the access of substrates, like bilirubin, to the enzyme by competition for passage through the phospholipoid membrane Equally, egress of the glucuronide formed could be inhibited Theoretically, the concentrating mechanism could share either of these as a common pathway It is unlikely that competition occurs at the point of access to the enzyme because high concentrations of unconjugated bilirubin in patients with familial non-hæmolytic hyperbilirubinæmia and in jaundiced Gunn rats do not inhibit the biliary excretion of bromsulphthalein and iodipamide In contrast indocyanine green and bilirubin glucuronide compete for excretion in the normal and in the jaundiced Gunn rat; this indicates that competition for the concentrating mechanism can only occur after the enzyme If the two processes are distinct, it must follow that bilirubin can only get to the secreting mechanism via the glucuronyl transferase pathway, and once conjugated it is prevented from escaping into the cell sap or medium by the inhibitors We postulate that the only exit is via the secreting mechanism, glucuronyl transferase inhibition is then produced by an accumulation of bilirubin glucuronide, this would explain the inhibitory effect of cholephils on conjugation by homogenates and microsomes It should be possible to demonstrate the accumulation of bilirubin glucuronide in inhibited microsomes, similar to the experiment of Table 5 with slices, but we have been unsuccessful in attempts to do this In view of the possible close relation between microsomes and the concentrating mechanism we examined the effect of cholephilic substances on two other microsomal enzymes, glucose-6-phosphatase and the hexobarbitone metabolizing enzyme Both were inhibited by some of these substances but less consistently than glucuronyl transferase and at relatively high concentrations

The evidence suggests that cholephilic substances act on a part of the secretory mechanism, but this is not necessarily the only site of action of these substances Some may affect the mechanism of uptake into the liver, the path of access to the enzyme or glucuronyl transferase directly Two cholephilic substances, bunamiodyl and novobiocin, produce an increased plasma concentration of unconjugated bilirubin in patients in certain circumstances In the case of novobiocin this could be due to a block in the uptake of bilirubin, inhibition of the conjugating enzyme, or it could be an effect on the secretory mechanism Bunamiodyl acts on the secretory mechanism We do not consider that the accumulation of bilirubin rather than bilirubin glucuronide excludes a block of the mechanism secreting cholephils Jaundice due to norethandrolone is associated with conjugated bilirubin in the plasma and it takes some days to develop It is probably not the immediate effect of enzyme inhibition, as suggested by Hsia, Dowben, Shaw and Grossman[34], but a more chronic and secondary effect on the concentrating mechanism limiting the movement of bilirubin glucuronide, and so allowing this to appear in the plasma

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6-AMINONICOTINAMIDE AND THE RADIOSENSITIVITY OF HUMAN LIVER CELLS IN CULTURE

By DR D L DEWEY and CHRISTINE HAWES

British Empire Cancer Campaign Research Unit in Radiobiology, Mount Vernon Hospital, Northwood, Middlesex

6-AMINONICOTINAMIDE is a structural analogue of nicotinamide, and the physiological disturbances which it causes in the animal organism are thought to be due to its incorporation into co-enzymes with a consequent loss of their normal function Herter *et al* [1] have shown that daily doses of 6-aminonicotinamide are not toxic to patients until the cumulative dose exceeds about 5 mg/kg body-weight However, in animal experiments the tolerated dose has been increased nearly nine times by the simultaneous injection of nicotinamide[2] Combination therapy using 6-aminonicotinamide together with irradiation has already been claimed to increase tumour regression in mice[3]

With a possible clinical use in mind, Dr L G Picciotto suggested testing the effect of 6-aminonicotinamide on the radiosensitivity of human cells in culture For these experiments a strain of human fœtal liver cells was used and the effect of radiation on colony-forming ability was measured In order to obtain accurate control of temperature and oxygen tension and to ensure a uniform dose distribution the irradiations were carried out in a water-jacketed nylon vessel at 37° C and the cells were suspended in phosphate-buffered medium with or without 6-aminonicotinamide at a concentration of 2 mM The suspension was rotated by a strong jet of air impinging on the surface and was also stirred with a motor-driven nylon paddle Samples withdrawn just before and immediately after irradiation with 200-kV X-rays were diluted if necessary and added to heavily irradiated feeder layers in glass bottles containing bicarbonate medium Cultures which had been exposed to 2 mM 6-aminonicotinamide were then treated with nicotinamide at a final concentration of 20 mM in order to reverse the inhibitory effect of the analogue on cell growth Complete reversal by nicotinamide was possible within an hour of adding the 6-aminonicotinamide The cultures were incubated at 37° C for 10 days and the number of colonies containing more than 32 cells at this time was taken as a measure of the number of surviving cells in the sample added

The log surviving fraction plotted against X-ray dose could be represented by a line with an initial shoulder followed by a straight portion. In the absence of 6-aminonicotinamide the dose giving 37 per cent survival on the straight line part was 140 rads, slightly greater than the 120 rads previously reported for the same cells irradiated at 20° C (ref 4) The effect of adding 6-aminonicotinamide to a final concentration of 2 mM shortly before irradiation is shown in Fig 1 The immediate impression conveyed by the result displayed in Fig 1 is that 6-aminonicotinamide sensitizes cells to radiation damage, and preliminary reports have already been published to this effect[5,6] More recent work using abnormal telophase figures as the criterion of damage suggested that the results shown in Fig 1 might not be due to radiosensitization[7]

Ehrlich ascites tumour cells of the mouse were used in the initial work on chromosome abnormalities, which were examined in telophase of the first post-irradiation mitosis The effect of 6-aminonicotinamide on the radiosensitivity of these cells *in vivo* was tested by irradiating 5-day ascites tumours in the mouse peritoneal cavity in the presence or absence of the analogue Two groups of mice were injected intraperitoneally with 0 65 ml of an 80 mM solution of 6-aminonicotinamide 0 5 h before they received 300 rads total-body irradiation The

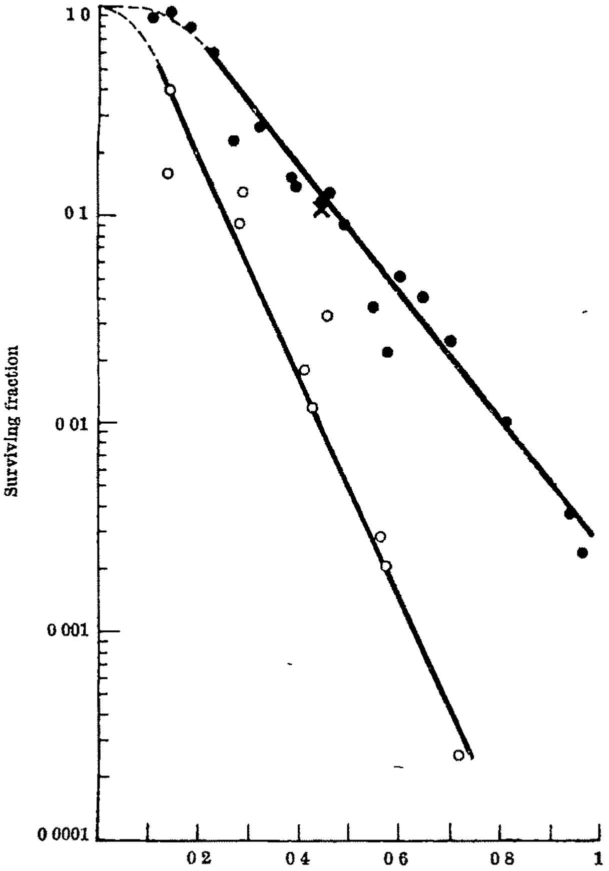

Fig 1 The colony-forming ability of human embryo liver cells in culture after exposure to 200-kV X-rays ●, Cells irradiated in suspension in a phosphate-buffered medium, ○, cells irradiated in medium containing 2 mM 6-aminonicotinamide, ×, 6-aminonicotinamide added to cells after irradiation

mice in one group were then injected with nicotinamide. Half an hour after irradiation the ascitic suspensions were transferred into unirradiated host mice and incubated for 15 h, at which time the proportion of abnormal telophase figures attained a maximum value The results given in Table 1 (experiment 1) show that 6-amino-nicotinamide had no effect on the observed amount of radiation-induced damage

In view of this negative result it was decided to repeat the chromosome damage experiment under controlled *in vitro* conditions Ascitic suspensions diluted with phosphate-buffered saline were brought into equilibrium with oxygen and irradiated in the 'Perspex' vessel described by Deschner and Gray[8] 6-Aminonicotinamide at a final concentration of 8 mM was added to the test sample 15 min before irradiation and chromosome damage was estimated as in the *in vivo* experiments The results of irradiating cells in the presence of 6-aminonicotinamide together with results of controls in which the analogue was absent or was added immediately after irradiation are given in Table 1 (exp 2) Again there was no evidence of any sensitization

The apparent lack of correlation between results obtained using colony-forming ability on one hand and chromosome abnormality on the other as criteria of damage required further investigation The possibility of explaining the discrepancy as a species difference was eliminated by repeating the chromosome investigations on the same human cell line which was used in the experiments measuring reproductive integrity The cells were grown on glass coverslips in small Petri dishes at 37° C for about 45 h before irradiation The entire experiment was carried out in a 'Perspex' gas-tight incubator designed to allow irradiation of the cultures through the closed lid All the Petri dishes were irradiated at the same time, half of them having received 6-aminonicotinamide at a concentration of 2 mM 26 min prior to irradiation Throughout this procedure, the incubator was gassed with air containing 6 per cent carbon dioxide. After the X-ray exposure, nicotinamide at a final concentration of 20 mM was added to the experimental cultures and the control cultures were incubated in 2 mM 6-amino-nicotinamide for an equivalent length of time before nicotinamide was added Previous experiments had shown that the percentage of abnormal telophase cells reached a maximum 23 h after irradiation At this time the coverslips were transferred to fixative and the cells were stained and examined As in the earlier experiments with the Ehrlich tumour the presence of 6-aminonicotinamide during irradiation had no effect on the radiosensitivity of the human cells as measured by chromosome damage (Table 1, exp. 4)

These results indicated a difference in the effect of 6-aminonicotinamide on radiosensitivity as measured by chromosome damage on one hand or reproductive death on the other, the possibility was investigated that this difference was due to a change in technique between the two types of experiment In the earlier experiments establishing the dose-response relations for the destruction of reproductive integrity (Fig 1), the cells were irradiated in suspension rather than in contact with the glass bottles to eliminate uncertainties in radiation dosimetry However, in the work on chromosome damage, the cells were growing on glass coverslips when they were exposed to 6-aminonicotinamide and radiation The survival investigations were therefore repeated on cells which were stuck to a surface at the time of treatment Cells growing in glass bottles were given the same dose of irradiation either in the presence or in the absence of 6-aminonicotinamide and the resulting colonies were counted in the normal way The results showed that 6-aminonicotinamide had no effect on radiosensitivity under these conditions

The essential difference between the earlier positive result shown in Fig. 1 and the later negative results is that in the former experiment 6-aminonicotinamide was added before the cells were allowed to attach to glass and the order was reversed in the later experiments The conclusion we drew was that 6-aminonicotinamide reduced the plating efficiency and this was later confirmed by an experiment designed to measure this effect directly

Table 1 CHROMOSOME DAMAGE INDUCED BY X-IRRADIATION IN THE PRESENCE OR ABSENCE OF 6-AMINONICOTINAMIDE (6 AN)

Exp	Material	Irradiation†	Abnormal telophases (per cent)		
			Control	6 AN before and during irradiation	6 AN after irradiation
1	Mouse ascites tumour cells	0 r	7 7 (2)	9 8 (2) 8 5 (1)*	
		300 r *in vivo*	76 3 (5)	76 8 (4) 74 7 (5)*	
2	Mouse ascites tumour cells	0 r	5 2 (3)	8 7 (4)	
		112 r *in vitro* gas phase oxygen	63 6 (4)	56 6 (4)	65 0 (2)
3	Human fœtal liver cells	0 r	7 2 (3)	8 6 (2)*	
4	Human fœtal liver cells	170 r. *in vitro* gas phase air		58 1 (6)*	59 5 (6)*

* Excess nicotinamide added within 1 h of adding 6 AN

† Dose measured in roentgens in air at the position of the irradiated sample

The figures in brackets refer to the number of observations made For each observation, a minimum of 200 telophase cells was scored from a single mouse or coverslip culture

In the earlier work, cells irradiated in suspension were afterwards diluted to give approximately the same number of viable cells per ml before plating As a result, 6-aminonicotinamide present in irradiated suspensions was diluted to a much greater extent after low doses than after high doses of radiation Thus the reduction of plating efficiency with increasing concentration of 6-aminonicotinamide gave an apparent survival curve of steeper slope than the control curve

The foregoing account has described the experiments in chronological order to illustrate how easily an experimental result can be misinterpreted In this case, it is interesting to note that the initial control in which 6-aminonicotinamide was added after irradiation (Fig 1) did not show the apparent sensitization, which may be accounted for by the fact that these cells were plated before the 6-aminonicotinamide was added

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CHANGES IN THE ELECTROPHORETIC PROPERTIES OF RABBIT SPERMATOZOA DURING PASSAGE THROUGH THE EPIDIDYMIS

By J. M. BEDFORD
Department of Physiology, Royal Veterinary College, London, N W 1

WHEN washed free of seminal plasma or taken directly from the vas deferens or cauda epididymis, motile mammalian spermatozoa exhibit a marked tendency to agglutinate by the head region if suspended in homologous heated serum, or in isotonic media containing free electrolytes[1] During examination of spermatozoa collected from different regions of the rabbit epididymis it was noticed that in 0 145 M sodium chloride, spermatozoa from the caput region show little capacity for head–head autoagglutination As sperm move toward the cauda region, however, an increasing percentage exhibits the capacity for autoagglutination (unpublished observations) Mammalian spermatozoa are known to mature with respect to their fertilizing ability, during passage from the caput to the cauda epididymis[2,3], and thus it seemed possible that the progressive increase in the capacity for autoagglutination during this passage might reflect surface changes of importance in the epididymal stage of the sperm maturation process

The surface characteristics of cells, as reflected in the ζ potential, have been investigated in several studies by means of controlled electrokinetic measurements[4-7] On the assumption that differences in genotype might be reflected in the sperm phenotype, and specifically in the surface character of spermatozoa, this method has also been applied by several investigators in attempts to separate male and female determining spermatozoa[8] In an attempt to obtain evidence for the existence of some change in the character of the sperm surface during the period of epididymal maturation, the electrophoretic behaviour of spermatozoa from different regions of the epididymis has been examined therefore, using an apparatus similar to that described by Bangham *et al* [9]

Adult male rabbits were killed by dislocation of the neck, and spermatozoa were immediately taken from the caput, corpus and cauda regions of the epididymis (Fig 1) by incising and squeezing these tissues in a medium of 0 145 M sodium chloride The sperm suspensions were then adjusted to a concentration of approximately 2 million/ml and were cooled to 10° C, at this temperature many spermatozoa from the corpus and cauda regions still showed definite though sluggish swimming movement The sperm were then transferred to the microelectrophoresis apparatus, the bath of which was maintained at 4° C. At this latter temperature the great majority of spermatozoa soon became immotile, though if the temperature were allowed to rise, independent sperm movement was again resumed As in earlier investigations[8,10], the sperm were immobilized by cooling in order that any movement of the sperm might be interpreted solely in terms of the response of a charged particle in a d c electric field All recordings were made at 50 V with a current of 1 0 m amp, at 4° C Under these conditions the resultant electrophoretic component was great enough to produce a measurable response in the spermatozoa, while any rise in temperature and drift due to convection currents were delayed long enough for an adequate number of readings to be obtained from each sample Accurate measurements were made of the electrophoretic mobility of individual spermatozoa and of the time taken for complete re-orientation of oriented sperm after reversal of the direction of the electric field Estimates were made of the time for any sperm population to reach a maximum state of orientation, and of the mode of orientation, that is, the percentage of spermatozoa oriented tail first, head first, or pointing vertically with respect to the attractive electrode In Table 1 it can be seen that, with the possible exception of rabbit 2, the mean mobility of sperm populations from the respective regions of the epididymis was similar in all animals, but in each animal the mean mobility of spermatozoa from the caput region was clearly lower than that of spermatozoa from the corpus and cauda regions, respectively From Fig 2 it is apparent that although caput spermatozoa generally exhibited relatively lower mobilities, a few caput sperm showed similar mobility to those sperm obtained from the cauda region This latter observation is consistent with the presence of a small percentage of fertile sperm in the caput region of the rabbit epididymis[11]

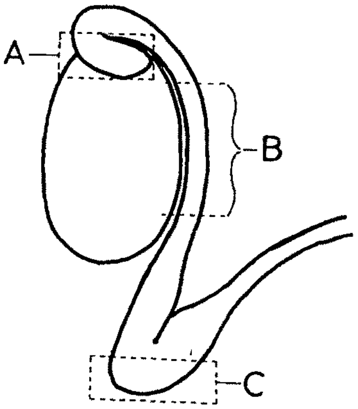

Fig 1 Diagram to show the regions of the caput (*A*), corpus (*B*), and cauda (*C*) epididymis, from which spermatozoa were recovered

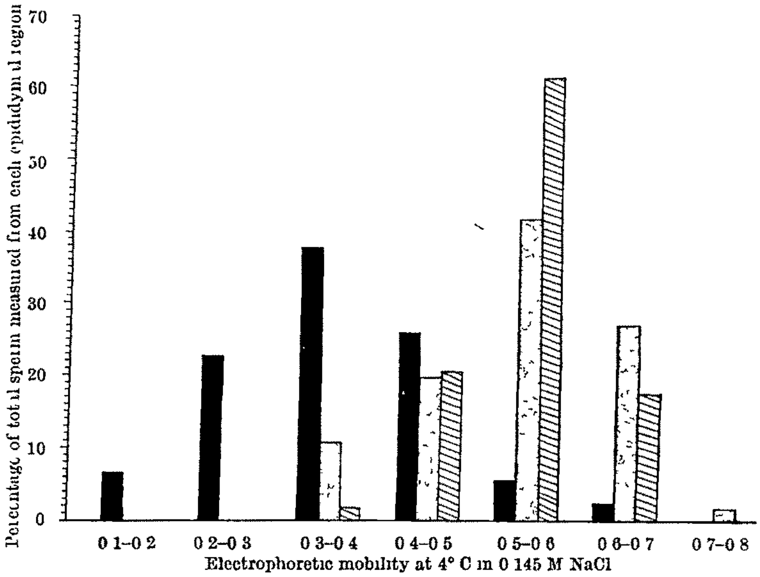

Fig 2 Histogram to show the distribution of electrophoretic mobilities (within a unit range) of spermatozoa from the different epididymal regions Black, caput spermatozoa, stippled, corpus spermatozoa, hatched, cauda spermatozoa

and with the observation that a few spermatozoa from the caput do show the capacity for autoagglutination

Unlike many other cell types which have been investigated by electrokinetic methods, the sperm cell is differentiated anatomically, and the surface membrane does not appear to exhibit uniform characteristics over the different anatomical regions[12] Thus, although the electrophoretic mobility may serve perhaps as one parameter for the measurement of sperm maturity, it seems that in order to obtain a true or complete indication of the state of the sperm surface, as reflected in the net surface charge, both the direction and speed of orientation must also be considered This is well demonstrated in a comparison of the measurements of sperm from the corpus and cauda regions Although the electrophoretic mobility was similar in sperm populations from both these regions (Table 1), the estimates of speed of initial orientation and the times for re-orientation following reversal of the electric field were markedly different in these sperm groups in each of the eight animals tested (Table 2) Under the influence of the particular field strength used, spermatozoa from the cauda region soon showed a definite orientation response, and after approximately 1–1 5 min the great majority of sperm were seen to be moving tail first toward the anode Spermatozoa taken from the corpus, on the other hand, orientated more slowly under identical conditions The majority of the spermatozoa from the corpus ultimately became orientated with the tail toward the anode, but some also remained vertical, and in several animals a small proportion of corpus spermatozoa became orientated with the head facing the anode Head anode orientation was seen more commonly, however, in spermatozoa from the caput region Compared with the behaviour of cauda spermatozoa, the tendency for orientation of caput spermatozoa was not immediately obvious, and in all caput samples the time allowed to elapse before the field could be deemed to have reached a state of maximum response to the electrophoretic component was relatively very much prolonged

Table 1 MEAN ELECTROPHORETIC MOBILITIES OF SPERMATOZOA TAKEN FROM CAPUT, CORPUS AND CAUDA EPIDIDYMIS, RESPECTIVELY EACH VALUE REPRESENTS THE MEAN OF NOT LESS THAN 12 SPERMATOZOA MOBILITY EXPRESSED AS μSEC^{-1} V^{-1} CM^{-1} AND MEASURED AT 4° C, IN 0 145 M SODIUM CHLORIDE

Rabbit No	Spermatozoa from Caput		Spermatozoa from Corpus		Spermatozoa from Cauda	
1	0 2782	(±0 036)*	0 5227	(±0 019)	0 550	(±0 012)
2	0 3461	(±0 036)	0 4229	(±0 019)	0 4574	(±0 015)
3	0 3550	(±0 024)	0 5759	(±0 024)	0 5394	(±0 010)
4	0 3778	(±0 015)	0 6172	(±0 015)	0 5619	(±0 012)
5	0 3405	(±0 010)	0 5616	(±0 025)	0 5674	(±0 020)
6	0 3176	(±0 022)	0 5178	(±0 016)	0 5492	(±0 014)
Mean	0 3468	(±0 019)	0 5363	(±0 027)	0 5371	(±0 025)

**S E*

In rabbit No 8 no true orientation response could be elicited in sperm samples from either the corpus or from the caput regions, although a normal response was obtained from the cauda spermatozoa in this animal In the other animals, the time for re-orientation of caput and corpus spermatozoa (mean of at least three sperm taken at random from each group) was clearly longer in each case than the time required for the re-orientation of cauda spermatozoa

Difficulty was experienced in judging the orientation response of spermatozoa in which the tail had become bent at the locus of the cytoplasmic droplet In media of low ionic strength, that is, 6 per cent fructose, it appears that the droplet possesses similar electrophoretic properties to those of the sperm head In this medium, both the head and the droplet are obviously more strongly attracted to the anode than is the distal part of the tail Such inference of similar surface properties over the region of the droplet and the head is perhaps strengthened by the additional observation that, in media of high ionic strength, motile spermatozoa appear to adhere to the surface of glass slides either by the anterior and lateral regions of the sperm head, or by the cytoplasmic droplet In the latter case, the head and the tail oscillate freely while the droplet remains apparently adherent to the surface of the slide (unpublished observations)

Mammalian fertilization is a complex process which shows considerable dependence on the environment and on the specificity of the participating gametes Several differences in spermatozoa from the respective epididymal regions have been reported[13–16] Yet in the present state of our knowledge of the mammalian fertilization process, the increasing capacity for motility[17], which may be related conceivably to changes observed in midpiece mitochondria[18,19], and possibly, though less clearly, also changes in the proportions of the acrosome cap of rabbit spermatozoa[11], are the only differences so far recognized which may reasonably be correlated with the disparity in fertilizing ability of spermatozoa from different epididymal regions It has been suggested[20] that changes in the

Table 2 ORIENTATION IN AN ELECTRIC FIELD (50 V, 1 0 M AMP IN 0 145 M SODIUM CHLORIDE AT 4° C) OF SPERMATOZOA FROM THE CAPUT, CORPUS AND CAUDA EPIDIDYMIS RESPECTIVELY ALL SPERMATOZOA ANODE POSITIVE

Rabbit No	Region of epididymis	% Head anode ~● +	% Vertical ● ~ + ~ ●	% Tail anode ●~ +	Time for orientation of field (min)	Mean time* for reversal of orientated spermatozoa (min)
7	Caput	75	20	5	4 3	4 0
	Corpus	—	5	95	3 3	2 3
	Cauda	—	—	100	0 85	1 1
8	Caput		Random		—	—
	Corpus		Random		—	—
	Cauda	—	5	95	1 25	1 1
9	Caput	90	10	—	5 3	2 25
	Corpus	10	60	30	4 6	2 8
	Cauda	—	10	90	1 0	1 4
10	Caput	40	30	30	5 0	4 5
	Corpus	10	20	70	3 25	4 0
	Cauda	—	—	100	0 85	1 0
11	Caput	60	25	15	6 3	2 25
	Corpus	—	15	85	4 0	2 0
	Cauda	—	15	85	1 5	1 1
12	Caput	75	15	10	2 25	2 8
	Corpus	5	15	80	3 0	2 0
	Cauda	—	—	100	1 4	1 4
13	Caput	80	10	10	4 0	3 3
	Corpus	10	5	85	2 0	2 5
	Cauda	—	10	90	1 1	0 9
14	Caput	40	50	10	6 6	2 8
	Corpus	—	5	95	5 3	2 5
	Cauda	—	5	95	1 5	1 25

* Three spermatozoa in each group

permeability of the sperm surface occur during passage through the epididymis The present demonstration of the occurrence of marked changes in the electrophoretic behaviour of maturing epididymal spermatozoa, with the development of an increasing capacity for autoagglutination, make it seem fairly certain that changes do occur over the surface of spermatozoa during epididymal maturation It is not unreasonable to suppose that such differences in the sperm surface may be responsible in part for the evident inability of the majority of caput spermatozoa to penetrate the ovum

The nature of the change in the sperm surface during epididymal passage is open to further investigation The orientation of many caput sperm with the head pointing toward the anode indicates the existence of a relatively higher net surface charge over the head region in these spermatozoa The change in direction of orientation and the relatively higher mobility and speed of orientation of cauda spermatozoa under the same conditions reflect an absolute increase in the net negative surface charge over the tail, as the sperm pass through the proximal regions of the epididymis These latter findings are in accordance with the report by Nevo *et al* [10] that isolated tails of mature bull spermatozoa exhibit a higher net surface charge than do the isolated sperm heads The relatively slower tail–anode orientation response of most corpus spermatozoa suggests that the change is a gradual process, those spermatozoa remaining vertical in the electric field probably represent the stage or condition in which the net surface charge over the head approximately equals that of the tail region The question now remains as to whether or not an absolute reduction in the negative surface charge density of the head region occurs during epididymal passage of the sperm

The mechanism and significance of the 'stickiness' of mammalian spermatozoa are not understood, although it is clear that for autoagglutination of mammalian spermatozoa to occur, the surrounding medium must be of a certain ionic strength[1] It has been suggested that the adhesion of cells may depend to a certain degree on the principles advanced in the Verwey-Overbeek[21] theory for the stability of lyophobic colloids, in which the energy of interaction is presumed to depend in part on a repulsive force proportional to the net surface charge In the present context, it is noteworthy that spermatozoa fail to exhibit head–head agglutination in media in which head–anode orientation was shown in the microelectrophoresis cell, in this investigation, and in other unpublished studies using media of low ionic strength From this fact it seems possible to speculate that in media of low ionic strength the increased net surface charge over the head region in all epididymal spermatozoa may exert a repulsive force great enough to negate any inherent attractive forces in the sperm surface Although most caput spermatozoa fail to agglutinate also in media of high ionic strength, their weak head–anode orientation response in the electric field, in this type of medium, renders it probable that the failure to agglutinate may be due equally to the absence of attractive forces over the immature sperm surface

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INDIVIDUAL AND SUB-GROUP ANTIGENIC SPECIFICITY OF BENCE-JONES PROTEIN

By Dr. STEFAN STEIN, Dr RALPH L. NACHMAN and
Dr RALPH L. ENGLE, jun
Department of Medicine, New York Hospital, Cornell Medical Center, New York City

BENCE-JONES proteins were classified immunologically into two antigenic groups, *A* and *B*, by Korngold in 1956[1] He identified antigenic groups among serum myeloma proteins as well[2] Mannik and Kunkel further classified all the immunoglobulins into two distinct antigenic groups[3] and demonstrated that the same antigenic determinants are involved in grouping immunoglobulins, serum myeloma proteins, and Bence-Jones proteins Thus, group 1 γ-globulins, Korngold group 1 myeloma proteins, and Korngold Type *B* Bence-Jones proteins share antigenic determinants and are all classified as group 1 Likewise, there is antigenic similarity among group 2 γ-globulins, Korngold group 2 myeloma proteins, and Korngold type *A* Bence-Jones proteins, these constitute group 2. This article will follow the nomenclature suggested by Mannik and Kunkel

Migita and Putnam demonstrated that the Bence-Jones proteins are related to the electrophoretically slow (*S*) fragment of a papain digest of the same antigenic type myeloma protein[4] In addition, they, as well as Mannik and Kunkel, showed that patients with group 1 serum myeloma proteins usually excrete group 1 Bence-Jones proteins, while those with group 2 serum myeloma proteins typically excrete group 2 Bence-Jones Edelman recently demonstrated the chemical and immunological correspondence between the Bence-Jones protein and light (*L*) chains cleaved from the myeloma protein of the same patient[5] The investigation reported here was undertaken to evaluate the antigenic nature of individual Bence-Jones proteins

Production of Group Specific Antisera to Bence-Jones Proteins Group specific Bence-Jones antibodies were produced in the following way Bence-Jones proteins were separated[6], typed[7], and injected into a series of New Zealand albino rabbits Each rabbit was injected with a total of 450 mg of a Bence-Jones protein from a

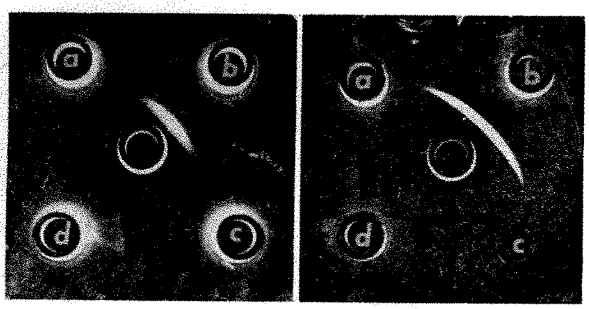

Fig. 1, left: Centre well, antibody against group 1 Bence-Jones (patient 40) absorbed with human serum. Peripheral wells: *a*, group 2 Bence-Jones (patient 20); *b*, group 1 Bence-Jones (patient 40); *c*, group 2 Bence-Jones (patient 21); *d*, human serum. Right: Centre well, antibody against group 2 Bence-Jones (patient 20) absorbed with human serum. Peripheral wells: *a*, group 1 Bence-Jones (patient 40); *b*, group 2 Bence-Jones (patient 20); *c*, group 1 Bence-Jones (patient 41); *d*, human serum

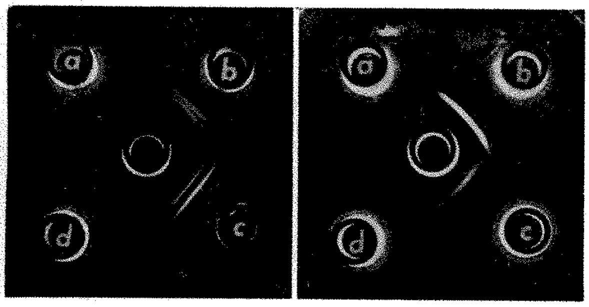

Fig. 2, left: Centre well, antibody against group 1 Bence-Jones (patient 40) absorbed with lyophilized γ-globulin. Peripheral wells: *a*, group 1 Bence-Jones (patient 42); *b*, group 1 (patient 40); *c*, group 1 Bence-Jones (patient 41); *d*, pooled γ-globulin. Right: Centre well, antibody against group 2 Bence-Jones (patient 20) absorbed with lyophilized γ-globulin. Peripheral wells: *a*, group 2 Bence-Jones (patient 23); *b*, group 2 Bence-Jones (patient 20); *c*, group 2 Bence-Jones (patient 21); *d*, pooled γ-globulin

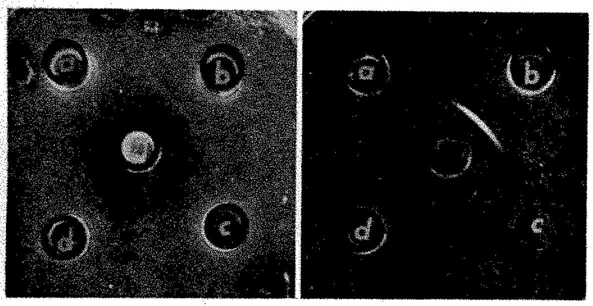

Fig. 3, left: Centre well, antibody against group 1 Bence-Jones (patient 40) absorbed with lyophilized normal human serum and group 1 Bence-Jones protein (patient 41). Peripheral wells: *a*, normal human serum; *b*, group 1 Bence-Jones (patient 40); *c*, group 1 Bence-Jones (patient 41); *d*, group 1 Bence-Jones (patient 42). Right: Centre well, antibody against group 2 Bence-Jones (patient 20) absorbed with normal human serum and group 2 Bence-Jones protein (patient 21). Peripheral wells: *a*, human serum; *b*, group 2 Bence-Jones (patient 20); *c*, group 2 Bence-Jones (patient 21); *d*, group 2 Bence-Jones (patient 23)

single patient using Freund's complete adjuvant, over a period of six months. Double immuno-diffusion agar-gel studies were performed[8] using 0·2 ml. antiserum in the centre well, and in peripheral wells, antigens consisting of 0·2 ml. of 0·1 per cent Bence-Jones proteins, 0·2 ml. pooled lyophilized human γ-globulin with concentrations varying from 1 to 160 mg/ml., or human serum diluted 1 : 10. The antisera were absorbed with normal human serum to remove antibody activity against non-specific contaminants present in the immunizing sample.

Antisera to group 1 Bence-Jones protein reacted with the group 1 protein, but not with group 2 proteins (Fig. 1, left). The typical double line of the group 1 reaction is seen. Identical specificity was demonstrated to group 2 Bence-Jones protein (Fig. 1, right). In no case did antisera to group 1 Bence-Jones proteins react with group 2 samples nor did group 2 antisera react with group 1 samples after absorption of the contaminants.

Demonstration of Antibody Potency following Absorption with γ-Globulin. Antisera were incubated at 37° C for 1·5 h with lyophilized pooled human γ-globulin (16 mg/ml. antiserum). The mixture was centrifuged at 13,000 r.p.m. for 30 min at 4° C. The clear supernate was used as absorbed antibody. These absorbed antisera reacted with the immunizing Bence-Jones protein as well as with other Bence-Jones proteins of the same antigenic group but from different patients. No reactivity was noted with γ-globulin (Fig. 2). Spur formation was observed in the group 2 system when the immunizing Bence-Jones protein was compared in adjacent wells with Bence-Jones proteins from different patients (Fig. 2, right). The spur formation suggests antigenic deficiency among the Bence-Jones proteins of the same group from different patients when compared with the immunizing Bence-Jones protein, and also suggests individual specificity of the immunizing Bence-Jones protein. No spur formation was demonstrable in the group 1 system.

Antisera to group 1 and 2 Bence-Jones proteins were individually absorbed with human serum (8 mg/ml.) as already described here. The supernate was further absorbed with 8 mg/ml. of Bence-Jones protein of the same group but from a different patient. In each case, the antibody retained activity against the immunizing Bence-Jones protein but lost potency to two other Bence-Jones proteins of the same group from different patients (Fig. 3). This reactivity was more pronounced in the group 2 system. These results further substantiate the evidence that Bence-Jones proteins are individually specific.

Relationship of Individual Bence-Jones Proteins of a Given Group to Each Other. As the γ-globulin absorption investigations indicate, Bence-Jones proteins of the same antigenic group react with the group specific Bence-Jones antibody despite γ-globulin absorption (16 mg/ml.) calculated to be greater than 10 times equivalence. These findings indicate that Bence-Jones proteins of the same antigenic group share a 'core' of determinants, which is common to the group. The fact that the 'common core' remains detectable after absorption of the antibody with γ-globulin suggests that it differs qualitatively or quantitatively from normal pooled human γ-globulin. According to present concepts, Bence-Jones proteins may be considered unincorporated components of the γ-globulin molecule and, therefore, may represent the product of asynchronous synthesis of a diseased plasma cell. Thus, any qualitative difference observed between the 'common core' shared by Bence-Jones proteins of a given group and normal pooled γ-globulins could be a function of: (1) preparative artefact; (2) unmasking of hidden antigenic sites; or (3) a true primary structural difference as might be expected of an abnormal protein. The proliferation of a clone of cells producing large amounts of a protein found in extremely small quantities in the normal spectrum of γ-globulins might explain a postulated quantitative difference. The data presented do not allow a clear determination of the foregoing possibilities.

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IN VITRO CELL TRANSFORMATION WITH CHEMICAL CARCINOGENS

By YOHEVED BERWALD and Prof. LEO SACHS

Section of Genetics, Weizmann Institute of Science, Rehovoth, Israel

THE possibilities for the examination of chemical carcinogenesis could be considerably enhanced by demonstrating that the change of normal to tumour cells can be obtained *in vitro* with chemical carcinogens. Although there have been several attempts to induce this change *in vitro*[1], in earlier experiments the cells had been grown in tissue culture for long periods. It was still difficult to decide from these earlier experiments whether the altered cells found after treatment had been induced by the carcinogen that had been added[1], or whether there had been a selection in culture of altered cells that had arisen independently of the treatment.

One of the first steps in the examination of chemical carcinogenesis *in vitro* thus seemed to be to determine whether hereditary changes can be directly induced. We have paid particular attention to changes in organization resulting in a random arrangement of the cells, which will be referred to as a decrease in contact inhibition[2]. This criterion had been successfully used to establish *in vitro* transformation with polyoma virus (PV) (ref. 3), and with PV it was further found that *in vitro* transformed cells could progressively grow as tumours when inoculated into animals. The aim of the work recorded here was to determine: (*a*) whether cell transformation *in vitro* resulting in a hereditary decrease of contact inhibition can be obtained by treatment with chemical carcinogens; (*b*) whether these carcinogens can directly induce this transformation. Three chemical carcinogens, 3,4-benzpyrene (BP), 3-methylcholanthrene (MC), and ethyl carbamate (urethane), were tested with hamster and mouse cells. In addition to the treatment of short-term mass cultures, the effects of these carcinogens were tested on cells plated for cloning, to investigate the hereditary nature of any observed transformation, the frequency of transformed cells, and the possibility that the carcinogen merely selects transformed cells that occur spontaneously.

1- or 2-day-old secondary cultures from golden hamsters and *C57 B1/6* mouse whole embryos were used as a source of normal cells. Cultures were grown in 50-mm plastic Petri dishes (Falcon Co.) with Eagle's medium containing a four-fold concentration of amino-acids and vitamins plus 10 per cent calf serum, in a humidified incubator with a constant flow of 10 per cent carbon dioxide in air. Cells were cloned on X-irradiated rat feeder cells[4]. For the assay of transformed clones, the plates were fixed and stained with May-Grünwald-Giemsa after 7 days of incubation[4]. Plates were seeded at 1×10^6 cells for mass cultures and at 1×10^4 or 5×10^4 cells per plate for cloning.

The carcinogens BP and MC were used in the form of paraffin disks made of 'Millipore' filters[5] (0·45μ porosity and 150μ thickness) dipped into a solution of melted paraffin (m.p. 56° C) containing 5 per cent (w/w) of the carcinogen heated to 180° C. After the wax had solidified, the filters were cut into disks (0·6 cm diameter), with a paper punch, sterilized with 70 per cent ethanol, and stored in the dark. For treatment with BP or MC, one disk was added per Petri dish. Urethane was added directly to the culture medium. For the experiments with PV, mass cultures were infected with the small plaque polyoma mutant *SP2* (ref. 4) at a virus: cell ratio of 10 plaque forming units per cell (2 h adsorption), before the cells were plated for cloning.

The first experiments were carried out using BP and hamster cells, and it was repeatedly found that in addition to a toxic effect on a proportion of the cell population, there existed, among the survivors, cells that were fusiform in shape and exhibited a random pattern of growth. Colonies of such cells were found both after treatment of mass cultures with BP, and after treatment of cells which had been plated for cloning. No such colonies were found in untreated control cultures, or after treatment with disks containing only paraffin. It was seen from the cloning experiments that a hereditary cell transformation resulting in a decrease of contact inhibition had been obtained by treatment with BP *in vitro*. The transformed clones were identified by the random arrangement of cells with a fusiform tendency, and they could be distinguished from clones of cells transformed by PV, in that they did not pile up on one another as much as the PV transformed cells (Fig. 1). As in the case of PV transformation[4], there was still some degree of contact inhibition. Transformed cells could be inhibited from forming clones when grown together with normal cells in confluent cultures.

Representative results on cloning efficiency and percentage of transformed clones (that is, percentage of transformed/total number of clones), after various periods of BP treatment are given in Table 1. The results include experiments with BP treatment before cloning, after cloning, or both before and after cloning. All the experiments in Exp. 4 were carried out on one batch of cells, and those in Exp. 1 on another batch of cells. As expected, there was a considerable decrease in cloning efficiency of the BP-treated cultures due to the toxic effect of the carcinogen.

Table 1. PERCENTAGE OF *in vitro* TRANSFORMED HAMSTER EMBRYO CLONES AFTER TREATMENT WITH BENZPYRENE AND/OR POLYOMA VIRUS

Exp. No.	Type of treatment*	Time of BP treatment†	Total No. clones‡	Total No. BP transformed clones	Total No. PV transformed clones	Per cent cloning efficiency	Per cent BP transformed clones	Per cent PV transformed clones
1*A*	Paraffin	—	872	0	0	0·2	0	0
1*B*	BP	9 days b.c.	310	55	0	0·08	17·8	0
4*A*	None	—	615	0	0	1·5	0	0
4*B*	BP	2 days b.c.	478	25	0	0·6	5·2	0
4*C*	BP+PV	2 days b.c.	336	28	4	0·4	8·3	1·2
4*D*	BP	2 days b.c. +0 a.c.	692	57	0	0·2	8·2	0
4*E*	BP+PV	2 days b.c. +0 a.c.	322	52	1	0·1	16·1	0·3
4*F*	PV	—	~2,400	0	31	3·0	0	1·3
4*G*	BP	0 a.c.	231	38	0	0·6	16·5	0
4*H*	BP+PV	0 a.c.	582	98	0	0·7	16·8	0
4*I*	BP+PV	1 day a.c.	678	178	1	0·8	26·3	0·2
4*J*	BP+PV	3 days a.c.	618	127	6	0·8	20·6	1·0

* BP = benzpyrene; PV = polyoma virus; PV always inoculated and adsorbed for 2 h before cloning.

† b.c. = before cloning; a.c. = after cloning; 0 a.c. = BP added at time of cloning. Where b.c. only, no BP added after cloning. Where a.c. disks with BP present for 7 days if added at 0 a.c., for 6 days if added at 1 day a.c., and for 4 days if added at 3 days a.c. All plates were fixed and stained at 7 days after cloning.

‡ Plates seeded with 5×10^4 cells in Exps. 1*A*, 1*B*, 4*D* and 4*E*, and with 1×10^4 cells in all other experiments. A total of 4 plates seeded in Exps. 4*A* and 4*G*; and 8 plates in all other experiments.

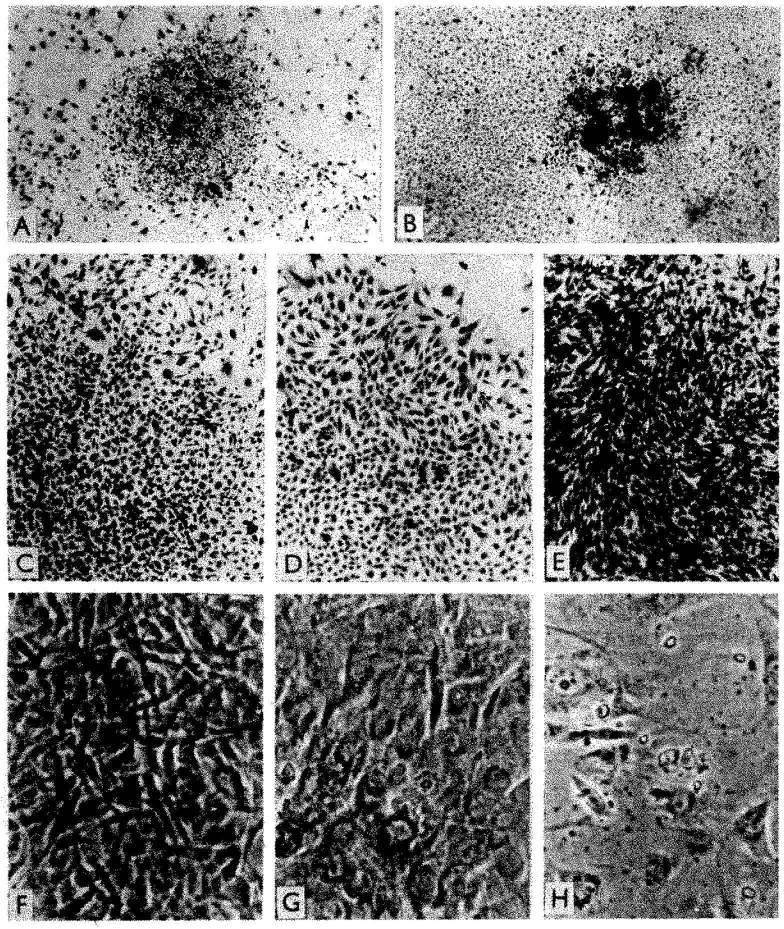

Fig. 1. All are photographs of cells derived from hamster embryos. *A–F* are clones and *G* and *H* are uncloned cultures. *A* = BP transformed (×*c.* 5·7). *B* = PV transformed (centre) and normal (×*c.* 5·7). *C* = BP transformed (×*c.* 18). *D* = normal (×*c.* 18). *E* = PV transformed (×*c.* 18). *F* = BP transformed (×*c.* 117). *G* = Normal (×*c.* 117). *H* = Same as *G* after 16 days of BP treatment to show toxic effects of carcinogen (×*c.* 117). *A–E* = fixed and stained; *F–H* = living cultures, phase contrast

In Exp. 1, mass cultures were treated with BP for 9 days and the cells then cloned. The results show that there were 17·8 per cent transformed clones in the experimental, and no transformed clones in cultures that had been pre-treated for the same period with disks containing only paraffin. A reduction of the period of treatment to 2 days before cloning (Exp. 4*B* and 4*C*) still gave, in two experiments, 5·2 and 8·3 per cent BP transformed clones in comparison to 1·3 per cent PV transformed clones. This percentage for PV transformation is comparable with that obtained in previous experiments with this PV mutant on hamster embryo cells grown under similar conditions[4]. The percentage of BP transformed clones in Exp. 4, with various times of treatment, ranged from 5·2 to 26·3 per cent.

It appeared unlikely, from the results of the 2-day treatment with BP before cloning, that the transformation was merely due to a selection by the carcinogen of transformed cells that had arisen spontaneously independently of the carcinogenic treatment. But in order further to establish this point, experiments were carried out on adding the carcinogen to cells that had been plated for cloning. The data show (Exps. 4*G*–4*J*) that when BP was added either at the time that the cells were plated for cloning, or even at 1 or 3 days after cell plating, there were 16·5–26·6 per cent BP transformed clones in the experimental cultures, and no such transformed clones in the untreated or PV infected cultures. Regarding the experiment where BP was added at three days after cloning, further cloning experiments are required to determine whether the transformed clones, obtained by adding carcinogen after cell divisions had presumably occurred in the clones, consist of a mixture of transformed and normal cells.

These results thus indicate that BP can induce *in vitro* cell transformation, and that this transformation, as in the case of PV induced transformation[4], was induced as a direct result of the action of the carcinogen, and not merely by the selection of spontaneously occurring transformed cells. Similar results have also been obtained after MC treatment of hamster embryo cells, and after BP and MC treatment of mouse embryo cells. The *in vivo* behaviour of the cells transformed by BP and MC *in vitro* is now being tested by cell inoculation into animals.

In contrast to the results with BP and MC, no transformed clones were found in a total of about 2,400[*] hamster embryo clones and 960 mouse embryo clones examined after treatment with urethane at 10 mg/ml. for 3 days before cloning. Urethane can produce different types of tumours *in vivo*, but it seems predominantly to produce tunours of the lung[6]. Further investigations should be able to establish whether these negative results with urethane are due to the absence of appropriate target cells in sufficient number in the embryo cultures used in the present experiments, or whether the absence of cell transformation *in vitro* may be related to the possibility that the carcinogenic effects *in vivo* are due not to urethane itself, but to some metabolic derivative that is produced in the animal but not in tissue culture. It should be noted that, although BP or MC applied as disks did not produce a toxic effect on cultures of two tumour-producing PV *in vitro* transformed cell lines (one from hamsters and one from *C57 Bl*/6 mice), whereas disks containing the same concentration of BP or MC produce a clear toxic effect on cultures of normal embryo cells, no such differential toxicity was found when the same cell lines were compared with normal embryo cells at urethane concentrations of 0·1, 1, 10 and 30 mg/ml.

Regarding the resistance to the toxic effects of carcinogenic hydrocarbons that has been observed with various tumours[5,7], treatment with BP at different times after PV infection (for example, Exps. 4*E*–4*J*) should enable one to establish exactly how long after virus infection PV transformed cells acquire this resistance.

A test on resistance to MC and BP and malignancy *in vivo* was also made with two cell populations of the long-term hamster tissue culture line *BHK*[8]. An uncloned population of *BHK* 21 gave, after 7 days' treatment of mass cultures with disks of MC or BP, 92 and 78 per cent, respectively, of the total cell count of paraffin-treated controls. However, clone 13, which was derived from an earlier passage of *BHK* 21 and showed a different growth pattern *in vitro*, gave after the same treatment 28 and 16 per cent, respectively, of the total cell count of the control cultures, and thus appeared to be as susceptible as normal hamster cells. One month after subcutaneous inoculation into 2-month-old hamsters, the uncloned *BHK* 21 inoculated at 2×10^4 cells per hamster had produced progressively growing tumours in 5/5 animals, whereas clone 13 had not produced tumours in 5 animals inoculated with 2×10^5 cells. The more resistant population of *BHK* was thus also more malignant *in vivo*. The mechanism of this resistance to carcinogenic hydrocarbons in the tumour cells tested is so far unknown. One possibility is that in resistant cells there may be a decreased penetration by the carcinogen owing to changes in the cell surface.

It can be seen from Table 1 that there was a survival of apparently normal-looking clones even in cultures treated with BP both before and after cloning. Further experiments including re-cloning are thus being carried out, to determine whether these apparently normal-looking clones have a hereditary resistance to the toxic action of BP or MC without a decrease in contact inhibition, and whether cells transformed by BP or MC are invariably resistant to the toxic action of these carcinogens.

The foregoing results seem to indicate the feasibility of studying cell transformation by chemical carcinogens *in vitro*, and a similar approach may prove useful to examine the *in vitro* effects of radiation. If the results of cell inoculations into animals will show that the cells transformed *in vitro* with chemical carcinogens can grow progressively as tumours *in vivo*, the present *in vitro* system may also prove useful as an additional test for carcinogens, and for determining whether the carcinogenic action of a compound *in vivo* is due directly to the compound itself or to some metabolic derivative produced in the animal.

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TEICHOIC ACID AND THE GROUP ANTIGEN OF LACTIC STREPTOCOCCI (GROUP *N*)

By DR. S. D. ELLIOTT

The Rockefeller Institute, New York, and Department of Animal Pathology, University of Cambridge

THE immunological activity of bacterial glycerophosphate polymers (teichoic acids) was first described by McCarty[1], who extracted these substances from a variety of micro-organisms and found them reactive with serum containing antibodies to the polyglycerophosphate of group *A* streptococci. Recently, serologically reactive teichoic acids of taxonomic interest have been isolated from several bacterial species[2,3], and it is the purpose of the present communication to report an example from the lactic streptococci (group *N*) in which a galactosyl glycerophosphate polymer appears to be the group antigen.

Sharpe[4] found serologically identical type-specific antigens in certain strains of enterococci and lactic streptococci. I have confirmed this observation and furthermore have found that the type-specific carbohydrate antigens of enterococci are located in the bacterial cell-walls[5]. The group-specific antigen of the enterococci (group *D*), on the other hand, is an 'intracellular' glucosyl glycerophosphate polymer[2], and it seemed possible that a chemically similar but serologically distinct intracellular substance might be the group antigen for the lactic streptococci (group *N*). Accordingly, intracellular teichoic acid was extracted from two different strains of group *N* streptococci and subjected to chemical and serological analysis.

The group *N* streptococci selected for investigation were strains 6681 and *B*209. The former is catalogued under this name in the National Type Culture Collection but is sometimes referred to as the 'Orla Jensen' strain. Strain *B*209 came from the collection of Dr. R. C. Lancefield, who received it under the designation *LE*1 from Dr. J. M. Sherman.

Precipitin tests revealed in dialysate broth culture filtrates of strain 6681 a small amount of group *N* antigen; it was absent from culture filtrates of strain *B*209. This extracellular group antigen was precipitated from the culture filtrates by the addition of ammonium sulphate to 0·8 saturation and amounted to about one-tenth the yield later obtained by disrupting the cocci. The intracellular group antigen was extracted from both strains and partially purified by the procedures already described for the isolation of group *D* antigen from enterococci[5]. Washed cocci were disrupted by shaking with glass beads (Ballotini No. 13) in a mickle disintegrator and the cell contents separated from the cell-walls by centrifugation for 2 h at 40,000*g*. The cell contents fraction reacted strongly with group *N* antisera of heterologous type and continued to do so after digestion with nucleases and proteinases followed by prolonged dialysis against distilled water. The group *N* antigen was then precipitated from the digest by adding 1 volume of ethanol. After washing in ethanol and ether, the dried precipitate contained in addition to the group antigen approximately 30 per cent of nucleic acid. Most of the latter was removed by dissolving the dried material in 1 per cent calcium chloride and adding one-fifth volume of ethanol[6]. The nucleic acid was precipitated by this procedure while the group antigen remained in solution from which it, in turn, was precipitated by the addition of 1 volume of ethanol. This precipitate was washed in ethanol and ether and the dried material used for chemical analysis.

Samples of 5–10 mg were examined by paper chromatography after hydrolysis in 4 N hydrochloric acid for 6 h at 100° C. The chromatographic procedures and the quantitative methods later used for the estimation of hexoses and phosphorus were the same as had been utilized in the analysis of group *D* antigen[2,5].

When examined by paper chromatography acid hydrolysates of group *N* antigen preparations from both strains were found to contain glycerol phosphate, galactose phosphate and galactose. In addition, all preparations were contaminated with nucleic acid varying in amount from 3 to 30 per cent as estimated by absorption at 260 mμ. The most highly purified material came from strain 6681 and contained, in addition to glycerol, 10 per cent of galactose, 3·53 per cent of phosphorus and 3 per cent of nucleic acid. It seemed possible that in the group antigen galactose was present only in the phosphorylated form and that part of this became degraded during the acid hydrolysis preceding chromatography. In the quantitative determination of galactose[7], therefore, the group antigen preparations were compared with standard solutions of galactose-6-phosphate (barium salt) since this showed greater absorbancy at 600 mμ than did equivalent concentrations of *d*-galactose. The proportions of galactose and phosphorus in the best preparation, 10 per cent and 3·5 per cent respectively, are equivalent to a molecular ratio of 6 . 11·4 Assuming that the group N antigen is indeed a glycerophosphate compound, this ratio could be accounted for by a polymer the units of which each contained one galactose phosphate and one glycerol phosphate sub-unit, adjacent units being linked through the glycerol phosphate groups

Further evidence for the participation of galactose phosphate in the structure of the group N antigen came from the results of quantitative precipitin inhibition tests These showed that when galactose-6-phosphate and purified group N antigen from strain 6681 were present in a ratio of 500 1 there was approximately 25 per cent inhibition of precipitation by group N antiserum. No inhibition could be detected when either *d*-galactose or dextrose was substituted for galactose phosphate Both galactose-6-phosphate and galactose-1-phosphate had inhibitory activity, but the latter was not tested on a quantitative basis

Table 1. PRECIPITIN REACTIONS OF STREPTOCOCCAL TEICHOIC ACIDS

Streptococcal teichoic acid from	Rabbit antiserum to streptococci of stated group		
	Group A	Group D	Group N
Group A (1 mg/ml)	++	—	—
Group D (1 mg/ml)	+	++	—
Group N (0 1 mg/ml)	+	—	++

The results of precipitation reactions between intracellular teichoic acids from streptococci of groups A, D and N and their corresponding antisera are shown in Table 1 It will be seen that whereas the group A serum, containing antibodies to the unsubstituted glycerophosphate polymer, precipitates with all three teichoic acids, the group D and group N sera react only with the homologous antigens There are two obvious reasons for the cross-reaction between group N antigen preparations and the antiserum to group A polyglycerophosphate First, it may be due to the presence of unsubstituted polyglycerophosphate in the group N preparations In support of this explanation, intracellular material from strain B209 was found to contain a mixture of glycerophosphate and galactosyl glycerophosphate polymers from which the former could, in part at least, be separated by virtue of its greater solubility in ethanol or trichloroacetic acid A similar mixture may also have been present in the group N preparations from strain 6681, although here a separation of the two components was not achieved Evidence suggestive of such a mixture, however, came from gel-diffusion experiments in which group N preparations from strain 6681 when tested against group A polyglycerophosphate antiserum formed two precipitin lines one of which was continuous with the line formed by group A polyglycerophosphate Secondly, the serological cross-reaction may be due to the glycerophosphate 'backbone' common to the structure of both the unsubstituted and the galactosyl substituted polymer In support of this explanation is the result of an absorption experiment in which purified group N antigen from strain 6681 (0 1 mg) was mixed with serum containing antibodies to group A polyglycerophosphate (1 ml) The group N antigen was completely precipitated from solution by the polyglycerophosphate antibodies, and at the end of the experiment the absorbed serum no longer reacted with the homologous glycerophosphate polymer

In summary, it appears that the group antigen of the lactic streptococci (group N) contains galactosyl glycerophosphate probably in a polymerized form Galactose phosphate constitutes part at least of the immunologically determinant group Some streptococci of group N contain a mixture of galactosyl and unsubstituted glycerophosphate polymers

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EVIDENCE FOR NON-ALLELISM BETWEEN GENES AFFECTING HUMAN SERUM CHOLINESTERASE

By PROF H. HARRIS, E. B. ROBSON and A. M. GLEN-BOTT
Medical Research Council Human Biochemical Genetics Research Unit and
Department of Biochemistry, King's College, London
AND
J. A. THORNTON
Department of Anæsthetics, University of Sheffield

AN examination of human serum cholinesterase by starch-gel electrophoresis, using sera from many different individuals, revealed that several components with the characteristics of that enzyme were regularly present, though one of these components, referred to as C_4, was very much more prominent than the others[1,2]. Furthermore, in some sera an extra component, C_5, was found This appeared to be an individual characteristic and to be genetically determined The C_5+ phenotype has now been observed in about 10 per cent of a random sample of British people, and family examinations indicate that most individuals of this phenotype are probably heterozygous for a gene which determines the presence of the component Another interesting feature is that C_5+ individuals have been found to have, on the average, slightly higher levels of serum cholinesterase activity than C_5- individuals[2], a finding which suggests that the C_5 component may have no detectable counterpart in the C_5- phenotype

It has also been shown that a gene which determines the occurrence of an atypical form of serum cholinesterase with unusual inhibition properties occurs with a frequency of about 0 02 in British and other European populations[3] This gene will be referred to as E_1^a and its common allele as E_1^u. The three genotypes $E_1^uE_1^u$, $E_1^uE_1^a$ and $E_1^aE_1^a$ are generally distinguished by determining what has been called the dibucaine number (DN)[4] This is the percentage inhibition of serum cholinesterase activity produced by the inhibitor dibucaine under certain standard conditions $E_1^uE_1^u$, $E_1^uE_1^a$ and $E_1^aE_1^a$ individuals have DNs of around 80 ± 2, 62 ± 4, and 20 ± 3 respectively, and are said to have the 'usual' (U), 'intermediate' (I) and 'atypical' (A) phenotypes as defined by dibucaine number determination[5].

The question arises as to whether the gene which determines the occurrence of C_5 is allelic or non-allelic to the genes E_1^u and E_1^a We have obtained information about this from the examination of two kindreds in

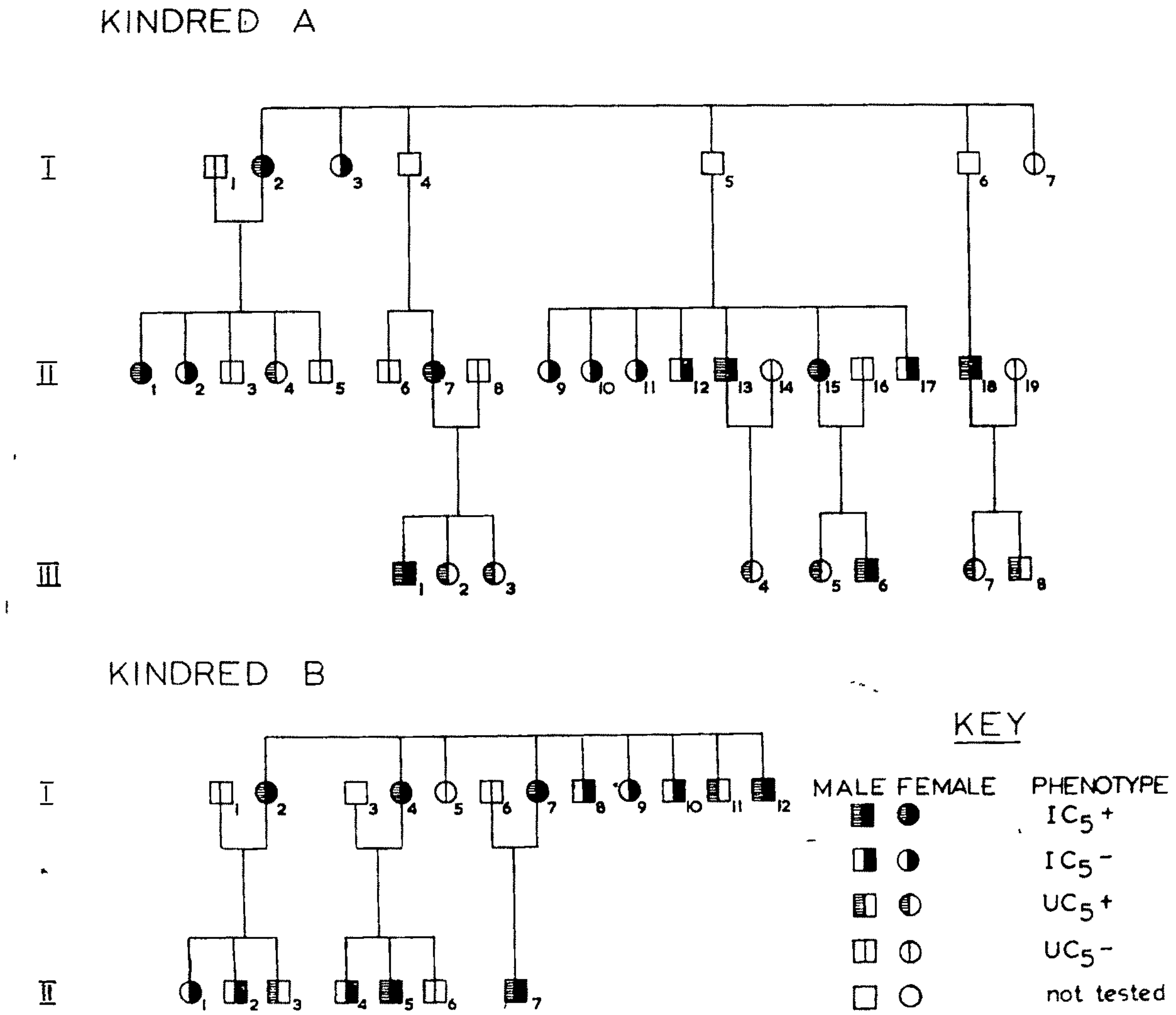

Fig 1 Pedigrees of two kindreds in which the four phenotypes UC_5-, UC_5+, IC_5- and IC_5+ are found

which all these genes are segregating Individuals of four different phenotypes could be identified by a combination of the results obtained from starch-gel electrophoresis with those obtained from DN determination using whole serum The four phenotypes will be referred to here as UC_5-, UC_5+, IC_5- and IC_5+ The critical families in testing for allelism or non-allelism are those where one parent is heterozygous for the gene E_1^a and also for the gene determining C_5 (phenotype IC_5+), and the other parent is homozygous for the usual allele or alleles (phenotype UC_5-) The relevant parts of the kindreds are shown in Fig 1 There are seven matings of the critical type where both parents could be examined, and among the seventeen children of these matings four phenotypes were observed with the following frequencies UC_5 − 2, UC_5 + 8, IC_5 − 3, IC_5 + 4 There was also one mating of a woman of the IC_5+ phenotype where the husband could not be tested but had a high probability of being of the UC_5- phenotype In this case the three children were respectively UC_5-, IC_5- and IC_5+ If the genes are non-allelic four different genotypes would be expected among the children of such matings and the four corresponding phenotypes were in fact observed If the genes are allelic only two genotypes (corresponding to the phenotypes UC_5+ and IC_5-) would be expected However, the interpretation is slightly complicated because it has been found that in occasional individuals, who, from their family relationships, would be expected to be carrying the gene determining the presence of C_5, the component cannot be demonstrated Such 'overlaps' might simulate non-allelism in the foregoing test, even if the genes were allelic However, if the genes were indeed allelic, it would be necessary, in order to explain the results, to postulate an unreasonably high rate of non-manifestation. Thus, to account for the IC_5+ individuals among the children in these families, it would be necessary, if the genes were allelic, to suppose that at least 4 out of these 7 UC_5- tested parents were in fact heterozygous for the gene determining C_5 They were all unrelated, and it has been estimated from other investigations that less than one in fifty of randomly selected individuals with the phenotype UC_1- in the English population are non-manifesting heterozygotes for the gene determining C_5 Thus, it is very likely that the genes are non-allelic

If the occurrence of component C_5 depends on a gene at a locus different from the locus of the genes E_1^u and E_1^a, it is of some interest to know whether E_1^u and E_1^a affect the inhibition characteristics of C_5 in the same way as they affect the inhibition characteristics of component C_4, which constitutes the major proportion of the serum cholinesterase in both C_5- and C_5+ individuals We have examined these points by comparing the inhibition characteristics of components C_4 and C_5 after their separation in starch gel

Horizontal unidimensional starch-gel electrophoresis was carried out using a *tris*/citrate buffer system at *p*H 5 0 (gel buffer 0 016 M succinic acid, 0 0184 M *tris* bridge buffer 1 6 M sodium hydroxide, 0 41 M citric acid) The zones of serum cholinesterase activity were detected, as previously described[2], using α-naphthyl acetate as substrate In each experiment the same set of serum

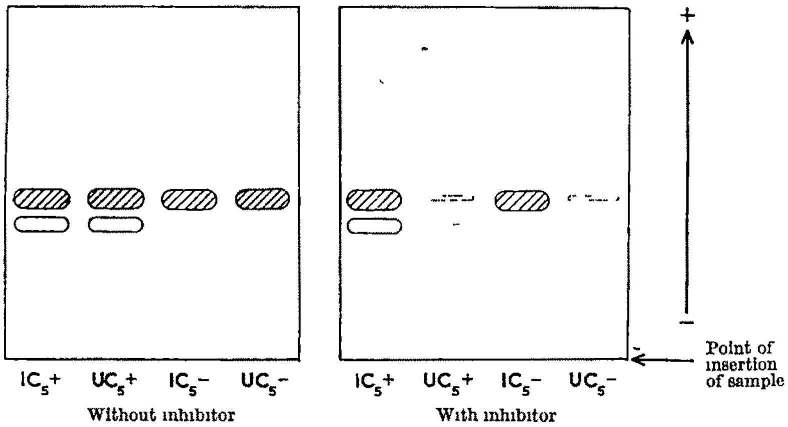

Fig 2 Diagram showing the effect of the inhibitor *RO* 2–0683 (10^{-7} M) on serum cholinesterase components C_4 and C_5 in individuals of the U and I phenotypes The C_4 and C_5 components were separated by starch-gel electrophoresis at *p*H 5 The sera in the gel without the inhibitor were diluted 1 in 4 with buffer, while the sera in the gel containing inhibitor were undiluted

samples was run on two separate gels in the presence and in the absence of inhibitor The inhibitor used was *RO* 2–0683 (Roche), which has been shown to give a somewhat greater discrimination between the 'usual' and 'intermediate' phenotypes than dibucaine[6] It was usually added to the appropriate gel and bridge buffers to a concentration of 10^{-7} M Sera of the four phenotypes UC_5-, IC_5-, UC_5+ and IC_5+ were used The degree of inhibition was established approximately by comparing undiluted serum on the gel with inhibitor with serial dilutions of serum on the gel without inhibitor

The results of a typical experiment are shown diagrammatically in Fig 2 Sera of types UC_5- and IC_5- give only a single zone of activity in the gel without inhibitor This is mainly C_4 The minor components C_1, C_2, C_3, etc , are not detected under these conditions In the gel with inhibitor (10^{-7} M) the IC_5- serum shows a single zone with about 25 per cent of the original activity, whereas the UC_5- zone is almost completely inhibited (less than 2 per cent of the original activity) Thus the 'usual' and 'intermediate' types of reaction to inhibitor can be readily distinguished visually Sera of types UC_5+ and IC_5+ show two zones of activity in the gel without inhibitor, the regularly present zone C_4 and the additional zone C_5 In the gel with inhibitor both zones in the UC_5+ serum are almost completely inhibited, and both zones in the IC_5+ serum are partially inhibited to the same degree (about 25 per cent of the original activity) In each case the degree of inhibition of the C_5 zone is essentially the same as that of the C_4 zone The same effect has also been observed with other concentrations of inhibitor Thus, the gene E_1^a affects the inhibition characteristics of the component C_5 in the same way that it affects the main serum cholinesterase component C_4 One may note that since there is evidence that in the sera of individuals who show the 'intermediate' DN phenotype there are actually present both the 'usual' and 'atypical' forms of the enzyme[7,8], it is likely that in IC_5+ individuals the C_5 zone may represent two distinct components, one having 'usual' type and the other having 'atypical' type DN characteristics

Although the molecular basis of the variations is unknown, one may conclude from these observations that the occurrence and the properties of the C_5 component are determined by genes at at least two loci

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OXYGEN DIFFUSION RATES AND NEMATODE MOVEMENT IN CELLULOSE SPONGES

By S. D VAN GUNDY and Dr L H STOLZY
Department of Nematology and Department of Soils and Plant Nutrition,
University of California, Riverside

THE influence of low oxygen diffusion rates in soils on nematode activity has been demonstrated by us[1] Efforts to examine different phases of nematode activity *in vitro*, however, have been hindered because no medium was available which provided an oxygen diffusion rate comparable to those recorded in soil studies A pure cellulose sponge ('O-cello', manufactured by General Mills) was selected as a synthetic medium because it was relatively inert, had structure, was made of pores of different sizes, and provided continuous and connecting surfaces. This is a report on the moisture and oxygen diffusion rate determinations of the sponge and on its use for investigating one phase of nematode activity, movement

Determinations of moisture and oxygen diffusion rate were made in 7 cm × 9 cm × 2 cm sponge blocks The sponges were washed in acetone and in water, dried at 60° C, and weighed They were saturated with tap water and placed on a double-walled ceramic suction plate Ten platinum microelectrodes[2] were placed in the sponge Water content and oxygen diffusion rate in relation to suction were determined from 0–90 millibars (mb) at a constant temperature of 25° C (Fig 1) The oxygen diffusion rate increased as the larger pores of the sponge

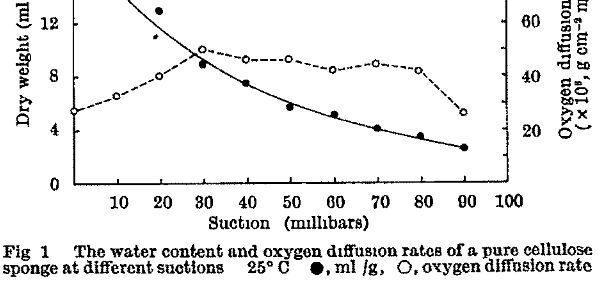

Fig 1 The water content and oxygen diffusion rates of a pure cellulose sponge at different suctions 25° C ●, ml /g, O, oxygen diffusion rate

drained. A suction of 30 mb appeared to drain a sufficient number of pores to allow maximum oxygen diffusion. The oxygen diffusion rate then remained nearly constant to 80 mb where the water films became too thin to wet the electrodes.

A method and apparatus for varying the oxygen concentration above the soil surface have been described[3]. The same technique was used to vary the oxygen diffusion rate in sponges except that wire screens were inserted to suspend the sponges above the bottom of the cylinder. The dried sponges were placed in the containers and equilibrated for 24 h at a constant temperature of 21° C prior to the addition of water. The water was equilibrated by bubbling with the same oxygen concentration. A hypodermic syringe and needle were used to transfer enough water to the sponges to maintain suctions of 20, 30, and 40 mb. Oxygen diffusion rates increased rapidly as oxygen concentration increased from a partial pressure of less than 1 to 25 mm mercury (Fig. 2). There was little change in oxygen diffusion rate from a partial pressure of 25–144 mm mercury. Oxygen diffusion rates at 40 mb were nearly identical to those at 30 mb, however, those at 20 mb were slightly lower, indicating that 30 mb of suction —or more—were necessary for optimum diffusion.

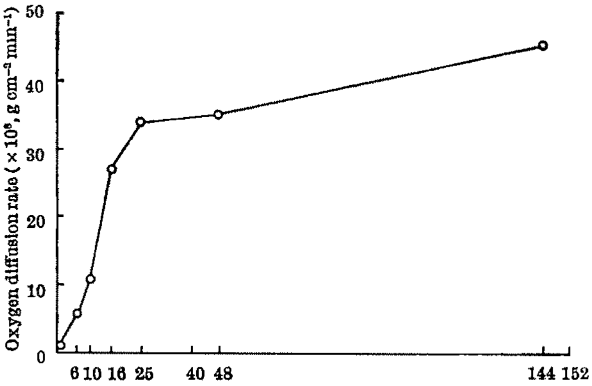

Fig. 2 Oxygen diffusion rates in pure cellulose sponges maintained at a moisture suction of 30 mb and exposed to different partial pressures of oxygen 21° C

Nematode investigations were conducted in 1 cm × 1 cm × 7 cm strips cut from large sponges. The sponge strips were washed in acetone and distilled water and dried at 60° C. The moisture suction of each sponge was adjusted by weighing and adding a calculated amount of water based on the water content–suction relationship in Fig. 1. Nematode suspensions of *Meloidogyne javanica* (Treub) Chitwood larvæ and *Hemicycliophora arenaria* Raski, all stages, were added to the sponge from a 1/2 ml hypodermic syringe. Nematodes were recovered from the sponge strips by cutting them into 1-cm cubes in sequence and placing in test tubes containing 10 ml of water. Since the sponges floated, gravity served as a natural separating force. Four replicate sponges were used in each treatment. One test was conducted on the recovery of *M. javanica* from sponge cubes in which 80 per cent of the nematodes were recovered in 1·5 h and 100 per cent in 3 h. Subsequent nematode determinations were made after a recovery time of 15 h.

Two tests were conducted to determine whether the nematodes were carried along the sponge in water currents set up by adding the nematode suspension to the sponge. Suspensions of *M. javanica* (17,000/0·5 ml) in one, and *H. arenaria* (1,300/0·5 ml) in the other, were syringed on the first cm of sponge strips. After 10 min the strips were cut into 1-cm cubes. Nematodes were not recovered from any of the cubes except that on which they were syringed. The sponge acted as a filter and held the nematodes in place while the water came to equilibrium in the sponge. Nematode suspensions in all subsequent tests were added in 0·05 ml of water to avoid any chance of nematode movement in the sponge.

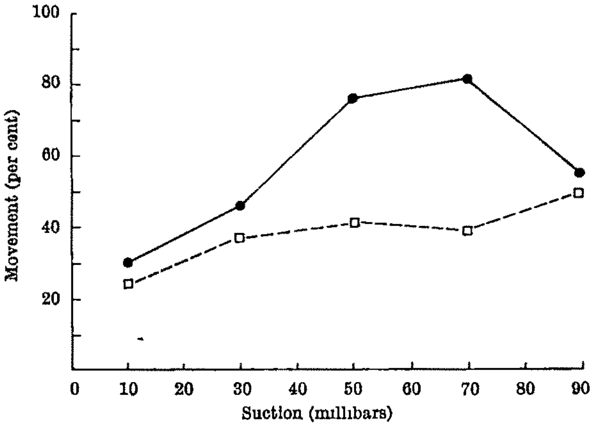

Fig. 3 Percentage movement of *M. javanica* larvæ and all stages of *H. arenaria* over a distance of 1 cm or more after 48 h in pure cellulose sponge strips of different suctions ●, *Meloidogyne javanica*, □, *Hemicycliophora arenaria* 27° C

The movement of *M. javanica* (2,200/0·05 ml) and *H. arenaria* (1,050/0·05 ml) was investigated in the sponge at suctions of 10, 30, 50, 70, and 90 mb maintained at 27° C for 48 h (Fig. 3). Optimum movement of *M. javanica* occurred at 50 and 70 mb and approximately 1, 3, 10, 22, 23, 19, and 21 per cent of the larvæ had moved 6, 5, 4, 3, 2, 1, and 0 cm, respectively. Movement of *H. arenaria* increased gradually from 10 to 90 mb, however, its total movements were less than *M. javanica*. The distribution of *H. arenaria* at 10–90 mb was 2, 3, 3, 4, 9, 28, and 51 per cent of the nematodes which had moved 6, 5, 4, 3, 2, 1, and 0 cm, respectively.

The effect of oxygen diffusion rate on nematode movement was studied at 50 mb of suction under a constant temperature of 21° C (Fig. 4). Although this temperature was not best for nematode movement, the oxygen diffusion rate of the sponge had already been determined at 21° C and a change in temperature would also change oxygen diffusion rate. Thus the percentage movement at 21° C was not so great as in the tests at 27° C. There was a linear relationship between movement of *M. javanica* (1,000/0·05 ml) and oxygen diffusion rate. The movement of *H. arenaria* (1,200/0·05 ml) was less responsive to oxygen diffusion rate than *M. javanica*. Observations in solutions bubbled with different oxygen concentrations indicated that there were no visible differences in movement of *H. arenaria* except at a partial pressure of 1 mm mercury where the nematodes were quiescent. Since this was a mixed population of all stages of the nematode, there may have been differences in susceptibility between larval stages and adults. Also the morphology of this nematode is quite different from *M. javanica* and may

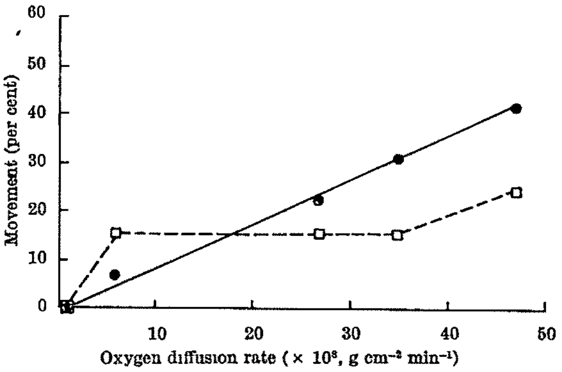

Fig. 4 Percentage movement of *M. javanica* larvæ and all stages of *H. arenaria* over a distance of 1 cm or more after 48 h in pure cellulose sponge strips maintained at different oxygen diffusion rates ●, *Meloidogyne javanica*, □, *Hemicycliophora arenaria* 21° C

account for the difference in responses to moisture and oxygen

Wallace[4] concluded from his studies that nematode movement in wet sand was optimum at the soil suction-level just sufficient to drain the pores Since this is also the point where air flows back into the soil pore spaces, oxygen could be responsible for the increased nematode movement It appears from the studies on *M javanica* larvæ that a sufficient supply of oxygen, as well as the proper moisture-levels, are essential for optimum nematode movement This effect of oxygen diffusion rate on movement may explain the reduction in secondary invasion of *M javanica* in tomato roots exposed to different oxygen concentrations[5] In the case of *H arenaria*, a nematode with a thick cuticle and heavy annules, there was little effect of moisture or oxygen on movement

The most important feature of this work was that the sponge proved to be a satisfactory substitute for soil in nematode–æration studies The thickness of the water films on the sponge surfaces and the oxygen diffusion rate in the water films can be easily determined and regulated *in vitro* The sponges may provide a synthetic medium useful for moisture and aeration investigations on other phases of the biology of nematodes, as well as for investigations on other organisms which reside in water films of soil

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PHOSPHOLIPID BREAKDOWN IN BAKER'S YEAST DURING DRYING

By J. S. HARRISON and W. E. TREVELYAN

Research Division, Distillers Co , Ltd., Epsom, Surrey

SINCE the Second World War an increasing proportion of baker's yeast has been marketed in the form of the very stable, so-called active dried yeast This is made from a specially prepared yeast cake, which is reduced to pieces of suitable size and dried in a stream of warm air until its moisture content has fallen to about 8 7 per cent (dry weight basis), at which level the dried yeast is in equilibrium with an atmosphere of approximately 33 per cent relative humidity

When yeast of an appropriate strain is cultured in various ways and dried, the product, after rehydration by admixture with water (reconstitution), gives a suspension of cells which may ferment sugar at nearly the same rate as the undried culture More commonly there is some loss in activity. For yeast dried in the laboratory to a moisture content of 8 7 per cent we have found, using a standard test[1], losses ranging from 7 to 100 per cent This loss in activity is associated with a loss in low-molecular-weight constituents of the cell, which escape into the medium during reconstitution[2] As these constituents include nucleotides and similar substances, which absorb light strongly at a wave-length of 260 mμ, the extent of leakage during reconstitution can be measured simply and reproducibly by a spectrophotometric method using such a test we have found a close correlation between leakage and loss of activity We have made[3] a preliminary examination of the extracellular nucleotides present in a suspension prepared from dried yeast, using the technique of ion-exchange chromatography They do not apparently differ chemically from those found inside a normal yeast cell, though the proportions of the various nucleotides differ in the two cases in a way which suggests that molecules which are smaller or less highly ionized diffuse from the cell more readily It seems to be agreed[4] that the loss in activity is due to some change in the permeability of the cell membrane, caused by chemical changes occurring during drying, and/or a failure of the membrane to re-form properly or quickly enough when the dried cell is rehydrated[2]

When dried yeast is mixed with water, the activity of the resultant suspension depends very much on the temperature at which rehydration is carried out For maximal retention of activity the temperature must be carefully controlled at a level which is governed by the moisture content of the dried yeast In one case, the optimum temperature was found to range from 21° at a moisture content of 23 per cent to 42 5° at 5 8 per cent moisture Even with this precaution, loss in activity may be severe or even total if the nitrogen content of the yeast has been allowed to exceed a certain limit Thus, one strain of yeast was found to lose only 15 per cent of activity after drying and reconstitution, provided the organism had been grown in such a way as to restrict its content of total (Kjeldahl) nitrogen to 6 4 per cent However, at 8·3 per cent nitrogen 40 per cent activity was lost, while at 9 8 per cent nitrogen the suspension of rehydrated yeast was totally inactive

Clearly the activity of a suspension made from dried yeast will depend, not only on the loss suffered during drying and reconstitution, but also on the activity the yeast had before it was dried In general, for a given strain of yeast grown under specified conditions, this is positively correlated with the nitrogen content Consequently the plot of dried yeast activity against nitrogen content shows a maximum, usually in the region 6–7 per cent nitrogen content

Undoubtedly, many factors govern the extent to which activity is lost during drying, and the relative importance of one or other of these may depend on the nitrogen content It is, for example, possible that a variable proportion of the loss in activity associated with drying/rehydration is due to chemical or enzymatic reactions occurring during the initial phase of drying That enzymatic reactions do go on then is shown by the conversion of yeast cell glycogen to trehalose[5] Poor-drying, nitrogen-rich yeasts often darken considerably when dried this was at first considered to be evidence of a Maillard-type reaction, which might cause loss of activity, but the idea was discarded when it was found that yeast with a very high nitrogen content sometimes did not discolour, and when it was found that the cell-wall became thinner and perfectly transparent when dried, so that the colour of the interior parts of the cell might in any event be expected to become more obtrusive in the dried yeast

The extent of enzymatic degradation occurring when yeast is dried would vary according to the amount of the relevant enzyme present This again might well depend on the nitrogen supplied during propagation Such a situation was found to apply in the case of the inducible enzyme α-glucosidase (maltase), the content of which diminished, when yeast was grown with a restricted supply of assimilable nitrogen, far out of proportion to the fall in fermentation activity An autolytic enzyme with similar properties was suspected to be responsible for part of the loss of activity observed when high-nitrogen yeast was dried.

Preliminary work on the effect of drying on the carbohydrates, proteins, and nucleic acid of the yeast cell did not provide evidence of hydrolytic degradation of a type or magnitude commensurate with the observed losses in activity. We therefore directed our attention to the lipids of yeast, more especially as the cell membrane is thought to be largely composed of lipid material[6].

To determine total fatty acids and ergosterol in pressed yeast, a sample of the latter was refluxed with methanolic potassium hydroxide, which simultaneously saponified and extracted the lipids. These were partitioned into fatty acids and non-saponifiable lipid by conventional methods, after which ergosterol was determined by ultra-violet spectrophotometry and total fatty acids by weight and by titration with sodium hydroxide in ethanol. Dried yeast was first rehydrated before applying this procedure.

No decrease in the content of total fatty acids or of ergosterol was noted after yeast had been dried. The main use of this saponification procedure, in fact, was in testing methods designed to extract lipids from yeast in an undegraded form. To do this efficiently it was found essential first to disintegrate the yeast cells, which was done by shaking a thick suspension of yeast for 2 h with 2-mm diameter glass beads. After this treatment, the suspension was stirred with 15 vol. of chloroform/methanol 2 : 1 for 24 h. The washed cell residue contained only trace amounts of lipid, as shown by the failure of the saponification procedure to remove significant quantities of total fatty acids or of ergosterol.

The solvent, applied to whole yeast cells, extracted only about a quarter of the total lipid. Procedures based on a lengthy preliminary extraction with approximately 70 per cent ethanol, for example, Hanahan's[7], were abandoned because of some evidence of autolysis. For example, chromatography on silicic acid-impregnated paper[8,9] showed the ethanol extract to contain appreciable amounts of lysolecithin and lyso-phosphatidyl ethanolamine.

The chloroform/methanol extract of disintegrated yeast was washed without loss of lipid by the Folch technique[10], using 0.1 M sodium chloride. It was then evaporated, after which the lipids were taken up in ether and partitioned into phospholipids and neutral lipids by addition of acetone and a little magnesium chloride[11], which precipitated 92 per cent of the lipid phosphorus. Ergosterol and ergosterol esters, which were found to be entirely in the acetone-soluble fraction, were determined by spectrophotometry of the digitonin complex[12] precipitated before and after hydrolysis with methanolic potassium hydroxide. Fatty acids were estimated, as before, after saponification of one or other fraction. Phosphorus was determined after digestion with perchloric acid, and nitrogen by a direct Nesslerization procedure after digestion by the Kjeldahl method, using copper and selenium catalysts[13]. Information on the relative amounts of individual phospholipids present was obtained by paper chromatography. The phospholipid mixture was resolved by chromatography on silicic acid-impregnated paper, and in addition was subjected to mild alkaline hydrolysis[14] and the water-soluble glyceryl phosphoryl derivatives chromatographed on acid-washed paper. Yeast phospholipid was suspended in a 1 : 1 mixture of chloroform and methanol, methanolic potassium hydroxide was added, and the mixture shaken for 15 min at room temperature. The reaction was stopped by saturating the mixture with carbon dioxide. The upper layer obtained after addition of water was applied to the paper as such, or after conversion with ion-exchange resins to the free acids or ammonium salts.

Examinations of the effect of drying have so far been largely confined to yeast containing 8 per cent of nitrogen, which loses about half its activity as a result of dehydration followed by reconstitution. No evidence of hydrolysis of sterol esters could be found. The phospholipid fraction was found to have a fatty acid/phosphorus molar ratio of about 2 and a nitrogen/phosphorus ratio of about 1. After drying, the amount of phospholipid was found to have decreased by 10–20 per cent as shown by determinations of total weight, fatty acid and phosphorus. The fatty acid content of the neutral lipid fraction increased by a corresponding amount. In one case, in which the phospholipid phosphorus had fallen after drying from 53 to 43 µmoles/g dry weight, the chromatographic procedures showed that this was due to a decrease in the content of lecithin and of phosphatidyl ethanolamine. Analysis of the glyceryl phosphoryl derivatives after paper chromatographic separation showed that lecithin had decreased from 21 to 11 µmoles phosphorus/g. Phosphatidyl ethanolamine, but not phosphatidyl inositol, was also lost.

Similar changes in yeast phospholipids were induced by autolysis. After incubation with toluene for 5 h at 38° the yeast was extracted with chloroform/methanol without prior disintegration of the cells by shaking with glass beads, as the latter step was found, in the case of autolysed yeast, to be unnecessary. The phospholipid fraction had lost half its phosphorus and the major component was now phosphatidyl inositol (56 per cent), a finding which, incidentally, suggests the basis of a method for the preparation of this substance.

It appears, therefore, that when yeast of this type is dried even at moderate temperatures, the nitrogen-containing phospholipids are partially hydrolysed to diglycerides by a lecithinase resembling that described by Hoffmann-Ostenhof, Geyer-Fenzl and Wagner[15]. Yeast may also contain a phospholipase *A* and an acyltransferase, which, acting in conjunction, hydrolyse lecithin to glycerylphosphorylcholine and fatty acid[16]. However, we have found that the increase in free fatty acid associated with drying is much smaller than the loss of total fatty acid from the phospholipid fraction, which suggests that phospholipase *A* action plays a relatively minor part.

A sample of yeast of the same strain, as grown for drying and containing only 6.1 per cent nitrogen, lost less than 20 per cent of its activity when dried and reconstituted. The content of alkali-labile phospholipid was 33 µmoles phosphorus/g before drying and 34 µmoles/g after drying. Lecithin phosphorus was estimated to be 14 µmoles/g in both cases.

Thus the severe loss of activity, and high leakage, shown by high-nitrogen baker's yeast after drying and reconstitution may be due in part, in any event, to the action of a lecithinase *C* on the phospholipids of the cell membrane. The enzyme is either absent from low-nitrogen yeast or is not activated by drying.

A loss in lipid-bound choline associated with drying has been previously reported[17] for *S. cerevisiae* and *T. utilis*, though the technique of drying is not described.

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SYNTHESIS OF BACTERIOCHLOROPHYLL BY *RHODOPSEUDOMONAS SPHEROIDES* UNDER DARK-AEROBIC CONDITIONS

By MASATAKA HIGUCHI and GORO KIKUCHI
Department of Biochemistry, Tohoku University School of Medicine, Sendai, Japan

COHEN-BAZIRE *et al* [1] found that, while the synthesis of bacteriochlorophyll by *R spheroides* cells was completely suppressed when the growing conditions were switched from the light-anaerobic to light-aerobic or dark-aerobic conditions by aeration, the synthesis could proceed even in the dark if the partial oxygen pressure was maintained at very low levels The dark semi-aerobic synthesis of bacteriochlorophyll by *R spheroides* was examined more precisely by Lascelles[2], who established the conditions which ensured that dark-aerobically grown cells formed bacteriochlorophyll rapidly in the dark We have found that the synthesis in the dark of bacteriochlorophyll in air by heavy suspensions of the dark aerobically grown *R spheroides* cells was markedly influenced, in terms of not only the rate of synthesis but also the intracellular status of the product, by the presence or absence of yeast extract or relatively high concentrations of iron in the incubation medium

R spheroides was grown dark-aerobically in the medium *S* of Lascelles[3] and the colourless cells were collected at an early stationary phase of culture The basal incubation medium used was similar to Lascelles's standard mixture[2] except that α-ketoglutarate was replaced by fumarate in our medium Collected cells were washed once and suspended in the basal medium so as to give a cell concentration of about 3 mg dry weight per ml The medium was supplemented with various additions according to the design of individual experiments 20 ml each of the suspension were placed in 100-ml Erlenmeyer flasks and incubated in the dark in air while being gently shaken (80 rev /min) at 30° Under these conditions, however, the oxygen pressure in the incubation mixture may be maintained at a considerably reduced level on account of the active respiration by the cells Synthesis of the pigment commenced without an appreciable lag period and proceeded at a constant rate, usually 6–8 h, until the chlorophyll-level reached maximum; this depended on the composition of incubation medium used Illumination had no effect on the rate of synthesis of bacteriochlorophyll under these conditions

The pigment formed was estimated spectrophotometrically by the method of Cohen-Bazire *et al* [1] In our basal medium, 1 5–3 μg of bacteriochlorophyll were synthesized per mg dry weight of cells at 6 h incubation under the conditions described (Table 1) The *R spheroides* strain used by us, however, excreted also a fair amount of porphyrin(s) when incubated in either Lascelles's standard mixture or our basal medium Similar phenomena were observed to occur by Lascelles when the dark-aerobically grown *R spheroides* cells were incubated semi-aerobically in the dark[2] or anaerobically in the light[4] in a medium containing no or little iron

The addition of yeast extract (Difco) into the basal medium resulted in marked increase in the synthesis of bacteriochlorophyll (Table 1, Exp 1) and suppression of accumulation of porphyrin A similar effect was observed also, even when Lascelles's standard mixture was used The stimulative effect of yeast extract cannot be attributed to such components in the extract as amino-acids, vitamins and nucleotides since the addition of casamino-acid, vitamin mixture and hydrolysates of yeast ribonucleic acid, separately or together, did not appreciably increase the yield of bacteriochlorophyll under comparable conditions (Table 1, Exp 1) The addition of casamino-acid however, resulted in considerable cell growth during incubation The optimal concentration of yeast extract to bring about the maximum synthesis of bacteriochlorophyll falls in the range 0 25–0 5 per cent It was also found that synthesis of bacteriochlorophyll was greatly stimulated by supplementing the basal medium with additional ferric citrate (Table 1, Exp 2) The optimal concentration of ferric citrate to be added was about 0 5 μmole/ml Manganese showed a similar effect but to a lesser extent These findings tempted us to measure the iron content in yeast extract, but the value found was too small to attribute the effect of yeast extract to the iron in it The stimulative effect of yeast extract was reduced to less than half the original activity by treatment with charcoal The iron content in the extract was also reduced considerably by the same treatment. The possibility remains that the yeast extract contains some organic iron compounds which may be responsible for the observed effect of yeast extract Carotenoid synthesis was also increased by these supplements (Fig 1)

Significant differences were noticed between the absorption spectra of cell extracts, particularly in the near infra-red region, according to whether the bacteria were incubated with or without yeast extract or ferric citrate (Fig 1) Cell-free extracts of *R spheroides* were prepared by 3 min sonication followed by 30 min centrifugation at 7,000*g* The extract of the cells incubated in the iron-free medium showed small absorption peaks of chlorophyll at 880 mμ and 590 mμ (Fig 1, *A*), while the extract of the cells incubated in the medium supplemented with 0 5 μmoles/ml of ferric citrate or 0 25 per cent yeast extract gave the chlorophyll peaks at 590, 800 and 850 mμ

Table 1 EFFECTS OF YEAST EXTRACT, FERRIC CITRATE AND OTHER FACTORS ON THE SYNTHESIS OF BACTERIOCHLOROPHYLL IN *R spheroides* AND THE CELL GROWTH

Additions	Final conc	Bacteriochlorophyll formed μg/ml medium	Bacteriochlorophyll formed μg/mg dry wt	Growth* mg dry wt of cells/ml medium
Exp 1				
None		7 7	2 6	+0 1
Casamino-acid	0 5%	9 6	2 4	+0 8
Vitamin mixture solution	0 5%	9 6	3 1	0
Acid hydrolysate of yeast RNA	0 5 mg/ml	9 3	2 9	+0 1
Inorganic salts solution	10%	18 8	5 9	+0 1
Yeast extract	0 25%	23 2	6 3	+0 2
Exp 2				
None		4 4	1 6	−0 1
Ferric citrate	0 2 μmoles/ml	21 1	7 4	−0 05
$MnCl_2$	0 1 μmoles/ml	11 8	4 1	0
Phosphate buffer	10 μmoles/ml	4 1	1 5	−0 1
Inorganic salts solution	10%	22 2	7 7	−0 1

* As measured by the optical density change at 680 mμ (ref 1)

Dark aerobically grown cells were suspended (3 mg dry wt /ml) in the basal medium supplemented with various additions as shown in the Table and incubated aerobically in the dark for 6 h at 30° Casamino-acid used was a commercial vitamin-free casein hydrolysate In this experiment the medium was also supplemented with 100 μg/ml each of L-tryptophan, L-cystine and DL-methionine Vitamin mixture solution used contained in 1 ml 2 mg each of thiamine, nicotinamide and pyridoxine, 5 mg of sodium pantothenate, 3 mg of riboflavin, 75 mg of ascorbic acid, 500 μg of biotin, 2 μg of vitamin B_{12}, 5,000 I U of vitamin A, 500 I U of vitamin D and 25 mg of L-lysine hydrochloride Acid hydrolysate of yeast RNA was prepared by hydrolysing a commercial yeast RNA sample with 1 N hydrochloric acid at 95° for 1 h and used after being neutralized with sodium hydroxide Inorganic salts solution used contained in 1 ml 1 μmole of $MnCl_2$, 2 μmoles of ferric citrate and 100 μmoles of phosphate buffer (*p*H 6 8) with a shoulder at 870 mμ (Fig. 1, *B* and *C*). In Fig. 1, *A*, also a peak at 423 mμ can be seen which is probably due to porphyrins. The spectrum of the extract of the cells incubated in the basal medium (which contained 0.01 μmole/ml of ferric citrate) comes in between the two types of spectrum cited here (Fig. 1, *D*). In spite of these marked spectral differences between extracts obtained from the cells incubated under different conditions, when the chlorophyll spectrum was examined after being extracted into methanol–acetone from the cells, no significant difference could be observed between those differently incubated cells; all the samples examined gave the typical bacteriochlorophyll spectrum showing a maximum at 770 mμ. These results indicate that the structure of bacteriochlorophyll itself has not been affected by the supplement of iron or yeast extract to the incubation medium. In contrast, it is highly probable that these additions had a profound influence on the status of bacteriochlorophyll in the living cells which presumably is dependent on some interactions of bacteriochlorophyll and other intracellular components.

Although the chlorophyll content in cells and the optical densities at 800, 850 and 800 mμ in cell-free extracts varied significantly according to incubation conditions when examined at 6 h incubation, the values of *E* 850 mμ/*E* 880 mμ and *E* 800 mμ/*E* 880 mμ (*E* stands for optical density) were found to increase linearly and in parallel with each other with the increase in chlorophyll content (Fig. 2). The values, however, were more or less variable in separate experiments. These results suggest that the component which gave an absorption maximum at 850 mμ (850 mμ component) may possess an additional absorption at 800 mμ. Or the 800 mμ peak may be an indication of a third component which could grow up in parallel with the formation of 850 mμ component. We have observed that when the cells were collected at an earlier phase of incubation in the medium rich in iron or yeast extract, the chlorophyll spectrum of the cell extracts was quite similar to that of cells collected at a later phase of incubation without the supplements. Moreover, when the dark-aerobically grown cells were pre-incubated for 2 h in the medium rich in iron, then washed and transferred to the iron-deficient medium, the same cells continued actively to synthesize bacteriochlorophyll even in the latter medium, giving rise to the formation of 850 mμ component as the major product. Either of these bacteriochlorophyll components in extracts of *R. spheroides* cells can be sedimented almost completely by 90 min centrifugation at 105,000*g*. This indicates that all of them are bound to chromatophores.

Considering these experimental findings, we are inclined to assume that the synthesis of bacteriochlorophyll in question would proceed in the following fashion: at earlier stages of incubation, the rate of 880 mμ component formation far exceeds that of 850 mμ component formation, but the rates change gradually during incubation, and at later stages the 850 mμ component is synthesized much more actively than the other, and this step may be sensitive to iron or some unknown components in the yeast extract.

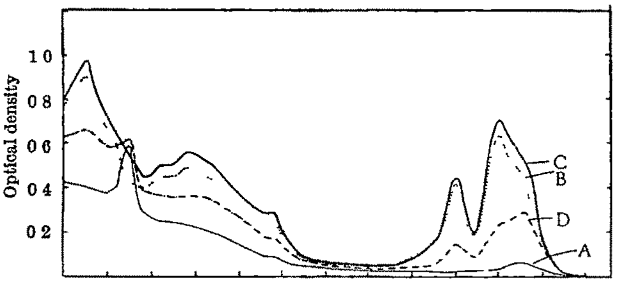

Fig. 1. Absorption spectra of the cell-free extracts of *R. spheroides* incubated dark-aerobically with or without yeast extract and ferric citrate in the medium. 20 ml. each of the suspension of dark-aerobically grown cells (2.1 mg dry wt./ml.) were incubated aerobically in the dark for 6 h at 30°. The composition of the ordinary basal incubation medium (containing originally 0.01 μmoles/ml. ferric citrate) was changed in individual experiments shown in the figure in the following way: (*A*) minus iron, (*B*) plus ferric citrate up to 0.5 μmole/ml., (*C*) plus 0.25 per cent yeast extract, (*D*) no change

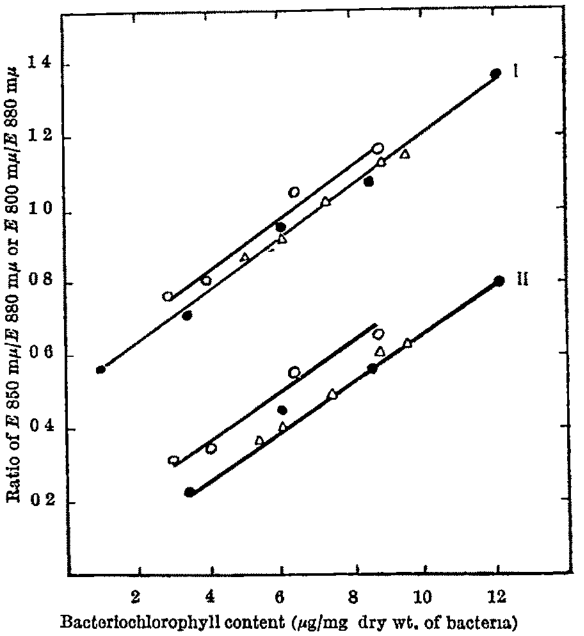

Fig. 2. Change of the values of *E* 850 mμ/*E* 880 mμ and of *E* 800 mμ/*E* 880 mμ as a function of the bacteriochlorophyll content in the *R. spheroides* cells. I, *E* 850 mμ/*E* 880 mμ; II, *E* 800 mμ/*E* 880 mμ. Experimental conditions were similar to those in Fig. 1, except for the composition of incubation media used. Values represented by ● in the figure were obtained by a series of experiments in which the basal media supplemented with various amounts of ferric citrate were used; ○, supplemented with yeast extract, treated or untreated with charcoal or ion exchange resin ('*IR* 120-H' type); △, pre-incubated in media poor or rich in iron, then transferred respectively into second media containing different amounts of iron from those used for pre-incubation

In vivo spectrum of bacteriochlorophyll in photosynthetic bacteria has been shown to vary according to the influence of various factors such as the light intensity during the growth of organisms[1,5], heat shock[5], cooling at liquid nitrogen temperature[9], and genetic variations of the bacteria[7], and various interpretations have been given with respect to the differences in *in vivo* status of bacteriochlorophyll[5-9].

Although the present investigation could not reveal which of these speculations is the more valid, our findings that the synthesis of 850 mμ component was more susceptible to the amount of iron or yeast extract in the incubation medium would provide an experimental way of controlling the *in vivo* status of bacteriochlorophyll. Such an approach may facilitate the solution of the role in bacterial photosynthesis of each chlorophyll component as distinguished by the infra-red absorptions of cell extracts.

We thank Dr. T. Mizuochi, Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, for his advice.

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LETTERS TO THE EDITOR

RADIOASTRONOMY

Radio Frequency Radiation from Cygnus *Y*

DURING mapping observations of radio source *HB*21 at 600 Mc/s using the new 260-ft. radio telescope[1] here, a highly complex region of radio radiation was revealed between declination −49° and + 51° extending from right ascension 20 h 30 m to 21 h 50 m. The region is situated about 8° north-east of the well-known radio source[2] Cygnus *X*. The new region, like Cygnus *X*, contains an unusual number of separate discrete radio sources of various sizes, and will be referred to as Cygnus *Y*.

The present observations were made at 600 Mc/s. The antenna has half-power beam-widths of 24 min by 80 min of arc in right ascension and declination, respectively. The effective collecting aperture of the antenna is 870 m². A sample record of the drift curve, obtained at declination + 50°, is presented in Fig. 1, which shows the presence of six discrete radio sources of various angular sizes superimposed on the slow rise due to the general galactic background radiation.

These sources are listed in Table 1. The present observations have verified all the 3*C* and *CTB* sources in the region; thus the previous discrepancies between the 3*C* and *CTB* sources have been clarified. The source *CTB*104 was resolved into two separate components (see sources *D* and *E* in Fig. 1). The flux density given in Table 1 refers to the observed peak flux density rather than the integrated flux density. The integrated flux density cannot be determined until the radio mapping of the whole region is completed.

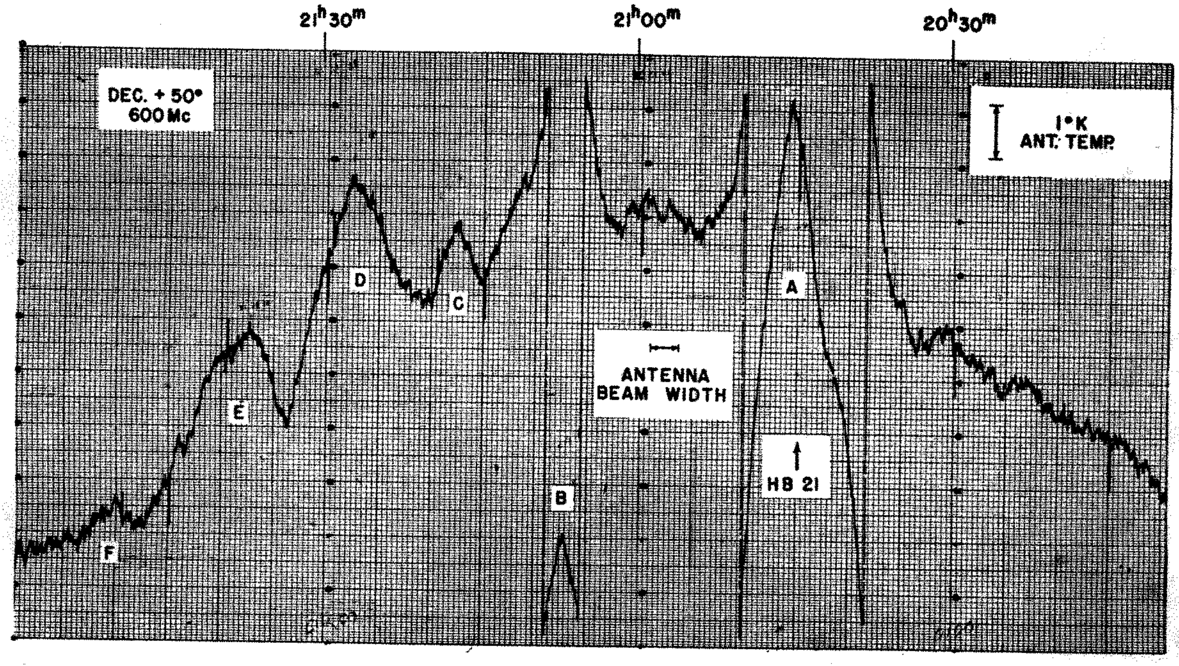

Fig. 1. Sample record of an observation taken at declination + 50°

A high-resolution radio map containing the Cygnus *Y* region was first made by Hanbury Brown and Hazard[3] at 158 Mc/s using a 2° antenna beam. They discovered the extended source *HB*21. A recent radio map of the same region made by Wilson and Bolton[4] at 960 Mc/s using a 0·8° antenna beam shows three discrete sources (*CTB*97, *CTB*101 and *CTB*104). Interferometric surveys of the region made at Cambridge[5,6] at metre wave-lengths show three discrete sources in the region (3*C*420, 3*C*428 and 3*C*431). However, the agreement among these sources is rather poor except for sources *HB*21 (same as 3*C*420 and *CTB*97) and 3*C*428 (same as *CTB*101).

Table 1

Source No.	Position (1950.0) Right ascension	Position (1950.0) Declination	Peak flux density 10^{-26} W m^{-2} $(c/s)^{-1}$	Angular size	Remarks
A	20h 45m 04s ± 10s	+ 50° 30′ ± 10′	46·9	1·9°	*HB*21 3*C*420, *CTB*97
B	21h 06m 54s ± 10s	+ 49° 45′ ± 10′	16·3	0·7°	3*C*428, *CTB*101
C	21h 17m 10s ± 10s	+ 49° 25′ ± 10′	6·7		3*C*431
D	21h 27m 11s ± 10s	+ 50° 30′ ± 10′	15·8	1·5°	*CTB*104
E	21h 37m 00s ± 10s	+ 50° 10′ ± 10′	7·8	1°	*CTB*104
F	21h 50m 24s ± 15s	+ 50° 15′ ± 15′	1·5		

The profile of source *A* in Fig. 1 clearly shows an unsymmetric structure. A further observation of the source at 1,400 Mc/s using an 11-min arc antenna beam has resolved the source into at least three components. This source has been identified with a supernova remnant[7]. Sources *B*, *D* and *E* are all extended sources of the order of one degree, and appear to have fine structure. There is a suggestion that source *E* consists of two components.

The Cygnus *Y* region, like Cygnus *X*, is one of the interesting and unusual parts of the sky because of its high source density and the complex structure. The region is now being mapped in detail at 600 Mc/s and 1,400 Mc/s to obtain the spectrum of the individual sources as well as the detailed structure of the region.

This work was supported in part by the National Science Foundation, the Air Force Cambridge Research Laboratories, and the funds for basic research at the Ohio

State University. I thank my colleagues here for their assistance.

H. C. Ko

Radio Observatory,
Ohio State University,
Columbus 10, Ohio.

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ASTRONOMY

Spectra of Meteor Wakes

With regard to the interesting communication by Rao and Lokanadham[1] I would direct attention to a possible consequence.

Hoffmeister[2] has collected various instances of auroral-like glows connected with meteoric showers, Perseids, Leonids, 1872 Bielids. Such was also noted in Germany and England during the famous Leonid shower of November 13–14[3]. It was indeed remarked it might be possible that "brilliant meteor streams electrify the higher regions of the atmosphere and produce phenomena analogous to the aurora borealis"[4].

The detection of *H* and the red line of *NI* is interesting in view of the observation by von Wrangel in Siberian waters 1820–24[5] that dark areas of aurora traversed by meteors lighted up and continued to glow.

Cicely M. Botley

5 Blatchington Road,
Tunbridge Wells.

[1] Rao, M. S., and Lokanadham, B., *Nature*, **197**, 169 (1963); **198**, 77 (1963).

[2] Hoffmeister, C., *Akad. Verlag.* (Leipzig, 1937).

[3] Fritz, H., *Das Polarlicht Brockhaus* (Leipzig, 1881).

[4] Philipson, M. T., *Meteors, Aerolites*, etc. (London, 1866).

[5] Dauvillier, A., *Les Aurores Polaires* (Alcan, Paris, 1954).

Miss Botley has brought to light some interesting observations of auroral-like glows of meteors during some meteoric shower periods[1–3] and during periods of auroral activity[4]. These observations indicate that this phenomenon is associated with the atmosphere, in which meteors appear, being in an ionized or excited condition. There is evidence for such a general increase of electron density in the *E* region during meteoric shower periods[5]. It is also known that the atmosphere above 80 km is ionized by auroral activity[6]. The predominant constituents of the atmosphere in this region are atomic oxygen and molecular nitrogen[7].

The main contributing feature in the auroral-like glow of meteors appears to be the forbidden line of *OI* (λ5577, known as the auroral green line), which has been observed in as many as 30 meteor spectra[8–10]. A possible explanation for the production of this line by a combination of the oxygen contained within the meteoroid and that in the atmosphere has been outlined by Halliday[11]. From the observations of Halliday, Millman[8] concluded that even moderate solar activity was not necessary for this green line to appear in meteor spectra. This is consistent with the foregoing picture that the increase of atmospheric ionization above normal may also be produced by meteor showers, like auroræ which occur during periods of high solar activity.

The appearance of the weak $H\alpha$ and *NI* lines in meteor spectra[10] may be mainly due to these elements present within the meteoroid particle. The mechanism of production of the former line may be similar to that in auroræ, wherein the same line is produced by protons of the corpuscular radiation from the Sun[12]. It may be noted in this connexion that the nitrogen bands observed in the red and yellow regions of the meteor spectrum by Cook and Millman[13] were connected with the molecular nitrogen in the atmosphere.

The foregoing explanation of auroral-like glow of any meteor is based on the fact that it is caused by the further local excitation of the atmospheric gases, already in a sufficiently excited condition, by the passage of the meteor through them. Such a glow should reveal itself in the wake spectrum of the meteor, as did the oxygen green line[8–10]. On the other hand, the $H\alpha$ and *NI* lines are found only in the primary spectra[10,14] of meteors.

M. Srirama Rao
B. Lokanadham

Meteor Research Laboratory,
Department of Physics,
Andhra University,
Waltair, India.

[1] Hoffmeister, C., *Akad. Verlag.* (Leipzig, 1937).

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PHYSICS

Intensity of Neutral Argon Emission Lines radiated by the Low-voltage – High-amperage Arc compared with Four High-voltage Type Arcs

The intensity of strong neutral argon emission lines radiated by the thermally excited argon shield gas surrounding and within the central d.c. arc plasma of the Heliweld thoriated tungsten electrode welding arc has been measured. This arc was operated at 15 V and 135 amp (d.c.), providing a good source for thermally exciting neutral argon emission lines at a voltage below the ionization voltage (argon = 15·8 V).

The purpose of the investigation was to see how the relative intensities of the strong neutral emission lines of argon varied in reference to each other for this thermally excited arc compared with microwave, glow discharge and hollow cathode type arcs. This information should add to the background of data on observations of spectral sensitivity of elements excited under varied conditions.

As W. F. Meggers[1] points out, the relative intensities of lines in any particular spectrum are fairly independent of conditions; successive spectra will show large differences as functions of excitation conditions. In order to get the best comparison of intensities between arcs, therefore, the intensities are normalized to the strongest line common to all arcs. The intensities for each arc and intensity values are then adjusted by ratio to this normalized intensity value (0·8115μ argon line) as shown in Table 1.

The values recorded for the tungsten arc were recorded by a Perkin–Elmer monochromator with suitable entrance optics and output recorder.

Table 1 shows the normalized intensity values for all these arcs. The low-voltage tungsten arc emission lines appear to differ in intensity to the same degree as the microwave, glow discharge and hollow cathode intensities vary from each other.

Table 1. RELATIVE INTENSITIES OF ARGON EMISSION LINES EMITTED BY A LOW-VOLTAGE HIGH-CURRENT ARC COMPARED TO THREE HIGH-VOLTAGE LOW-CURRENT ARCS

Wave-length (μ)	Low-voltage tungsten arc*	Microwave excitation†	Glow discharge (60 a.c.)‡	Hollow cathode discharge§
0·6965	102	108	115	56
0·7067	105	90	102	56
0·7278	67	36	51	17
0·7384	167	163	104	107
0·7504	180	181	224	791
0·7515	196	145	116	117
0·7635	337	272	339	229
0·7724	162	235	276	155
0·7948	172	181	135	135
0·8006	198	162	170	115
0·8104	180	308	204	200
0·8115	361	361	361	361
0·8265	108	253	191	118
0·8408	212	344	229	224
0·8425	233	315	224	302
0·8521	108	235	151	123
0·8668	35	69	33	44
0·9123	212	415	—	361
0·9224	145	217	—	155
0·9658	126	290	—	229

* Tungsten arc 135 amp, 15 V, atm. pressure. 16 cm ft./h argon flow.

† Microwave excited, end view, 6·5 mm pressure, *Amer. Inst. Phys. Handbook*, 7–68.

‡ Glow discharge 60 cycle a.c., 60 m.amp, 3 mm pressure, *Amer. Inst. Phys. Handbook*, 7–61.

§ Hollow cathode discharge, iron electrodes, 150 m.amp, 1 mm pressure, *Amer. Inst. Phys. Handbook*, 7–61.

Table 2 gives the ratio of the tungsten arc to the other arc intensities for the various emission lines. It is apparent, even though all the intensity scales for each arc are normalized to the strong 0·8115μ argon line, that the same wave-length lines do vary from each other to a large degree from arc to arc.

Table 2. INTENSITY RATIOS OF ARGON I EMISSION LINES OF THE LOW-VOLTAGE TUNGSTEN ARC TO THE HIGH-VOLTAGE ARCS

λ (μ)	Energy-levels	Tungsten arc to microwave	Tungsten arc to glow dis.	Tungsten arc to hollow cath.
0·6965	$1S_5-2p_2$	0·95	0·88	1·82
0·7067	$1S_5-2p_3$	1·16	1·03	1·88
0·7273	$1S_4-2p_2$	1·85	1·32	4·00
0·7384	$1S_4-2p_3$	1·03	1·61	1·56
0·7515	$1S_4-2p_5$	1·35	1·70	1·70
0·7635	$1S_5-2p_6$	1·24	0·99	1·47
0·7724	$1S_5-2p_7$	0·69	0·59	1·04
0·7948	$1S_3-2p_4$	0·95	1·28	1·28
0·8006	$1S_4-2p_6$	1·22	1·16	1·72
0·8115	$1S_5-2p_9$	1·00	1·00	1·00
0·8265	$1S_2-2p_2$	0·43	0·56	0·92
0·8408	$1S_2-2p_3$	0·62	0·93	0·95
0·8425	$1S_4-2p_8$	0·73	1·04	0·77
0·8521	$1S_2-2p_4$	0·46	0·72	0·81
0·8668	$1S_3-2p_7$	0·51	1·05	0·79
0·9123	$1S_5-2p_{10}$	0·51	—	0·59
0·9224	$1S_2-2p_6$	0·67	—	0·93
0·9658	$1S_4-2p_{10}$	0·44	—	0·55

It would appear that, even taking into account the rough accuracy of these intensity values, the intensities between one line and another for each arc are not the same for different types of excitation. This cannot be attributed entirely to pressure differences between arcs since the tungsten low-voltage arc at atmospheric pressure did not vary from the other arcs for any particular emission line any more than the glow discharge arc at 3 mm pressure varied from the hollow cathode arc at 1 mm pressure. It is obvious that the electron temperatures are different between these arcs, and this is probably the main factor in determining the intensity ratios for various wave-length lines compared with the strong 0·8115μ line.

Table 3. SHIELD GAS ENVELOPE VERSUS CENTRAL TUNGSTEN ARC ARGON LINE INTENSITIES

Wave-length (μ)	Ratio of shield gas glow to central arc intensity lines
0·7504	0·127
0·7515	0·138
0·7635	0·216
0·7724	0·127
0·7948	0·145
0·8006	0·101
0·8115	0·154
0·8265	0·254
0·8408	0·282
0·8425	0·248
0·8521	0·223
0·9123	0·625
0·9224	0·500

The intensity of the argon lines emitted at the outside edge of the tungsten arc shield gas envelope were measured. The intensities of these lines were compared with those emitted by the central arc and are given in Table 3.

In this experiment the intensity ratios of the 0·9123μ and 0·9224μ lines show a definite increase in intensity for the glowing shield gas envelope. This would indicate a definite increase of emission intensity for the $1S_5-2p_{10}$ (0·9123μ) and $1S_2-2p_6$ (0·9224μ) energy-levels for the outside glowing shield gas envelope. This emitting portion of the arc is obviously at a lower temperature than the central arc column.

C. MITCHELL

Barnes Engineering Co.,
Stamford, Connecticut.

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Difficulties in obtaining Uniform Dose Distribution throughout Electron-irradiated Plastic Mouldings

THE published ranges of fast electrons and the associated depth/dose curves have been measured in slabs of material irradiated with initially mono-energetic electrons. Such figures give an indication of the maximum thickness of bulk material which can be usefully irradiated with an electron beam of a particular energy.

In practice, objects to be irradiated would probably be complicated mouldings with inserts of various materials rather than homogeneous large slabs. Such mouldings give rise to a variable depth/dose relationship due to non-complementary scattering from discontinuities. Electrostatic repulsion may be a factor in this effect.

By examination of the scatter and absorption images produced on a paper with a radiation-sensitive coating, it has been shown that very large differences in absorbed dose are indicated on a complex moulding irradiated in a mono-energetic unidirectional electron beam. The thickness of material presented to a 4-MeV electron beam was nowhere greater than 50 per cent of the range as measured using the same beam with a slab of material. Irradiation from both sides of the sample reduced the spread of indicated dose by a factor of approximately two, leaving the ratio of maximum to minimum doses about 3 to 1. This would involve serious over-dosing and inefficiency in cases where a specified minimum dose is required.

The original work on the problem of uniformity of dose throughout a plastic moulding was done using self-adhesive paper with a radiation-sensitive coating developed by Vale and Farrell[1] as an indicator label for radiation-sterilized articles.

The colour change on the indicator label paper with increasing dose was from yellow through orange to red. This gave satisfactory dose discrimination for the first experiments, but as the dose ratio was reduced by techniques described later, greater resolution was required. Vale and Farrell, by an alteration in the sensitive dye used in the coating, produced a paper that changed from bright green through brown to purple and then pink. Since four definite colours and their associated transition shades covered only 3 × 10^6 rads, quite good dose estimation could be obtained by visual inspection.

It has been shown by Trageser[2] that a forward-scattering foil in the electron beam path degrades the mono-energetic stream and the lower energy components produced almost eliminate the dose build-up at the front face of a sample.

At this laboratory a foil has been used not only to degrade the electron beam energy but also to produce a multidirectional effect. In order to obtain the greatest effect from the foil it needs to be as close as practicable to the moulding.

Further reduction in the dose ratio was obtained by utilizing back-scattered electrons from the sample support.

As expected, the number of back-scattered electrons was increased by increasing the atomic number of the backing plate and, as with the forward scatter foil, the widest scatter angles were presented to the moulding at the closest practicable distance.

The best results were obtained using a forward scatter foil of 0·5 mm aluminium and a back scatter plate of 1·5 mm lead over the sample support tray.

Final dose distribution checks were made using the improved indicator paper above, below, and inside the moulding. The dose ratio within the moulding irradiated from one side was then found to be 1·2.

Owing to the increase in overall efficiency on using the scattering foils, the minimum indicated dose under the conditions quoted was close to the mean exposure dose as measured in the first 1·2 g/cm^2 of a slab of material irradiated by the 4-MeV beam without scattering foils.

J. D. McCann
F. Rogers

Radiation Branch, Isotope Research Division,
Wantage Research Laboratory,
U.K. Atomic Energy Authority,
Wantage, Berks.

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Dielectric Loss Factor of Polytetrafluoroethylene under Irradiation

The effect of ionizing radiation on steady-state conductivity of polymeric dielectrics has been already examined by several authors. I have shown[1] that radiation affects not only the steady-state conductivity of polytetrafluoroethylene but also the transient conductivity, deduced from the current flowing through the specimen after the application of d.c. voltage. Recently I have carried out measurements of dielectric permittivity and dissipation factor under irradiation.

As a source of radiation, an X-ray equipment was used, giving dose rates in the range 0·01–100 r./sec. Circular specimens, 40μ thick, were cut from commercial polytetrafluoroethylene sheet and aluminium electrodes were evaporated on to both sides. A 'sandwich' type arrangement of two specimens was used. The dielectric permittivity and dissipation factor were measured at 50 and 1,000 c/s, 100 V and at about 21° C by the aid of a Wien's bridge (the four capacitances bridge) provided with a Wagner's earth.

The effect of radiation on the dissipation factor of polytetrafluoroethylene is much less pronounced than on the steady-state conductivity. The dissipation factor begins to rise at 50 c/s with a dose rate of 1 r./sec, at 1 kc/s even with 10 r./sec. The dielectric permittivity remains nearly constant in the whole range of dose rates used. From these a.c. measurements the loss factor was deduced. Fig. 1 shows the dependence of loss factor on dose rate over the range 0·01–100 r./sec. The loss factors at low frequencies were deduced from d.c. measurements[1] by Hamon's method[2]. The results indicate that the variation in the loss factor with dose rate is not caused solely by the rise of steady-state conductivity.

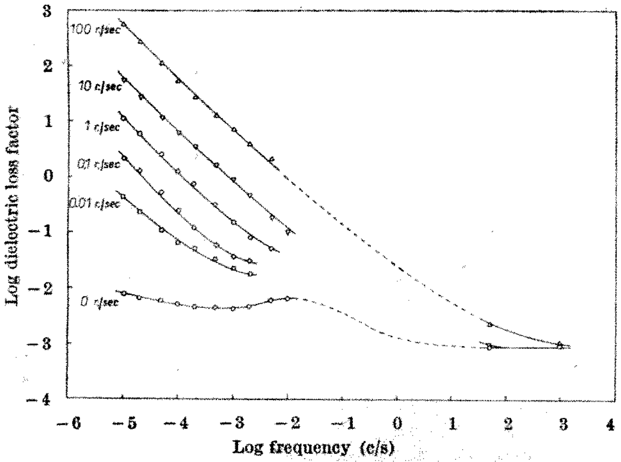

Fig. 1. Frequency-dependence of loss factor for polytetrafluoroethylene at various dose rates

V. Adamec

Výskumný Ústav Káblov a Izolantov,
Bratislava, Czechoslovakia.

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Heat Liberation in Alloy-junction Silicon Diodes

In a recent communication[1], Melehy and Jarmoc have reported interesting observations of the distribution of temperature in silicon diodes carrying a relatively large current in the forward direction. It is shown here that their observations are readily explained in terms of junction theory, when account is taken of the difference in electron and hole mobilities in silicon.

Consider a p^+–n–n^+ structure biased sufficiently in the forward direction so that the injected carrier density in the n-region is greater than the equilibrium density of electrons, and there are equal densities of electrons and holes[2]. When the width of the n-region is comparable with the diffusion length, recombination of electrons and holes is known to occur[3] predominantly in the end regions, at the metal-semiconductor contact.

For convenience we consider the end regions to be degenerate. An electron which surmounts the potential barriers between n^+- and n-regions, and between n- and p^+-regions, extracts heat[4] from the system at these two planes; the total energy abstracted per electron is to a first approximation $q(E_g - V_f)$, where qE_g is the band-gap energy and V_f the forward bias voltage on the diode. On arrival in the p^+-region the electron recombines with a hole and liberates energy qE_g, which may be assumed to be taken up by the lattice as heat energy. We neglect the kinetic energy of the charge-carriers, as well as heat dissipation associated with the flow of current in the n-region.

In the same way there is a transfer of heat energy by holes from the p^+–n and n–n^+ junctions to the n^+-region. However, because the electrons have greater mobility in the electric field which exists in the n-region, and the densities there are equal, the electron current in the device will exceed that of the holes; the transfer of heat energy to the p^+-region will therefore outweigh that to the n^+-region in the ratio $b \simeq 2{\cdot}8$ at room temperature, and we may expect the temperature in the p^+-region to rise above that in the n^+-region, as observed[1].

Furthermore, if the mobility ratio b is sufficiently large the heat extracted by electrons in the vicinity of the n^+-region may exceed that liberated by holes arriving there, and the temperature at this side of the diode will fall below the ambient. With a suitable semiconductor (for example, indium arsenide) a p^+–n–n^+ structure may thus act as a thermoelectric refrigerator by virtue of the flow of non-equilibrium charge-carriers.

L. W. Davies

A.W.V. Physical Laboratory,
Amalgamated Wireless (Australasia) Ltd.,
Sydney.

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METALLURGY

Recrystallization and Grain Boundary Sliding in a Magnesium Alloy

During tensile deformation of magnesium and its dilute alloys in the temperature range 100°–350° C, cells form preferentially close to the grain boundaries[1]. Afterwards irregular boundary migration occurs and evidence has been presented for the grain boundaries being pinned at their intersections with the cell walls[2]. This migration results in an increase in grain boundary area and thus the driving force must arise from the difference in stored energy between neighbouring grains. It is unlikely that sliding can occur to any appreciable extent at these irregular boundaries and in these circumstances it is probable that the shearing forces in the vicinity of the grain boundaries are accommodated by increases in the degree of misorientation across the cell walls. Eventually the irregular migration leads to primary recrystallization[2-6] along the old grain boundaries and an example is illustrated in Fig. 1.

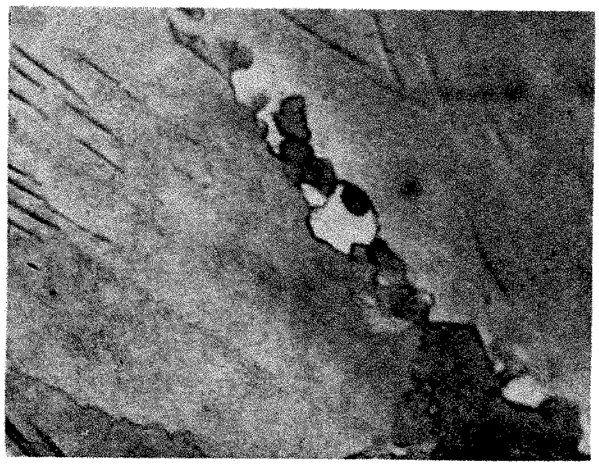

Fig. 1. Specimen deformed to failure at a rate of 0·1 per cent/h at 200° C. A network of new grains has formed along the old grain boundaries (× *c.* 270)

Stacey[3,4] has suggested that the observed increase in tensile ductility with decreasing strain rate of a magnesium/0·8 wt. per cent aluminium alloy at 150° C is due to such primary recrystallization. On the other hand, Greenwood *et al.*[7] and Brookes *et al.*[8] attribute similar increases in ductility to non-basal slip systems becoming operative preferentially at the slower rates.

Primary recrystallization is *per se* a recovery process and its occurrence would relieve stress concentrations at the original grain boundaries. In addition, ductility may be improved by sliding occurring at the new grain boundaries; it was to test this latter possibility that these experiments were carried out.

A 0·06-in. thick sheet specimen of a magnesium/0·8 wt. per cent aluminium alloy was polished chemically, deliberately scratched using 6μ diamond paste and then pulled to fracture at a rate of 0·1 per cent/h in air at 200° C. As a consequence of continuous migration and recrystallization during deformation there was no clear evidence for sliding at the new grain boundaries. Accordingly, a mixed strain-rate test was carried out. A similar specimen was polished and scratched as before and then deformed 15 per cent at a tensile rate of 18 per cent/h and a further 10 per cent at a rate of 180 per cent/h at 300° C. The initial slow deformation had resulted in a network of new grains being formed along the original grain boundaries and the subsequent rapid rate was used to minimize boundary migration during the second part of the test.

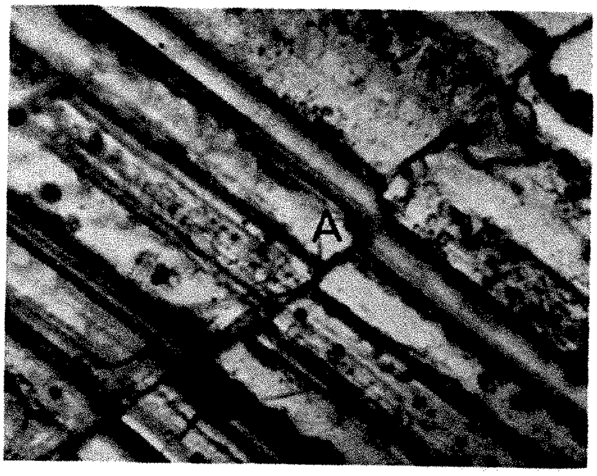

Fig. 2. Mixed strain-rate specimen deformed at 300° C. Where the fiducial lines cross the location of the old grain boundary two distinct steps are seen, for example at *A* (× *c.* 1,070)

Evidence for sliding at the new grain boundaries is presented in Fig. 2. Where the fiducial lines cross the locations of the old grain boundaries they show two distinct steps, for example at *A* (Fig. 2). This result lends support to the suggestion that primary recrystallization leads to improved tensile ductility.

J. E. Harris

Berkeley Nuclear Laboratories,
Central Electricity Generating Board,
Berkeley, Gloucestershire.

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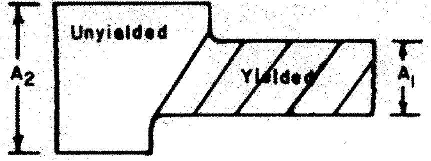
A Method of Determining the Stress Effective during Lüders Band Propagation

In materials which exhibit a well-defined yield-point drop followed by non-uniform yielding at a nearly constant stress, this stress is given by the Petch equation[1] as:

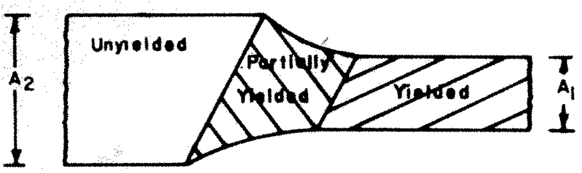
$$\sigma_y = \sigma_i + k_y d^{-\frac{1}{2}} \tag{1}$$

where σ_y is the lower yield stress, σ_i is the friction stress, k_y is a term originally thought to be associated with an unlocking stress and $2d$ is the grain diameter. It is desirable to have an accurate value of the macroscopic stress, σ_y, in order that the slope of the lower yield stress vs. grain size curve, k_y, be as meaningful as possible. In addition, the dependence on temperature of the lower yield stress is an important property in theoretical considerations. Difficulties may arise, however, in determining what value of the lower yield stress to use in such situations. This communication describes a method which can be used to determine, at least in an approximate manner, whether the magnitude of any corrections to the nominal yield stress is sufficiently large to warrant consideration.

One source of error is that due to the change in cross-sectional area as one proceeds from the yielded to unyielded material in a specimen. The magnitude of this effect can be rather large for a small grain size[2] or at low temperatures[3]. Change in area can be taken into account by using the value of the Lüders strain to calculate the area behind



(a) Very sharp front



(b) Diffuse front

Fig. 1. Sharp and diffuse Lüders band fronts in a tensile specimen

the front, but this procedure will result in an overestimate of the stress because the Lüders front is normally more or less diffuse. Fig. 1 illustrates two types of Lüders fronts. In Fig. 1*a* the stress causing flow is increased by the reduction in area from A_2 to A_1; however, in Fig. 1*b* flow occurs in a region where the area varies from A_2 to A_1 in a continuous manner, hence yielding occurs over a spectrum of stresses. The effective lower yield stress will, therefore, be somewhere between those values of load divided by A_1 and A_2 with the actual value being weighted towards the regions where most of the flow occurs as the band advances. This in turn is a sensitive function of Lüders band profile. Work by Hahn[4] indicates that this 'integrated' area lies nearer A_1 than A_2. In addition to the change in area there is a stress concentration due to the 'notch' produced by the inhomogeneous flow. The enhancement of stress in this case will be fairly localized. Both factors discussed here tend to increase the effective value of σ_y; but the magnitude of these effects is variable.

Some idea of the effective yield stress value can be gained from a plot of nominal lower yield stress and flow stress for various strains versus $d^{-\frac{1}{2}}$ used in conjunction with a plot of Lüders strain versus $d^{-\frac{1}{2}}$. It has been shown that in some materials[5,6] the flow stress curves for various strains are parallel and have a smaller slope than the lower yield stress curve as shown schematically in Fig. 2. Also, each grain size represents a certain Lüders strain as exemplified in Fig. 3 for Armco iron[3]. It is interesting to note from Fig. 3 that a small grain size can have an appreciable Lüders extension at −45° C. Picking a value of 10 per cent Lüders strain for illustrative purposes, the value of the grain size with which this strain is associated is found from Fig. 3. A vertical line is then drawn through $d^{-\frac{1}{2}}_{10\%}$ (Fig. 2) until it intersects the 10 per cent flow stress curve as shown by the asterisk. The difference in stress between this point and the lower yield stress curve represents the stress increase which can be ascribed in part to the sources discussed here. A second corrected yield stress point can be obtained in the same manner as that shown for the 10 per cent strain. This allows one to draw a corrected yield stress curve. The correction factor will decrease as $d^{-\frac{1}{2}}$ becomes smaller, because of the decreasing amount of Lüders strain. In the case where there are no grain boundaries—that is, a single crystal—a correction factor will be required if there is some kind of barrier which can adequately substitute for the missing grain boundaries.

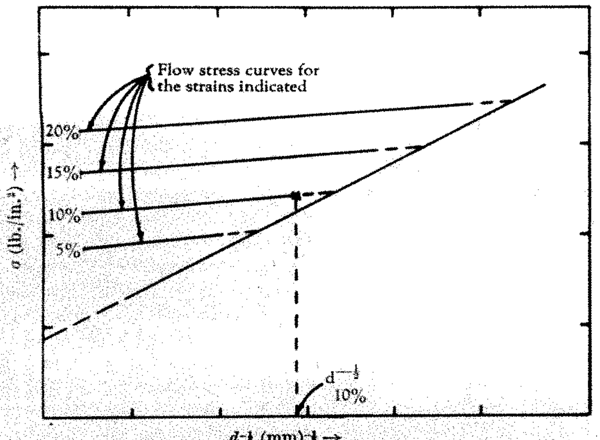


Fig. 2. Schematic illustration of nominal lower yield stress and flow stress at various strains versus grain diameter $(2d)^{-\frac{1}{2}}$

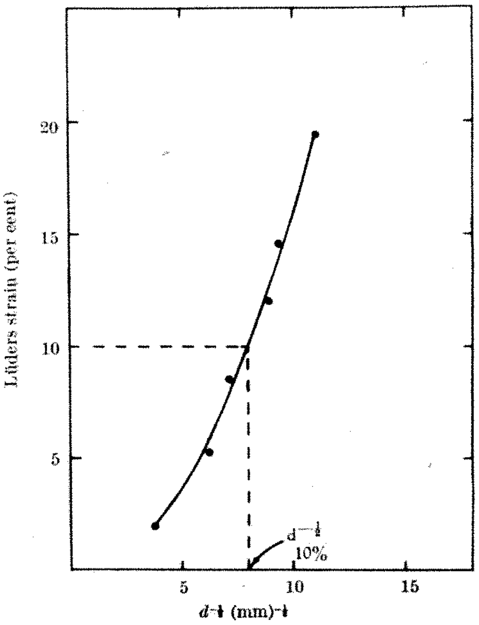


Fig. 3. Lüders strain versus grain size $(2d)^{-\frac{1}{2}}$ for Armco iron at −45° C. Data taken from ref. 3

Some recent results[2,7] support the presence of a discrepancy between nominal lower yield stress and flow stress; but it would be desirable to conduct a series of carefully controlled experiments in order to obtain quantitative values.

It would be anticipated that the suggested correction would be relatively negligible where the Lüders strain is small, but that it could be significant in certain circumstances. In the case of Armco iron at −45° C (Fig. 3), for example, the slope of the σ_y versus $d^{-\frac{1}{2}}$ curve would be increased after making the correction; whereas at room temperature in the same material, where the Lüders strain is much smaller and less grain size dependent[3], the slope would not be affected to nearly the same degree. Thus one's interpretation of the effect of temperature on k_y would necessarily be modified.

The foregoing method allows some idea of the effective lower yield stress to be obtained without resorting to complex analytical techniques. The initial steep slope observed at the completion of yielding may be due, in part, to the removal of the stress concentration factor when the Lüders front disappears. A complicating factor which could be present is that the nominal lower yield stress curve represents the propagation of different numbers of Lüders bands at the different grain sizes. It is known that for a given strain rate the yield stress decreases as the number of bands participating in the lower yield extension increases[8]. For sufficiently precise determinations, however, the stress associated with a fixed number of bands can be determined and used to plot the nominal lower yield stress versus $d^{-\frac{1}{2}}$ curve.

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C. P. SULLIVAN

Advanced Materials
Research and Development Laboratory,
Pratt and Whitney Aircraft,
North Haven, Connecticut

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CHEMISTRY

A New Continuous Solid-fluid Contacting Technique

A NOVEL technique for continuous countercurrent processes involving contact between particulate solids and fluids has been developed here. This has been carried out on a small-scale apparatus using several different systems from which the essential features of the technique have been verified. The technique is directly applicable to operation on an industrial scale as well as in the laboratory, and has wide potential application in the processing industries.

In its most general sense the technique is a method of contacting a particulate solid phase in countercurrent to a fluid phase with the view of achieving transfer of some soluble component from one phase to the other. The essential novelty of the idea lies in the use of a periodically modulated flow rate of fluid to a cascade of contacting stages by which solid is transferred in a direction countercurrent to the nett flow of fluid. The only necessary property of the system for the technique to be operable is that the dispersion of solid in fluid settles in a reasonably short time. During one part of the operating cycle, fluid flows through a series of fluidized or expanded beds of solid, during another part of the cycle, reversing the direction of fluid flow transfers equal increments of the settled solid beds between adjacent stages in a direction countercurrent to the nett flow of fluid.

Unique advantages of the proposed technique are the reliability and flexibility of operation. Positive control of both solid and fluid flow rates is achieved by direct control of external flows to the contactor, and does not require a fine hydrostatic balance or close tolerances in construction of the equipment. Although maximum flow rates are determined by the actual size of the stages, it is possible with any given unit to obtain a ratio of solid flow rate to fluid flow rate varying from zero to infinity by simple adjustment of the controls.

The main characteristics of the proposed technique may be illustrated by considering the results of one of the laboratory experiments on an ion exchange system.

In this experiment a 0·1 M aqueous solution of cæsium chloride tagged with cæsium-137 was passed at an average flow rate of 33·3 ml./min through a cascade of four 20-mm diameter contacting stages in countercurrent to cationic ion exchange resin ('Zeokarb 225', particle size −14+52 mesh, hydrogen form) moving at an average flow rate of 4·0 ml. of settled resin per minute. The concentration of the solution leaving was found by radiochemical analysis to be 0·004 M in cæsium, giving a cæsium recovery of 96 per cent. A McCabe–Thiele diagram drawn for this system gave the same recovery of cæsium for 3·5 ideal stages. Hence the stage efficiency was about 85 per cent. Details of the modulated flow-cycle are illustrated in the series of sketches, Figs. 1 *a*–*c*, which show the phenomena occurring in the stages corresponding to the instantaneous value of liquid flow rate outlined in Fig. 2. The stages shown in the figures are simplified for clarity and do not represent optimum designs.

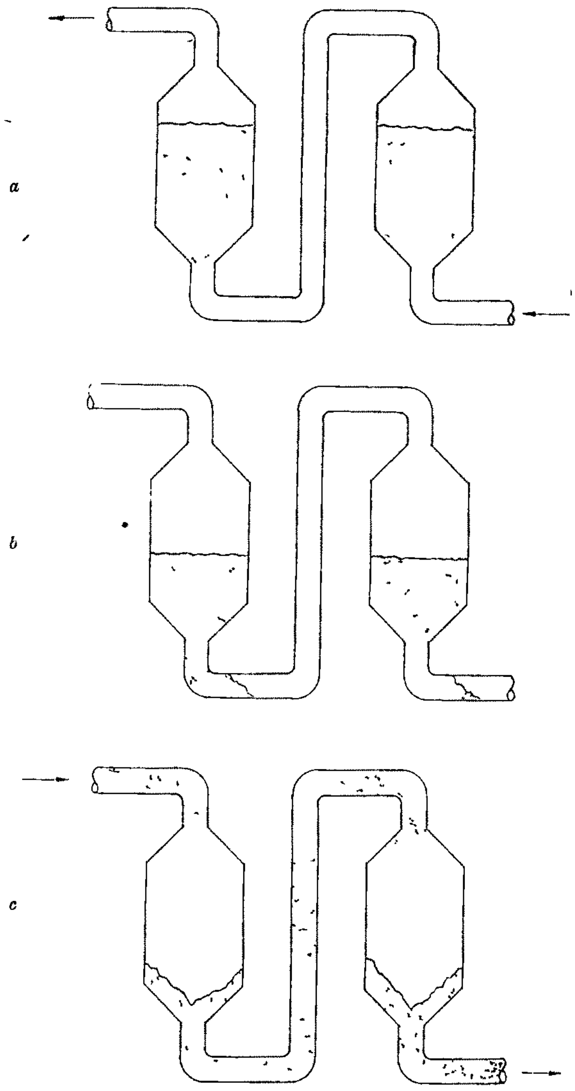

Fig. 1. *a*, Liquid upflow, expanded beds, *b*, no liquid flow, settled beds, *c*, reversed liquid flow, solids transfer

From the brief description given here the technique may be seen to be a hybrid between a series of dense phase moving beds and a multi-stage fluidized bed carried out in specially designed stages. Both the multi-stage fluidized bed and the dense phase moving bed are familiar concepts in the processing industries[1] and require no further description here. There may be an apparent similarity between this concept and the "pulsed sieve tray column" evolved from Swinton and Weiss's original idea in the use of pulsations in the liquid feed[2,3]. However, Swinton and Weiss merely superimposed a high-frequency low-amplitude vibration in the liquid feed to a multi-stage fluidized bed with conventional downcomers in order to promote the ability of the solid to flow. In the proposed technique the fluid feed is controlled in a manner such that the expanded or fluidized beds are settled, and then transferred between stages in the condition known as dense phase flow[1].

The potential industrial applications of this technique are considered to be very wide due to the extreme sim-

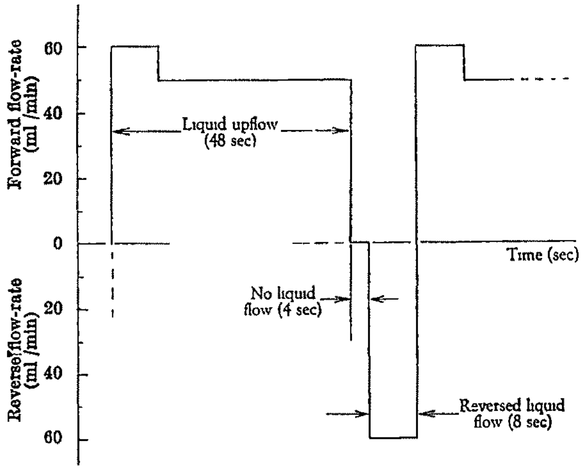

Fig. 2. Flow cycle. Average liquid flow-rate, 33.3 ml./min, average solid flow-rate, 4.0 ml./min.

plicity of its operation and applicability to construction of large-scale equipment.

In the liquid–solid field one could apply this technique to leaching of ores, purification of water, ion exchange recovery of metallic ions and chromatographic separation. For gas–solid systems the scope is more limited, as in most applications this technique would appear to have few economic advantages over a conventional multi-stage fluidized bed, although it would be equally feasible in all cases. In the processing of radioactive or highly corrosive materials this technique would have advantages over alternative methods owing to its simplicity and adaptability for remote operation.

F. L. D. Cloete
M. Streat

Department of Chemical Engineering
and Chemical Technology,
Imperial College of Science
and Technology,
London, S.W.7

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Use of a Digital Computer for Evaluating Ultracentrifuge Data

Fujita and MacCosham[1] have integrated Lamm's differential equation[2] for the ultracentrifuge, and have given analytical solutions which describe the integral and differential forms of the solute boundary for the sedimentation of a two-component system. They suggest that their equations may be of practical value in sedimentation analysis, but until recently the use of these equations for the routine estimation of molecular weights has not been possible because of the time required to perform the necessary calculations. This limitation no longer applies if the arithmetic is carried out by digital computer, and this communication shows how the equations can be used to evaluate apparent molecular weights from centrifuge data that would normally be treated by the method of Archibald[3]. The limitations inherent in the method arise chiefly from the assumption of Fujita and MacCosham that the sedimentation and diffusion coefficients, S and D, of the solute are independent of concentration. This may restrict the usefulness of the method to a particular class of solutes.

Fujita and MacCosham's differential equation for the gradient of concentration below the meniscus reads:

$$\frac{dc}{dr}=\frac{C_0}{r_0}\frac{e^{-\tau}}{\varepsilon}\left\{\left[1-\Phi\left(\frac{z+\tau}{2(\varepsilon\tau)^{\frac{1}{2}}}\right)\right]\left(2+\frac{z+\tau}{\varepsilon}\right)\exp(z/\varepsilon)-\frac{2}{\sqrt{\pi}}\left(\frac{\varepsilon}{\tau}\right)^{\frac{1}{2}}\exp-\left[\frac{(\tau-z)^2}{4\varepsilon\tau}\right]\right\} \qquad (1)$$

where $\tau = 2\omega^2St$ (ω = radial velocity of rotor, t = time), $\varepsilon = 2D/r_0{}^2\omega^2S$ (r_0 = meniscus distance from the centre of rotation), $z = 21 \log r/r_0$ (r = distance from centre of measured dc/dr), c_0 = initial concentration of solute, $\Phi(x)$ = error function defined as $2/\pi^{\frac{1}{2}}\int_0^q \exp(-q^2)dq$.

If values of dc/dr are measured in an experiment at two values of r within the boundary region at a given time of sedimentation and substituted in (1), the constants τ and ε here can be determined by solving the two resulting simultaneous equations. Then from τ and ε the coefficients S and D, and hence[4] the molecular weight, M, can be calculated.

For this purpose the computer is programmed to solve equation (1) by iteration, using a data tape on which are printed the two experimental values of $(dc/dr)_1$ and $(dc/dr)_2$ at r_1 and r_2, respectively, the time of sedimentation, and the various other parameters set out here. To begin the iteration τ is set to an initial value, τ_0, by choosing an arbitrary value of S such that $S > S_{\text{actual}}$. The other constant, ε, is set equal to unity initially, and then the computer calculates from equation (1) the apparent value of dc/dr, say $(dc/dr)_{1\text{app}}$, for $r = r_1$, and for these values of τ and ε. It then compares $(dc/dr)_{1\text{app}}$ with the experimental value $(dc/dr)_1$, and if the two values differ by more than 10^{-5} it decreases ε by successive chosen increments until agreement is reached. In this way a value of ε say ε_1, is obtained for the data at r_1 for τ_0. Next the calculation is repeated, but this time using the experimental value $(dc/dr)_2$ at r_2 to obtain a value ε_2 for $\tau = \tau_0$. The values of ε_1 and ε_2 are now compared, and if $|\varepsilon_1 - \varepsilon_2| > 10^{-4}$, the whole calculation is repeated with successively smaller values of τ until $|\varepsilon_1 - \varepsilon_2| \leqslant 10^4$. When this has been achieved, values of τ and ε, and hence D and S, have been found which satisfy equation (1) for the data at r_1 and r_2 to within the prescribed limits.

The programme has been tested using data obtained from ultracentrifuge experiments on sucrose. This substance was chosen because its molecular weight and diffusion constant[5] are known accurately, and the effect of concentration on S and D is minimal. A Spinco model E analytical ultracentrifuge fitted with an RTIC temperature controlling mechanism set at 20° C was used to examine a solution of 'AnalaR' sucrose (1.39 per cent) in a 30 m/m cell, with a second cell as counter-poise containing water to produce a base line to the schlieren pattern. The rotor speed was 50,740 r.p.m. and photographs were taken at intervals of approximately 16 min.

The mean molecular weight, sedimentation and diffusion coefficients together with their associated errors, are shown in Table 1. The molecular weight obtained was approximately 0.5 per cent lower than the true value (342), but the uncertainty of the estimate, as indicated by the standard error, was ± 2.7 per cent of the mean figure. The estimates of S and D were erratic and higher than the accepted values[5] ($D = 4.57 \times 10^{-6}$ cm sec^{-1}, $S = 0.24 \times 10^{-13}$ sec^{-1}), showing that the main factor controlling the shape of the differential and integral boundaries is the ratio of these two coefficients rather than their absolute values. It must be remembered that in calculating S from τ the value of the time t is an assumed one, and some inaccuracy may be introduced here due to uncertainty in making allowance for the period of acceleration. This in turn affects the calculation of D from the ratio S/D. Further experiments will be required before any comment can be made on the magnitude of the standard errors of these results. The computer programme will be available to others as required, and it is hoped that this will allow a rapid test of the method.

Table 1 Molecular weight, sedimentation (S) and diffusion coefficients (D) of sucrose, determined from pairs of co-ordinates taken from five exposures Allowance was made in the exposure time for the period of acceleration to 50,740 r p m , r_0 = 6 074 cm

Corrected exposure time (sec)	r_1 (cm)	r_2	(dn/dr)$_1$*	(dn/dr)$_2$*	Mol wt	S/D (cm^{-1})	S (×10^{13}) (sec)	D (×10^6) (cm sec^{-1})
665	6 094	6 162	0 897	0 462	322 8	5 26	0 47	8 9
1,625	6 100	6 160	0 930	0 759	386 0	6 29	0 58	9 2
2,590	6 100	6 232	0 869	0 424	320 5	5 22	0 36	6 9
2,590	6 100	6 144	0 869	0 660	322 2	5 25	0 23	4 3
3,035	6 100	6 208	0 945	0 528	349 1	5 69	0 31	5 4
4,025	6 110	6 224	0 885	0 451	341 0	5 55	0 22	3 9
				Mean	340 3	5 54	0 36	6 4
				S E ±	9 4	0 13	0 05	0 85

* dn/dr = refractive index gradient × tan θ, where θ = angle of schlieren phase-plate

It is essential in this method of molecular weight assessment to measure co-ordinates with a high degree of precision Thus the frequently used procedure of tracing an enlarged image on to graph paper is insufficiently accurate, and a micrometer method must be used In the present experiment the parameters were measured using a digital scanner ('Oscar', Benson and Lehner, England) which automatically prints the data in a form suitable for immediate insertion into the computer The use of this device greatly reduces the time required for accurate measurement, and in the present case each exposure required only about 1 min for measurement The digital computation took an average of 6 sec on a Ferranti *Atlas* computer

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S P Spragg

Department of Chemistry,
University of Birmingham

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Asymmetric Synthesis of α-Amino-acids by the Strecker Synthesis

THE non-enzymatic asymmetric synthesis of α-amino-acids has long been of considerable stereochemical interest However, the method of catalytic hydrogenation is the only one appearing in the literature[1-8] In this communication, a new method of asymmetric synthesis of α-amino-acids in solution is described

The method involves (*a*) the reaction of an aldehyde with hydrogen cyanide and D-(–)-α-methylbenzylamine ($[\alpha]_D^{27} = -40\ 6°$ in benzene) in methanol, (*b*) hydrolysis of the resulting amino nitrile to the *N*-(α-methylbenzyl) amino-acid, (*c*) catalytic hydrogenolysis to remove the methylbenzyl group A typical example of the asymmetric synthesis is as follows sodium cyanide, 1 47 g (0 03 mole), and D-(–)-α-methylbenzylamine hydrochloride, 4 73 g (0 03 mole), were dissolved in 5 ml of cold water To this, 20 ml of methanol and 1 0 g of free D-(–)-α-methylbenzylamine were added Then, acetaldehyde, 1 32 g (0 03 mole), was added to the cold methanolic solution The clear solution was kept at room temperature for five days The solvent was evaporated *in vacuo* The residue was dissolved in 50 ml of 1 N hydrochloric acid and the solution was extracted twice with ether To the aqueous solution was added 12 N hydrochloric acid to adjust the normality of the acid to approximately 5 N The solution was then refluxed for 6 h The hydrochloric acid was evaporated *in vacuo* and the residue was dried over sodium hydroxide under reduced pressure The crude *N*-(α-methylbenzyl) alanine hydrochloride was dissolved in 200 ml of 50 per cent alcohol and the *p*H was adjusted to 6 0 with sodium bicarbonate To this solution, 3 5 g of palladium hydroxide catalyst[8] was added Hydrogenolysis was carried out for 10 h to remove the methylbenzyl group The catalyst was filtered and washed with hot water The combined filtrate was evaporated to about one-third volume and acidified with dilute hydrochloric acid to about *p*H 1 The solution was evaporated to dryness and the alanine hydrochloride was extracted from the dry residue with absolute ethanol three times (total 50 ml) The solution was kept at –5° C overnight to precipitate the contaminated salt After filtration, pyridine was added to the alcoholic solution to precipitate crude alanine Yield, 0 71 g $[\alpha]_D^{27} = +10\ 01°$ in 6 N hydrochloric acid c = 2 18, m p 235°–243° dec A single spot was observed following paper chromatography The crude alanine which contains salt was dissolved in 2 5 ml of water, the *p*H was adjusted with pyridine to 5 5–6 0, and 10 ml of absolute ethanol was added A yield of 0 45 g of L-alanine was obtained (17 per cent) $[\alpha]_D^{27} = +13\ 13°$, in 6 N hydrochloric acid, c = 2 32, asymmetric yield, 90 per cent, m p 290° dec Anal calcd for $C_3H_7NO_2$: C, 40 44, H, 7 92, N, 15 72 Found C, 40 53, H, 8 07, N, 15 78

The intermediate *N*-(α-methylbenzyl)alanine was isolated using the reaction conditions here After hydrolysis and evaporation of the hydrochloric acid, the residue was extracted with absolute ethanol Pyridine was added to the solution and the ethanolic solution was kept at 5° C for 2 days to complete the precipitation of *N*-(α-methylbenzyl) alanine A yield of 0 85 g (15 per cent) of material was obtained m p 277° dec , $[\alpha]_D^{27} = -41\ 96°$ in 6 N hydrochloric acid, c = 1 91 The product was purified by sublimation[8] (0 1 mm mercury at 200° C), 0 68 g of pure *N*-(α-methylbenzyl) alanine was obtained m p 281° dec , $[\alpha]_D^{27} = -43\ 9°$ in 6 N hydrochloric acid c = 2 15, anal calcd for $C_{11}H_{15}NO_2$ C, 68 37, H, 7 82, N, 7 25 found C, 68 21, H, 7 81; N, 7 26 Hydrogenolysis of this gave L-alanine, $[\alpha]_D^{27} = +13\ 99°$ (95 per cent optically active)

The mechanism of the asymmetric synthesis has not been clarified However, the synthesis may involve several sterically controlled reactions[9]

The Strecker synthesis of amino-acids has been considered as one of the modes of formation of amino-acids in the primordial Earth[10-12] The asymmetric synthesis described here is therefore interesting in connexion with the formation of optically active amino-acids on the primitive Earth This type of asymmetric synthesis is under further investigation

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Kaoru Harada

Institute for Space Biosciences
and Department of Chemistry,
Florida State University,
Tallahassee, Florida

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Molecular Formula and Degree of Unsaturation

A RELATION stated without proof or reference by Soffer[1] deserves to be better known. Ideas more or less equivalent to it seem to be widely known to organic chemists, but workers in related fields may find the result useful when dealing with organic compounds. This communication presents a proof, together with some remarks on interpretation and use (supplementary to Soffer's remarks).

For a start, consider a molecule the molecular (numerical) formula of which is known, and the atoms of which can safely be regarded as covalent with predictable valencies. Thus we can calculate the sum V of the valencies of all the atoms. The number of bonds in the structure must be $\frac{1}{2}V$ because each bond satisfies two valencies.

But if there are N atoms then as few as $N-1$ bonds are sufficient to link them together—one bond would serve to link two atoms, a second bond would allow a third atom to be added, and so on.

If this were fewer than $\frac{1}{2}V$, then each of the extra bonds must link atoms which are already linked either directly or indirectly in the molecule. If indirectly, then such an extra bond completes a ring; if directly, it gives us a double bond (or increases the order of a multiple bond). Always we get what Soffer calls a "cyclic element of structure". (A triple bond is to be counted as two cyclic elements.) Thus the number of these cyclic elements is predicted as the number of 'surplus' bonds

$$\rho \text{ (say)} = \tfrac{1}{2}V - N + 1 \qquad (1)$$

Also 2ρ is the number of added hydrogens required to saturate the molecule (eliminating all bond-duplications and rings) because each pair of hydrogens eliminates one of the 'surplus' bonds.

(1) We see that two molecules have the same ρ-value whenever their molecular formulæ are identical (isomeric); or when the formulæ differ only by multiples of CH_2, NH and/or O, because these combinations contribute zero to $(\frac{1}{2}V - N)$.

(2) Equation (1) may be written

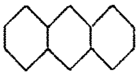
$$\rho = \tfrac{1}{2}\Sigma v n_v - \Sigma n_v + 1$$

where n_v is the number of atoms of valency v, and rearranged to give Soffer's form

$$\rho = 1 + \tfrac{1}{2}\Sigma n_v(v - 2) \qquad (2)$$

(that is, $\rho = 1 + \frac{1}{2}(2n_4 + n_3 - n_1)$ if no higher valencies are present). This gives a quicker calculation if it can be remembered.

(3) Soffer's definition, "in counting the number of cyclic structures in a polycyclic system no cyclic structure is counted if it is made up entirely of elements of other cyclic structures which themselves have been counted", can give ambiguous results in some cases. Thus tetradecahydroanthracene, $C_{14}H_{24}$, gives $\rho = 3$ and has indeed three six-membered rings



but could be counted as having only two rings, the middle one and the 'outside'. In such a case, we must say that ρ predicts the number of bonds that must be cut to eliminate all the cyclic structures. (For formally planar structures it is sufficient to count non-overlapping rings.)

(4) The equation holds if the true structure has the atoms in their predicted covalencies, or if an acceptable model of the structure has this property. Thus aromatic nuclei may be considered as having Kekulé structures as Soffer apparently intends when he cites benzylpenicillin and strychnine. (Alternatively we may arbitrarily call each aromatic (π) electron-pair a 'cyclic structure'.) A benzene ring is a possible explanation of four units of a calculated unsaturation.

Similar considerations apply to the groups $-NO$ and $-NO_2$ (count one cycle each), but substituted ammonium is more difficult to deal with as it appears 'supersaturated' until we calculate again using valency five for the nitrogen.

(5) The equation may be generalized to a set of atoms other than a single complete molecule. By adding equations for M separate molecules we obtain

$$\rho = \tfrac{1}{2}V - N + M \quad \text{or} \quad \rho - M = \tfrac{1}{2}V - N$$

where N, V, ρ are the total number of atoms, valencies and cyclic elements in the M molecules. Here M replaces the 1 of equation (1) (or (2), similarly). On the other hand, we must subtract $\frac{1}{2}$ from the same figure for each valency either not satisfied (as in a free radical), or not satisfied within the set of atoms (such as the monomeric unit of a dimer or polymer, or 'structural units' of coal[2]).

DAVID A. LAWS

Dominion Physical Laboratory,
Lower Hutt,
New Zealand

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Near Ultra-violet Absorption Spectra of o-, *m*-, and *p*-Fluorophenol Vapour

THE absorption spectra of *o*-, *m*-, and *p*-fluorophenol in vapour phase have been examined with a Hilger E_1 large quartz spectrograph. A number of plates were taken with different cell lengths and at temperatures ranging from 26° C to 60° C, while the temperature of the reservoir containing the substance varied from −15° C to 60° C. The bands in each case appear well defined with the cell of length 70 cm maintained at temperature 26° C. A hydrogen lamp was used as the source of continuum for the ultra-violet region. Ilford special rapid N 40 plates were used to photograph the spectra in about half an hour.

Nearly 300 bands have been photographed in each case. The intense bands are double headed, similar to those observed in the case of phenol. The mean doublet separation in all the three spectra is found to be of the order of 10 cm^{-1}.

o-Fluorophenol. This molecule belongs to symmetry class *Cs* to a first approximation and its absorption spectrum is due to the electronic transition ${}^1A' \leftarrow {}^1A'$. The most intense band at 36,802 cm^{-1} has been chosen as the (0, 0) band, and the entire spectrum analysed in terms of the frequencies 239, 415, 566 and 766 cm^{-1} in the ground state and 291, 506, 718, 945, 1,040 and 1,128 cm^{-1} in the excited state. The ground state frequencies correspond fairly closely to the known Raman frequencies.

m-Fluorophenol. The *meta*-derivative of the molecule can also be taken to belong to the symmetry class C_s and the bands may be attributed to the electronic transition ${}^1A' \leftarrow {}^1A'$ which is an allowed transition. The intense band at 36,620 cm^{-1}, which is observed with still greater intensity at low temperature, is taken to be the (0, 0) band. The entire spectrum is analysed in terms of two ground state frequencies, 427 and 741 cm^{-1}, and six excited state frequencies, 146, 206, 254, 713, 976 and 1,175 cm^{-1}.

p-Fluorophenol. The *para*-derivative belongs to C_{2v} symmetry class approximately, and the observed spectrum involves the electronic transition ${}^1A_1 \leftarrow {}^1B_1$, which is allowed with the transition moment lying in the molecular plane and perpendicular to C–OH bond. The most intense band at 35,132 cm^{-1}, which appears with increased intensity at lower temperatures, has been taken to be the (0, 0) band. The bands have been analysed in terms of three ground state frequencies 251, 466 and 865 cm^{-1}, and seven excited state frequencies, 356, 413, 569, 838, 875, 1,205 and 1,273 cm^{-1}. Satisfactory correspondence of the ground state frequencies has been found with the Raman frequencies of the molecule

A comparison of these spectra with those of phenol and of the corresponding chlorophenols has revealed many interesting features These are under investigation with spectrographs of higher dispersion and resolution. A detailed investigation of these spectra and their analyses will be published shortly

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S K TIWARI

Department of Spectroscopy,
Banaras Hindu University,
Varanasi-5

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Surface-active Characteristics of Sodium Salts of Anacardic Acids of Varying Unsaturation

CASHEW nut (*Anacardium occidentale* Linn) shell liquid chiefly consists of anacardic acid, an *ortho*-carboxymeta-alkyl phenol. Earlier workers[1–3] have shown that the unsaturated alkyl side-chain in the acid consists of 15 carbon atoms and that the acid is a mixture of saturated and mono-, di-, and tri-olefinic compounds, in proportions that give, on average, about two double bonds per molecule Properties of the sodium salt of the whole anacardic acid have been reported earlier[4–6] However, considerable interest remains in the examination of surface-activity unsaturation relationship in the anacardic acid series The work recorded here is an extension of previous work[5] on whole anacardic acid.

In the present investigation, low-temperature fractionation and chromatographic resolution were used as tools for segregating anacardic acid into fractions Anacardic acid with iodine value 124 7 (as determined by the method of Kale and Kamath[7]) and corresponding to 1 689 double bonds per molecule (on the basis of iodine value of mono-olefinic anacardic acid which is 73 82) was taken as the starting material and was separated into five different fractions *A*, *B*, *C*, *D* and *E* having their iodine values 50 66. 103 00, 125 80, 140 90 and 162 4, respectively

For evaluating comparative surface-active properties, tetrahydro-anacardic acid (THA) with zero iodine value (obtained by hydrogenating anacardic acid in alcoholic medium in presence of platinum catalyst, followed by repeated crystallization from ligroin) and the fractions *B* (nearly mono-unsaturated acid), *D* (nearly di-unsaturated acid) and *E* were chosen

The acids were separately converted into disodium salts[5] and their various surface-active properties were examined

(*a*) Surface tension at 30° C (determined by Du Nouy's tensiometer) of 0 01 per cent solutions of the disodium salts of THA and of the fractions *B*, *D* and *E* were 31 2, 41·0, 43 0 and 45 0 dynes/cm respectively

(*b*) While the interfacial tension of water against *n*-heptane was 39 39 dynes/cm at 30° C (determined by the Stalagmometer method), 0 01, 0 02, 0 05 and 0 10 per cent sodium tetrahydroanacardate in aqueous solution lowered the values to 24 33, 14 33, 8 53 and 4 79 dynes/cm respectively The values for fraction *B* were 28 38, 15 81, 5 18 and < 1 00 dynes/cm and those for fraction *E* were 37 38, 25 85, 12 38, 8 73 dynes/cm respectively

(*c*) The critical micellar concentration of the fractions *B*, *D* and *E* as determined by the conductance-concentration curve method (for details, see previous communications[5]) were 0 032, 0 052 and 0 06 per cent solution

(*d*) Emulsification test (with *n*-heptane) and foam-height experiments were conducted according to procedures already outlined[6] The volumes of emulsified layers after shaking 15 ml of 0 1 per cent solutions of sodium salts of THA and of the different fractions with 5 ml of *n*-heptane for 1 h were 5 4 ml , 5 6 ml , 5 5 ml , and 5 4 ml respectively Foam heights 1 min after the complete discharge of the foregoing solutions were 12 4, 14 8, 14 5 and 15 2 cm respectively.

The results presented here reveal that whereas at low concentration, namely, 0 01 per cent, saturated- or tetrahydroanacardic acid is most surface-active, surface activity of unsaturated products is more manifest at comparatively higher concentrations The rule that critical micellar concentration increases with unsaturation in a homologous series[8] is found valid in the sodium anacardate series

As unsaturation increases, emulsifying and foaming characteristics undergo a slight increase and then a slight fall Neither the saturated nor the most unsaturated anacardate gives the best surface-active and bulk properties, optimum hydrophilic–lipophilic balance lies near the mono- and di-unsaturated anacardate The whole anacardic acid as such is a good surfactant as shown before[5,6], its unsaturation falls more or less in the optimum hydrophilic–lipophilic balance zone

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ARUN K BISWAS*
ANIL B RAY†

Department of Applied Chemistry,
92 Acharya Prafulla Chandra Road,
Calcutta, 9

* Present address Indian Institute of Technology, Kanpur

† Present address Department of Pure Chemistry, University College of Science, Calcutta 9

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Thermal Decomposition of Benzyl Borate

IN recent years, the mechanism of the thermal decomposition of esters has been thoroughly investigated, and at the present time extensive evidence has been presented to support a *cis* elimination pathway[1] A similar mechanism was also suggested to explain the pyrolysis of borate esters[2–4], however, the recent work of Chapman and Borden[5] has disclosed the carbonium ion nature of this decomposition

In an investigation of the secondary decomposition mechanisms in the pyrolysis of esters, the degradation of the borate of benzyl alcohol was attempted This carbinol is of some interest, since it cannot undergo the normal elimination reaction The experimental method developed by O'Connor and Nace[3] was used in the decomposition A mixture of benzyl alcohol (5 00 g, 0 046 mole) and boric acid (2 85 g, 0 046 mole) was slowly heated to 140° The mixture was then kept at this temperature for 2 h After this time, the temperature of the heating bath was slowly raised to 300°–310° Decomposition was complete after 1 h The crude products were then dissolved in benzene and chromatographed on alumina Elution with benzene–hexane afforded anthracene (0 860 g, 21 per cent), and a yellow gum (2 73 g)

In contrast to the borate, the thermal decomposition of benzyl acetate[6] and benzoate[7,8] has been investigated and the major products of these decompositions are benzaldehyde, toluene, and the corresponding anhydride There is some evidence of a bimolecular decomposition in these esters[8,9] with the formation of benzyl ether as an intermediate Although very little is known about the pyrolysis of these esters, it is evident that the borate and benzoate of this alcohol pyrolyse via different reaction pathways

GERALD E RISINGER

Department of Chemistry,
Louisiana Polytechnic Institute,
Ruston, Louisiana

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Polarography of Cadmium Transfusion–Gelatin Mixtures

PROTEIN solutions, generally used as maxima suppressors in polarographic work, are known to cause abnormal decrease in the diffusion current of metal ions due to factors such as adsorption[1,2], viscosity[3] and metal protein interaction[4–6] The effect of increasing concentration of serum albumin on the limiting current of cadmium ion has been systematically investigated by Tanford[4], who explained the marked decrease in the current in view of cadmium-protein complex formation Investigations in this direction have not, so far, been undertaken in the case of gelatin, although the latter has been most widely used as the maximum suppressor To meet this end, transfusion gelatin, a modified product of bone gelatin, with its well-characterized nature (in terms of hydrogen ion equilibria[7] and mol wt [8]) was chosen and the effect of factors like *p*H, metal and protein concentration on the limiting current of cadmium was investigated

Chemically pure samples of cadmium sulphate, potassium chloride, ammonium chloride, ammonia and transfusion gelatin (kindly supplied by the Director, National Chemical Laboratory, Poona) were used to prepare solutions in conductivity water and the metal content determined gravimetrically[9] Ammoniacal as well as Walpole acetate buffers were made from 0 1 M solutions and their *p*H measured by Beckman *p*H meter model *G* Polarographic measurements were carried out with a Fisher electropode in conjunction with a Multiflex galvanometer type MGF_2 in the external circuit, using an H-shaped polarographic cell[4], triply distilled mercury (for D M E) and a water thermostat (Townson and Mercer, Croydon) maintained at 25° ± 0 1° C Capillary characteristics determined by Lingane's method[10] were

$$m^{2/3}t^{1/6} = 1\ 87,\ h = 52 \text{ cm and length of capillary, } l = 15 \text{ cm}$$

Nitrogen gas (purified by passing through alkaline pyrogallol and chromous chloride solution) was used to maintain the inert atmosphere The diffusion currents were measured by extrapolation method The mixtures prepared were (1) containing 0 333 mM Cd^{++}, ammoniacal buffer (*p*H 9 6), potassium chloride (to maintain ionic strength, $\mu = 0\ 15$) and varying concentrations of protein, namely, 0, 0 30, 0 40, 0 60, 0 65, 0 71, 0 75, 1 00, 1 20 and 1 35 per cent, (2) containing 2 5 per cent protein, buffer (*p*H 9 6) potassium chloride ($\mu = 0\ 15$) and different concentrations of Cd^{++} (2 664, 2 331, 1 998, 1 665, 1 332, 0 992, 0 666 and 0 499 mM), (3) containing 1 0 per cent protein, 0 333 mM of Cd^{++}, buffers of different *p*Hs 11 0, 10 0, 8 50, 6 8, 6 35, 5 95, 5 85, 4 50, 4 01, 3 80, 3 65, 3 00 and 2 65 and potassium chloride ($\mu = 0\ 15$) A set similar to (2) was also prepared without protein The reversibility of the waves was checked as described by Kolthoff and Lingane[3] Values of diffusion currents, *id* and *ido*, in presence and absence of the protein are given in Fig 1 (plots of *id*/*ido* versus *p*H, metal or protein concentrations) The limiting value of *id*/*ido* from the three curves (*A*, *B*, *C*) came out to be 0 73

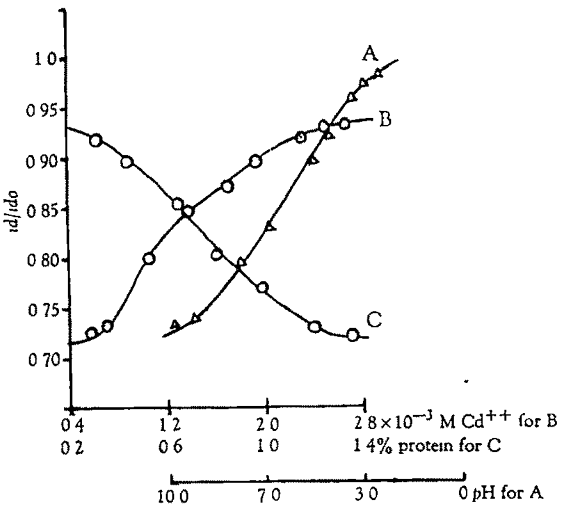

Fig 1 Effects of *p*H, metal ion and protein concentrations on diffusion current depression Curves *A*, *B* and *C* represent respectively the effects of *p*H, metal ion and protein concentrations on diffusion current decrease of cadmium ion at a total ionic strength of 0 15

From Fig 1 it is evident that the ratio *id*/*ido* decreases with increase in *p*H and protein metal ratios and attains a limiting value (not zero), showing thereby that non-specific forces like viscosity and adsorption are not responsible for the considerable decrease in the diffusion current in the case of cadmium Therefore, the metal-protein interaction is the only way to account for these observations Furthermore, the fact that it did not vary exactly as $\sqrt{h}$ shows that the limiting current is not entirely diffusion controlled[11] and a process slower than diffusion is involved in the reduction of cadmium ion bound to transfusion gelatin

The values of V_M calculated at different *p*Hs by the help of Tanford's equation were found to be approximately 2 at *p*H 8 5, where imidazole groups (in addition to carboxyl ones) offer principal sites[8] for metal ion binding Since the value of V_M at *p*H 5 8 is 1, it may be concluded that one cadmium ion is bound to the protein through its imidazole group Similar results have been reported by Tanford[4] and Gurd[12] in the case of serum albumin The value of log *K* calculated from Scatchard's[13] equation comes out to be 3 03 and $-\Delta F$ = 4 118 kcal/mole (at Cd^{++} concentration 0 333 mM)

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WAHID U MALIK
SALAHUDDIN

Chemical Laboratories,
Aligarh Muslim University,
India

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BIOCHEMISTRY

Role of Deoxyribonuclease in Synthesis of Deoxyribonucleic Acid

ALTHOUGH a considerable amount of work on DNA synthesis has been done in recent years in different laboratories, the exact mechanism of DNA replication in a living cell is not known even to-day. It is also not known whether or not a DNA molecule as a whole, with 10,000 nucleotides, or a part of it or smaller fragments of it, acts as a template for DNA or RNA synthesis.

Based on the observations that concentrations of both DNase activity and DNA are considerably higher in the liver of young rats than normally found in the same tissue of 2–10 months old rats, Devi *et al.*[1,2] suggested a possible role of DNase in synthesis of DNA. The recent reports on the ability of DNase 1 to increase the rate of mitosis in sea-urchin eggs[3] and DNA synthesis in many crude enzyme systems[4,5] led us to investigate the effects of DNase and sonications on the priming efficiency of two-stranded and heat-denatured DNA in DNA-synthesizing systems, previously described by us[6]. In this connexion, it should be mentioned that we[6] have recently found that two-stranded DNA acts more efficiently than heat-denatured DNA as a primer in the system where soluble extract of young or hepatectomized rat liver is used as the source of polymerase; when a soluble extract of old rat liver is used, heat-denatured DNA acts best as a primer. The results of our investigation on the role of DNase in DNA synthesis are reported in this communication.

It is known that if DNA is exposed to the action of different amounts of DNase for the same length of time, it undergoes degradation without denaturation to smaller fragments of various chain lengths having different molecular weights[7,8]. Similarly, when DNA is exposed to sonic vibration it also undergoes degradation without denaturation. In this way, a series of homologous DNA samples was prepared. The samples all differ in molecular weight[7] but retain double-helical structure, so far as can be judged from the shape of their melting point curves. They appear to be identical to the melting point curve of two-stranded, undegraded calf thymus DNA.

The action of DNase on DNA synthesis was examined by adding different amounts of DNase to the assay system before the addition of high-speed supernatant fraction of rat liver. The conditions of the experiment and the method of the preparation of soluble extract are similar to those previously described by us[5,6] and briefly mentioned at the bottom of Tables 1–3. In the second series of experiments, addition of DNase was omitted and all the samples of DNA, obtained after exposing the native, two-stranded DNA to sonication, were added to the assay system to act as primers. The assay method involves the incorporation of ^{14}C-TMP into primer DNA. The results obtained are summarized here.

The results in Table 1 show that addition of DNase to the assay system leads to an increase in the incorporation of ^{14}C-TMP into added DNA. This effect of DNase is observed when either of the two DNA's, two-stranded DNA or heat-denatured DNA, acts as a primer. This effect of DNase can also be noted when the soluble extract, prepared from either young or old rat liver, is used, for example, as the source of polymerase. It is, however, of considerable interest to note that two-stranded DNA, which acts as a poor primer in old rat liver enzyme system, exhibits even greater priming activity than heat-denatured DNA in the same system if the measurements are carried out in the presence of 20 μg of DNase. It can also be seen from our results that when heat-denatured DNA acts as a primer much smaller quantities of DNase are needed. If the concentration of DNase is increased further, the extent of incorporation of ^{14}C-TMP into fragmented DNA falls rapidly.

Table 1. EFFECT OF DNASE ON THE INCORPORATION OF ^{14}C-THYMIDINE INTO TWO-STRANDED AND HEAT-DENATURED DNA, CATALYSED BY SOLUBLE EXTRACTS OF YOUNG AND OLD RAT LIVER

Amount of DNase added (μg)	Soluble extract of 3–4 weeks old rat liver: Two-stranded DNA	Soluble extract of 3–4 weeks old rat liver: Heat-denatured DNA	Soluble extract of 10–12 months old rat liver: Two-stranded DNA	Soluble extract of 10–12 months old rat liver: Heat-denatured DNA
	Counts/min/ml.		Counts/min/ml.	
0	2,272	808	832	1,640
10	2,196	2,288	1,688	3,448
20	4,288	1,556	3,208	2,008
50	448	688	144	280
100	168	364	—	—

Conditions of the experiment: the incubation mixture contained in a volume of 0·4 ml., μm.moles each of *d*ATP, *d*CTP, *d*TTP and 2-^{14}C thymidine (10,000 c.p.m.), 2 mM of mercaptoethanol, Mg-acetate 8·0 μmoles, *tris* acetate 75 μmoles (*p*H 8·5), 350 μg of calf thymus DNA as indicated, Ca^{++} 25 μmoles, 2·5 mg of enzyme protein and 0·1 ml. water; incubation time 1 h at 37° C. DNA polymerase assay was carried out by the disk procedure (ref. 3).

Table 2. EFFECT OF SONIC VIBRATION ON THE PRIMING EFFICIENCY OF TWO-STRANDED DNA IN THE PRESENCE AND ABSENCE OF DNASE IN DNA-SYNTHESIZING SYSTEMS INVOLVING THE USE OF SOLUTE EXTRACT OF YOUNG AND OLD RAT LIVER

Time of sonication (sec)	Soluble extract of young rat liver: Without DNase	Soluble extract of young rat liver: With DNase 20 μg	Soluble extract of old rat liver: Without DNase	Soluble extract of old rat liver: With DNase 20 μg
	counts/min/ml.		counts/min/ml.	
0	2,346	4,163	620	1,994
30	2,674	3,218	790	2,554
60	2,853	5,800	1,200	2,674
120	3,340	2,656	1,888	2,220
180	2,116	1,973	985	720

Conditions of the experiment were the same as given at the bottom of Table 1.

If the priming efficiency of the various homologous samples of DNA, obtained when the latter was exposed to sonic vibration for different lengths of time, are compared it becomes evident that DNA, exposed to sonication for only 2 min, can act best as a primer; in other words, maximum incorporation of ^{14}C-TMP into this DNA occurs. The molecular weight of DNA, exposed to sonication for only 2 min, is approximately one million. These results are illustrated in Table 2 and demonstrate that if DNA is first subjected to the action of sonic vibration and then the incorporation of ^{14}C-TMP into DNA is measured in the presence of DNase, a further increase in the incorporation of carbon-14 is noted. This increase in the incorporation of carbon-14 is observed only if the time of exposure to sonic vibration is less than 2 min and when the measurements are carried out in the presence of only 20 μg of DNase or less. If the concentration of DNase or the time of exposure to sonication is increased, the incorporation of ^{14}C-CMP into fragmented DNA drops rapidly. Such a drop in incorporation of carbon-14 is also observed when DNA is exposed to sonication for more than two minutes.

All this information seems to suggest that degraded DNA with an approximate molecular weight of one million, and having a double-helical structure, can act best as a primer in DNA synthesis. It is conceivable that in a living cell the DNA, possessing a molecular weight of six millions or more, at first undergoes a limited degradation, thus resulting in the release of smaller fragments with appropriate chain-lengths of molecular weight of one million. These act as primers in DNA synthesis thereafter. The assignment of such a role to DNase *in vivo* cannot be ruled out, simply on the ground that influenza virus DNA loses its genetic activity if it is acted on by a small amount of DNase which does not even produce any change in its viscosity[9]. Contrary to this, Veldhinsew *et al.*[10] have recently reported that the transforming principle of T_4 DNA remains unaffected even when it is subjected to the action of DNase, so long as the molecular weight of the fragmented DNA does not drop below 50,000 (5×10^5). When these results are considered together with the findings of Devi *et al.*[1,2], mentioned earlier, regarding the DNA content and DNase activity in the liver of rats of different ages, one is led to conclude that DNase should have some specific role in DNA synthesis. If stepwise breakdown of hydrogen bonds between base pairs of the two polynucleotide chains of DNA and its synthesis occur simultaneously[11,12], it is at least conceivable that such a process becomes feasible and can occur more rapidly if fragmented DNA instead of undegraded DNA acts as a primer. If our efforts succeed in demonstrating the retention of biological activity by such fragmented

Table 3. PRIMING EFFICIENCY OF DNA PRESENT IN RAT LIVER NUCLEI AND CALF THYMUS NUCLEOHISTONE, CATALYSED BY SOLUBLE EXTRACT OF YOUNG OR OLD RAT LIVER

Source of DNA	Soluble extract of young rat liver (3–4 weeks old) counts/min/ml.	Soluble extract of adult rat liver (10–12 months old) counts/min/ml.
Rat liver nuclei (prepared at *p*H 7·0 in 0·44 M sucrose medium containing 0·004 M Ca^{++})	2,250	820
Nucleohistone (without heating)	2,475	860
Nucleohistone (after heating at 100° C for 10 min)	750	1,680

Conditions of the experiment were the same as given at the bottom of Table 1, the only difference being that instead of purified DNA either rat liver nuclei or calf thymus nucleohistone was added as a primer.

DNA's, then it will be possible to imagine that in a living cell DNA is first degraded by the action of DNase to proper chain lengths and acts as a primer for DNA or RNA synthesis thereafter.

We have also found that DNA present in rat liver nuclei, prepared from calf thymus in 0·25 M sucrose medium containing 0·004 M Ca^{++}, and nucleohistone, prepared from calf thymus, can also act equally well as a primer in DNA-synthesizing systems, although the extent of ^{14}C-TMP incorporation is much less than that which can be obtained when two-stranded DNA, degraded by sonic vibration, acts as a primer in the presence of DNase, under the best conditions of experiment. It is therefore clear from the results of Table 3 that histone cannot prevent, to any measurable extent, the synthesis of DNA. Bonner *et al.*[13] reported that DNA of pea embryo chromatin, complexed with histone, was inactive in the support of DNA-dependent RNA synthesis. In rat liver nuclei or calf thymus nucleohistone this does not seem to be the case, as DNA in them can act freely and efficiently as a primer in our DNA-synthesizing system. Sarkar and Dounce[14] previously reported that calf thymus nuclei exhibit very high proteolytic activity. It is therefore possible that histone, part of the DNA–histone complex, undergoes rapid hydrolysis, releasing the DNA to act as a template in DNA synthesis.

All the foregoing results clearly indicate that DNase does have a definite role in DNA synthesis.

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N. K. SARKAR
M. A. MUKUNDAN
ANIMA DEVI

Departments of Biochemistry, Faculty of Medicine,
Laval University, Quebec, Canada,
and Post-Graduate Institute for Medical Research,
Chandigarh, Punjab, India.

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Heterogeneity of Human Intestinal Alkaline Phosphatase

INVESTIGATORS who have examined the distribution of alkaline phosphatase after starch-gel electrophoresis of extracts of human small intestine have found the main enzyme activity concentrated in a single, rather diffuse zone in the haptoglobin region, with occasionally one or two minor, slow-moving components also present[1-4]. Chromatography on substituted cellulose columns has, however, revealed considerable heterogeneity in the main phosphatase peak of some intestinal preparations[5,6]. In order to resolve this apparent discrepancy between the results with the two techniques, intestinal phosphatase fractions obtained by column chromatography have now been compared directly with those seen on starch gels.

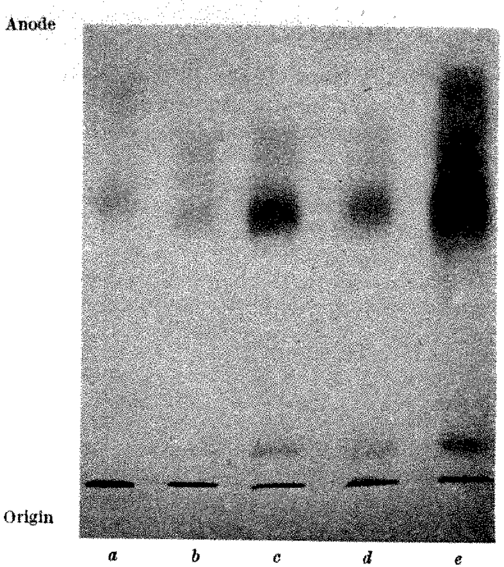

Fig. 1. Alkaline phosphatase zones after starch gel electrophoresis of intestinal extracts: *a*, autolysed for 4 months; *b*, *c*, *d*, butanol extracts of duodenum, jejunum and ileum; *e*, butanol extract of small intestine stored for 4 months

Extracts of specimens of human small intestine obtained within 24 h of death were made by Morton's butanol method[7]. Phosphatase was eluted from columns of DEAE-cellulose by an increasing concentration of chloride ion in *tris* buffer[5]; 3-ml. fractions were collected and

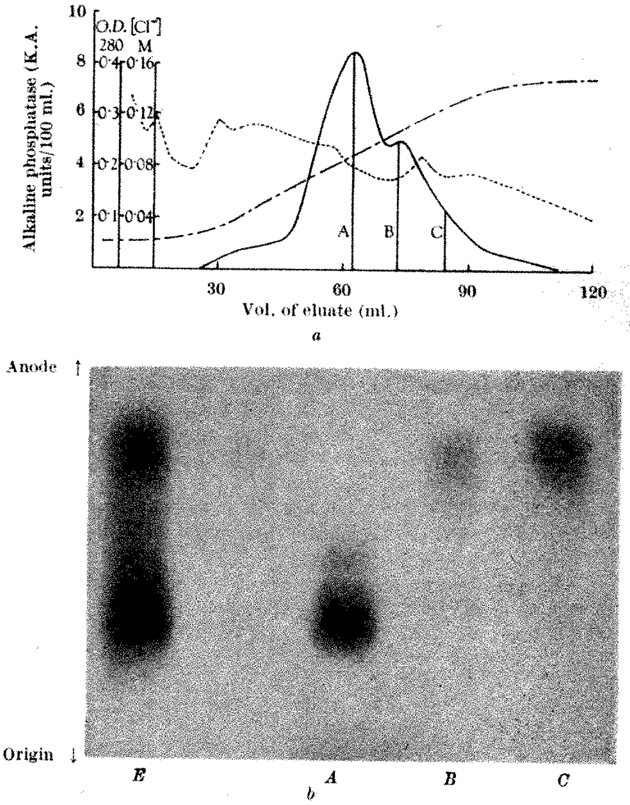

Fig. 2. *a*, Chromatogram of fresh butanol extract of small intestine on DEAE-cellulose column. (——, Phosphatase activity; ......, optical density at 280 mμ; —·—, chloride concentration); *b*, Starch-gel electrophoresis of column fractions *A*, *B* and *C* and whole extract *E*

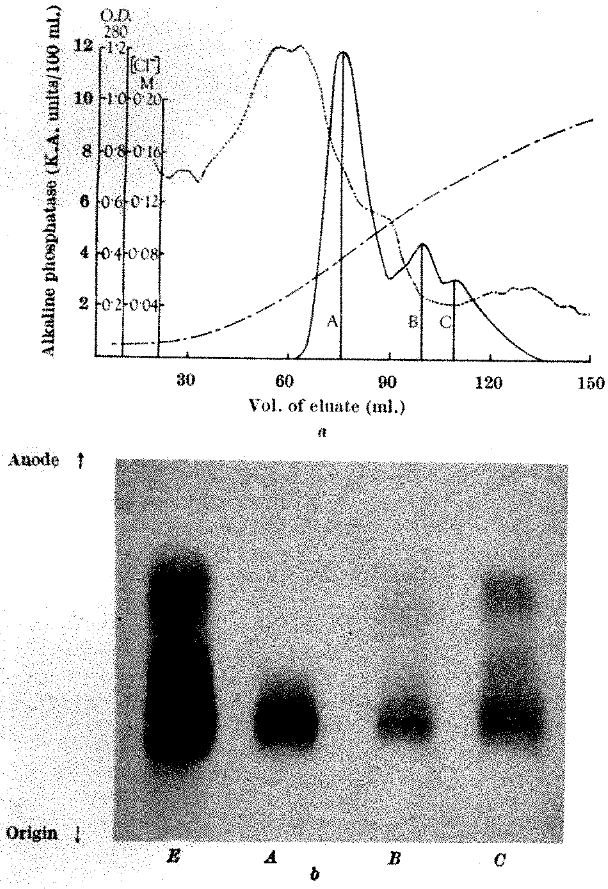

Fig. 3. *a*, DEAE-cellulose chromatogram of butanol extract stored for 4 months at −20° C (key as for Fig. 2*a*); *b*, Starch-gel electrophoresis of column fractions *A*, *B* and *C* and whole extract *E*

alkaline phosphatase activity, optical density at 280 mμ and chloride concentration measured in each. Horizontal starch-gel electrophoresis was carried out in the discontinuous buffer system of Poulik[8] and was continued until the visible junction of the two buffers had moved some 16 cm past the sample slots; the leading edge of the phosphatase zones was then about 10 cm from the origin. Phosphatase bands were located on the gel by the method of Estborn[9].

The single, broad phosphatase band previously found[3] was resolved into at least two components by the extended electrophoretic run (Fig. 1); these components maintained their relative mobilities when re-run on starch gel. In fresh butanol extracts the slower of the two main zones was stronger, the ratio of the relative activities in the slower and faster zones (determined by eluting the enzyme from the gel) varying between 2 : 1 and 4 : 1 in different preparations. Tissues taken from different segments of the intestine all showed these two bands, but no correlation was found between the relative proportions of the bands and the anatomical site of the specimen. When fresh butanol extracts of small intestine were chromatographed, the electrophoretically slower zone was eluted from the column first, the faster zone appearing as a shoulder on the main enzyme peak (Fig. 2). A third active component of intermediate mobility could also be detected eluting with the slower zone.

During storage at −20° C for about 4 months the electrophoretic pattern of the phosphatase in butanol extracts changed, the mobility of the faster zone increasing and the presence of a third zone becoming more apparent (Fig. 1). The chromatographic pattern of the stored extract was similarly more complex (Fig. 3). Prolonged autolysis of an intestinal homogenate (4 months at 4° C) also increased the mobility of the faster zone (Fig. 1), but in this sample the intermediate zone was less apparent.

The several alkaline phosphatase fractions which can be separated by chromatography or electrophoresis may represent structurally different, but functionally similar, enzymes. They may, however, be derived from a single enzyme; different enzyme molecules may remain attached during extraction to other substances, with which they are associated as a result of differences in intracellular localization, or the enzyme molecule may be partly modified after death or during the extraction process. If post-mortem modification of the phosphatase molecule is involved the process might be expected to go to completion during prolonged autolysis, one component vanishing if all the enzyme is degraded. Though there were alterations in electrophoretic mobility of the phosphatase components in the homogenate allowed to autolyse for 4 months, they did not change very strikingly in relative intensity.

When eluted from 'Sephadex *G*–200' with 0·9 per cent saline the phosphatase activity in the tissue extracts appeared as a single, smooth peak with no resolution into separate bands; the different mobilities on starch gel would therefore appear to be due to differences in charge rather than in molecular size.

D. W. Moss

Department of Chemical Pathology,
Postgraduate Medical School of London,
Ducane Road,
London, W.12.

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Gamma-Hydroxybutyrate, a Normal Brain Metabolite

THE finding that γ-aminobutyric acid undergoes an omega transamination to succinic semialdehyde in brain[1] and the subsequent description of a specific dehydrogenase for succinic semialdehyde[2,3] indicated a complete pathway parallel to the Krebs cycle in the brain, which by-passed α-ketoglutarate dehydrogenase—a slow step in the cycle[4]. It was reported in the initial investigation, which demonstrated succinic semialdehyde as an intermediate, that this compound could also effect the oxidation of reduced DPN in brain homogenates, or in the presence of crystalline lactic dehydrogenase. The most likely product of this reaction would be γ-hydroxybutyric acid (1).

$$\text{succinic semialdehyde} + \text{DPNH} \rightleftharpoons \gamma\text{-hydroxybutyrate} + \text{DPN} \quad (1)$$

We have found this new intermediate in extracts of brain tissue of rat, pigeon and man. Brain tissue was ground with 5 per cent trichloroacetic acid and extracted exhaustively with ether. The ether extract was saponified with 1 normal alkali to hydrolyse esters, anhydrides, and lactones. It was then acidified to 2 N in hydrochloric acid and heated to 100° overnight to reform lactones. The lactones were extracted three times with two volumes of ether and the concentrated ether extract was neutralized carefully to prevent saponification and treated with hydroxylamine in dilute alkali[5] to form the hydroxamic acids of the lactones. After the addition of five volumes of methanol to precipitate inorganic salts, the clear methanolic solution of hydroxyamic acids was subjected to ascending paper chromatography in several solvents. The spots were brought out with a spray of ferric chloride. A single major spot was found which corresponded to standard γ-hydroxybutyrohydroxamic acid. Mixtures of unknown with standards gave homogeneous spots. Table 1

Table 1. APPROXIMATE R_F VALUES OF HYDROXAMIC ACIDS CHROMATOGRAPHED

	Acetyl radical of hydroxamate				
Solvent system	Brain extract	γ-OH-butyryl	γ-OH-valeryl	Acetyl	Carbamyl
Butanol, water saturated	0·45	0·45	0·60	0·52	0·19
Phenol, water saturated	0·65	0·65	—	0·68	—
Isobutyric acid, water saturated	0·50	0·50	—	0·55	0·52
Butanol/isopentanol/formic acid/water, 2:2:1:3	0·32	0·32	—	0·40	0·21
Butanol/formic acid/water, 3:1:3	0·40	0·40	0·55	0·50	0·32

gives R_F values of the unknown and standards in various solvents.

The amount of γ-hydroxybutyrate in brain tissue was estimated by comparing the recovery of standard material carried through the same procedures. Rat brain is approximately 1–2 millimolar in γ-hydroxybutyrate and a human brain (no central nervous system disease) taken 3 h *post mortem* was 0·3 millimolar.

The successful use of the sodium salt of γ-hydroxybutyrate in anæsthesia has been reported by Laborit *et al.*[6]. We have confirmed this effect in mice and rats (500 mg/kg IV) and humans (100 mg/kg IV). In all species sleep begins within 15 min and lasts for about 90–120 min. Preliminary tissue investigations in rats show that the sleep period corresponds to an increment of γ-hydroxybutyrate in brain of about 2 millimoles/kg and lasts as long as this level is exceeded. This corresponds to an initial blood-level of about 3 millimolar. Preliminary data on human beings show sleep to occur while the blood-level exceeds 1 millimolar.

The significance of these findings that a normal brain intermediate in relatively small doses can produce sleep is being investigated.

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SAMUEL P. BESSMAN
WILLIAM N. FISHBEIN*

University of Maryland School of Medicine,
Baltimore, 1.

* Trainee in neurochemistry, grant 2*B*-5246, U.S. National Institute of Health.

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Lipase Formation by Micro-organisms grown on Hydrocarbons

WE have been investigating the utilization of hydrocarbons by micro-organisms, and have already described formation of amino-acids from kerosene by a fermentation process[1]. On the other hand, it is reported[2] that some authors have been examining the production by micro-organisms of feed supplements, fatty acids and other oxidation products from hydrocarbons. Recently, we investigated whether some enzymes are formed by micro-organisms grown on hydrocarbons as a sole carbon source, and have found that extracellular lipase is formed by 5 strains out of 26 micro-organisms tested.

Cultures were grown in the basal medium containing 2·5 per cent kerosene, 0·2 per cent urea (or 0·3 per cent NH_4NO_3) and mineral salts ($MgSO_4 \cdot 7H_2O$ 0·1 per cent; K_2HPO_4 0·1 per cent; Na_2HPO_4 $12H_2O$ 0·05 per cent; KH_2PO_4 0·05 per cent; $FeSO_4$ 0·002 per cent, $MnSO_4$ 0·001 per cent), at 26·5° C on the reciprocal shaker. Though the amounts of lipase formed by those strains are small (Table 1), it seems that fungi which utilize hydrocarbons all show lipase. Lipase activity in the cultured broth was determined by Nord's method modified according to K. Yamada and Y. Ota[3]. One unit is the amount of enzyme which liberates 1 μequiv. acid/min.

Table 1. LIPASE-FORMING STRAINS AND UNITS OF LIPASE FORMED

Strains	Period of cultivation (days)	Nitrogen source	Lipase activity (unit/ml.)
*S*169*M*1 (mould-like)	7	urea	0·4
*S*190*M*3 (mould-like)	7	urea	0·35
*S*192*Y*1 (yeast-like)	2	NH_4NO_3	0·6
*S*200*M*1 (mould)	3	NH_4NO_3	0·3
*S*209*M*1 (mould)	7	urea	0·85

Table 2. EFFECTS OF 'TWEEN-20' AND OLIVE OIL ON LIPASE FORMATION BY *S*192*Y*1 STRAIN

Medium	Lipase activity (unit/ml.)		Period of cultivation	
			2 days	3 days
Basal			0·55	0·4
Basal	'Tween-20'	0·005 per cent	0·95	0·4
Basal	'Tween-20'	0·05 per cent	0·4	0·25
Basal	Olive oil	0·05 per cent	0·3	0·05

Effects of 'Tween-20' and olive oil on formation of lipase by *S*192*Y*1 strain were tested. As shown in Table 2, formation of lipase seems to be slightly stimulated by adding 0·005 per cent 'Tween-20' to the basal medium, while it is prevented when 0·05 per cent of 'Tween-20' or olive oil is added.

Identification of those lipase-forming strains are yet to be made.

JOJI TAKAHASHI
YUKIO IMADA
KOICHI YAMADA

Department of Agricultural Chemistry,
Faculty of Agriculture,
University of Tokyo.

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Inhibition of Pyruvate Metabolism in Mitochondrial Suspensions by Cobalt

IN a recent publication, Strickland and Goucher[1] claim that, in brief polarographic experiments, cobalt does not inhibit the oxidation by mitochondrial preparations from rat liver of keto-glutarate, β-hydroxybutyrate or pyruvate. On the basis of these findings, the authors suggest that our observations[2] on the particular sensitivity of keto-acid oxidation to inhibition by cobalt may have been artefacts caused by prolonged incubation of the mitochondria in the absence of a phosphate acceptor.

We have confirmed that in the medium used by Strickland and Goucher[1], and in a modification of this (medium II, Table 1), even relatively high concentrations of cobalt produce no significant inhibition of pyruvate oxidation by isolated mitochondria during the first 20 min of incubation in conventional manometric experiments. Strickland and Goucher's[1] incubation medium, however, differs from that used by us not only in the presence of ADP, but also in the contents of inorganic ions, particularly orthophosphate. In our previous papers[2,3] it was emphasized that in studies of this type it is essential to control the phosphate content of the medium, since the latter limits the concentration of free, ionic cobalt that can be maintained in solution. It seems that the failure of Strickland and Goucher[1] to observe the inhibitory action of cobalt on pyruvate oxidation was due to the high (20 mM) concentration of orthophosphate in their medium. Thus, as shown by the data of Table 1, the inhibition of pyruvate metabolism is immediately apparent when the phosphate content is reduced from 20 mM (media I and II) to 8 mM (medium III). In the experiments at the lower phosphate concentration, the buffering capacity of the medium was maintained by the addition of 10 mM *tris*, *p*H 7·4. This prevented the fall-off in oxidation rate that occurred in the presence of 8 mM phosphate as the only buffer, but did not affect the inhibitory action of the cation. Under these conditions, the inhibition produced

Table 1 ACTION OF COBALT ON PYRUVATE OXIDATION BY MITOCHONDRIA FROM RAT LIVER IN DIFFERENT MEDIA

Cobalt concentration (μg/ml)	Incubation medium					
	I (ref 1)	II (Medium I with ADP replaced by 2 mM ATP, and without cytochrome *c*)			III (Medium II with the phosphate content decreased to 8 mM, and the addition of 10 mM *tris*)	IV (ref 2)
	Rate of oxygen consumption (μl /h)					
0	118	162 (162)	135 (135)	111 (77)	145 (145)	134
6			141 (119)			
10		182 (182)		123 (99)	128 (105)	85
15			138 (93)			
30	105	156 (145)	122 (87)	92 (68)	92 (60)	63
60		145 (110)		87 (60)	67 (43)	47
90			109 (63)			

Mitochondria were isolated in 0 25 M sucrose[4], washed and resuspended in 0 25 M sucrose at a density of 3 mg mitochondrial nitrogen/ml[2] Each Warburg flask contained the mitochondrial suspension (0 4 ml) and other components of the experimental system in a total volume of 2 0 ml The flasks were equilibrated for 10 min at 37° before the initial readings The values in each vertical column of the table were obtained with the same preparation of mitochondria, and are calculated from readings taken over the first 20 min of incubation Rates of oxygen consumption after 60 min are given in parenthesis

at a given concentration of cobalt was similar to that observed in our original medium (medium IV, Table 1)

In all media listed in Table 1, the presence of either ADP or ATP was essential for oxidative activity by the mitochondria, but neither the rates of pyruvate metabolism in the absence of cobalt nor the inhibition produced by the cation in media III and IV were affected by replacement of the diphosphate by the triphosphate Cytochrome *c* was unessential, and was omitted from the modification (medium II) of Strickland and Goucher's[1] medium

In the presence of 20 mM phosphate (media I and II) the low concentrations of cobalt (6–10 μg/ml) consistently gave slight stimulation of oxygen uptake, and even at concentrations of 60 μg/ml little inhibition of pyruvate metabolism was obtained until after about 20 min incubation, that is, after termination of the measurements described by Strickland and Goucher[1] Both these observations indicate that in these media the concentration of available cobalt is considerably less than that added Thus, as previously reported[2], stimulation of mitochondrial pyruvate oxidation occurs at sub-inhibitory concentrations of cobalt (less than 1 μg/ml) The delay in the onset of inhibition at the higher levels is possibly related to the reduced rate of cobalt uptake by the mitochondria that must occur if the concentration of free cation is limited by the solubility of a phosphate (complex). It seems unlikely that this delay reflects mitochondrial damage, since, in the absence of cobalt, the rate of oxygen consumption usually remained constant for at least 60 min (Table 1, media II and III)

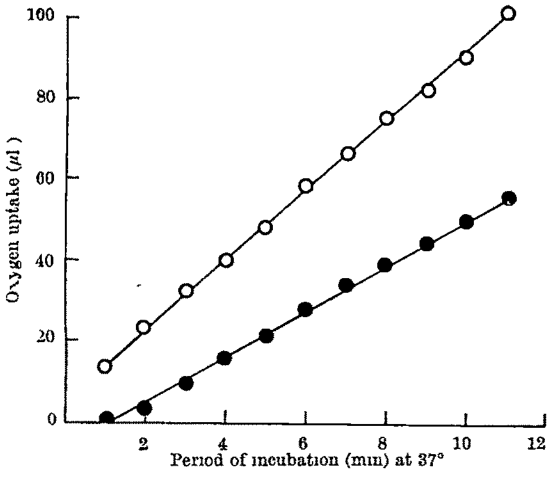

Fig 1 Inhibition of mitochondrial pyruvate oxidation by cobalt. Experimental conditions as described in the text O, control, ●, 1 μg atom Co^{2+}/ml

Certainly the criticism that the observed sensitivity of keto acid oxidation to cobalt under the appropriate conditions is an artefact due to the prolonged incubation, is invalid, since inhibition by the cation can be observed within very short periods To demonstrate this manometrically, two flasks of similar constants, both prewarmed to 37°, were used for each experiment Each contained the components of medium IV in the main compartment and 5 N sodium hydroxide (0 2 ml) in the centre well At zero time, the mitochondrial suspension (3 0 mg N/ml , 0 5 ml) was added to one flask, and 0 25 M sucrose (0 5 ml) to the other After the introduction of the filter paper strips into the centre wells, the flasks were attached to the manometers and inserted into the water bath within 40 sec, the manometer taps being closed after a further 60 sec Readings of the two manometers were taken 2 min from zero time, and at 1-min intervals for 11 min thereafter

Oxygen consumption by the mitochondria was then derived from the difference between the volume changes in the experimental and control systems The rates of oxygen uptake determined by this method were linear and reproducible Control mitochondrial suspensions consumed 8 6 μl oxygen/min This was reduced to 5 5 μl oxygen/min in the presence of 1 μ-atom cobalt/ml (Fig 1) Although the inhibition (36 per cent) observed under these conditions is less than that (53 per cent) produced by the same Co^{2+} concentration over a 20-min period (Table 1, medium IV) the results (Fig 1) leave little doubt that, provided adequate levels of free cation are maintained in solution, inhibition of keto acid oxidation by cobalt occurs at times when degenerative changes in mitochondrial organization and metabolism appear improbable

J T DINGLE
M WEBB

Strangeways Research Laboratory,
Cambridge

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Utilization of Alpha-Ketoglutarate by Red Blood Cells for Glutathione Synthesis

INVESTIGATIONS in this laboratory have indicated that glutamic-oxaloacetic transaminase activity is increased in young red blood cells Subsequent ageing is associated with a marked decrease in activity[1-3] In an attempt to provide some understanding of the possible physiological role of this enzyme in red cells, we were led to an examination of the metabolism of glutathione Sufficient information has already become available to indicate that erythrocytes possess the enzymatic machinery for synthesizing glutathione[4-6] The relatively rapid half-turnover time of glutathione, 65 and 96 h respectively in rat and human red cells[4,7], suggests that this mechanism plays some part in their metabolic economy In addition, it indicates that adequate supplies of substrate amino-acids are available to the erythrocyte In view of the relative impermeability of red cells to glutamic acid[8] there is some doubt as to the exact mechanism by which supplies of this amino-acid are made available to the red cell under physiological conditions Consideration was therefore given to the possibility that glutamic acid might be supplied through transamination of another metabolic intermediate after its entry into the erythrocyte This communication gives data indicating that α-ketoglutarate, the most obvious possibility, can be incorporated into glutathione *in vitro* by human red cells

Assay conditions for this investigation were identical to those used with rat erythrocytes[6] except for the inclusion of amino- or keto-acid labelled with carbon-14 Glutamic acid, cysteine and glycine were present at a final concentration of 0 03 M with incubation under nitrogen for 3 h at 37° C in a Dubnoff metabolic shaker The adequacy of these conditions for the promotion of glutathione synthesis[6] was confirmed, but no attempt was made to determine whether they were also optimal for human erythrocytes When α-ketoglutarate was used, it was substituted for glutamic acid at the same final concentration.

At the end of the incubation period, the red cells were washed three times with Krebs-phosphate buffer and hæmolysed in 10 ml of distilled water After the addition of 20 mg of carrier, glutathione was isolated by precipitation as the copper mercaptide[9] followed by reduction with hydrogen sulphide[4] The resultant solution was analysed for glutathione[10] and a representative portion was dried on stainless steel planchets Counting was done in a Nuclear Chicago gas flow counter with suitable corrections for self-absorption. Control experiments indicated the absence of significant non-specific radioactivity in the isolated material When estimates of red cell uptake of substrate amino- or keto-acids were desired, washings were carried out with incubation mixture devoid of radioactivity. After hæmolysis, a small portion of the hæmolysate was plated and counted directly in addition to the usual estimate of radioactivity in the isolated glutathione

Table 1. UPTAKE OF LABELLED AMINO- OR KETO-ACIDS BY RED CELLS

	Final conc in media (μM/100 ml)	Final conc in red blood cells (μM/100 ml)	
		Exp 1	Exp 2
Glycine-^{14}C	3,000	485	523
Glutamic acid-^{14}C	3,000	39	33
Alpha-ketoglutarate-^{14}C	3,000	305	213

The uptake of glutamic acid, glycine and α-ketoglutarate by erythrocytes after 3 h of incubation is presented in Table 1 The results demonstrate the relative impermeability of red cells to glutamic acid despite the high concentrations in the surrounding medium

Glutathione synthesis was estimated as the amount of incorporation of radioactive amino-acid into the isolated glutathione Quantitation was based on the specific activity of the added ^{14}C-amino- or keto-acid The results of three typical experiments comparing incorporation of each of these substrates are presented in Table 2 together with the mean values from a larger series of separate studies with each acid Although the results were somewhat variable in individual experiments, the mean α-ketoglutarate incorporation was approximately 70 per cent of that for glutamic acid The results for glycine indicate that much greater amounts of this amino-acid are incorporated This discrepancy is no doubt due to the demonstrated differences in permeability of the red cell to each of these amino-acids In all cases, synthesis was estimated from the specific activity of the amino-acid in the medium No attempt was made to correct for dilution by endogenous material While red cell uptake of glycine was sufficient to justify disregarding the negligible dilution by unlabelled amino-acid, this was apparently not true for glutamic acid In the absence of appropriate analysis, no adequate estimate of the effect of endogenous glutamate on the final specific activity appears possible However, an absolute minimum of a two-fold dilution must be involved since the uptake of labelled glutamic acid by the red cell is in the range of endogenous levels of this dicarboxylic acid reported by several investigators[11,12] The decreased incorporation of α-ketoglutarate can be explained in a similar manner Although this keto-acid apparently enters the human red cell much more easily than glutamate, since it appears in the isolated glutathione it is probably converted to glutamic acid by transamination The rate and amount of this conversion are unknown Unless transamination is extremely rapid and complete, the labelled glutamate so formed would also be extensively diluted by endogenous amino-acid

Table 2. INCORPORATION OF AMINO- OR KETO-ACIDS INTO GLUTATHIONE BY RED BLOOD CELLS *in vitro*

Exp No	Glycine-^{14}C	Glutamic-^{14}C	α-Ketoglutarate-^{14}C
		(μmoles 100 ml red cells)	
1	67 0	14 0	17 0
2	68 2	15 4	6 1
3	60 6	12 7	5 4
Mean	69 7 (16)*	16 0 (9)	10 8 (10)
range	(39–95)	(7 7–23 5)	(5 4–21 3)

* No of experiments

The present results provide experimental evidence of the erythrocyte's ability to utilize α-ketoglutarate in the synthesis of glutathione. The amount of incorporation appears to be consistent with recent results on red cell utilization of α-ketoglutarate[13] Additional study will be required to determine whether this pathway represents the normal physiological mechanism involved in glutathione synthesis Full appreciation of its physiological significance will depend, to a large extent, on the degree to which glutathione is itself further implicated in the metabolic economy of the red cell

MARTIN D SASS

Medical Research Laboratory Medical Service,
Veterans Administration Hospital,
Brooklyn 28, New York.

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Bound Carnitine in Bovine Brain

RECENT evidence for the presence of carnitine in phospholipids of yolk sac has been given by Mehlman and Wolf[1] Accordingly, it was proposed by these authors that phosphatidyl carnitine could represent a bound form of carnitine in avian tissue In contrast to the results of Strack *et al*[2] and Hosein and Proulx[3], their investigations failed to show the presence of any bound carnitine in the mammalian species In this latter case, analyses were performed in the dialysable material obtained from trichloroacetic (TCA) extracts no study was made on the TCA precipitates nor on the non-dialysable residues It is quite conceivable that carnitine could have been present, in the fractions not investigated, either bound to or as a component of phospholipid

In the work recorded here, evidence was sought for the presence of carnitine in the phospholipids of bovine brain The brains were first homogenized in cold 50 per cent aqueous acetone to extract the free carnitine After filtration, the residue was extracted for phospholipids according to a specific method by Erickson and Lands[4] This material, dissolved in a minimal amount of chloroform, was washed several times with water The chloroform was then evaporated under reduced pressure and the phospholipids were hydrolysed in 6 N methanolic hydrochloride[1] After removal of fat with ether, the aqueous phase was decolorized with 'Norit-A' A portion of the final solution, adjusted to *p*H 8 5, was treated with 5 per cent methanolic reineckate to precipitate choline Following filtration, the mother liquor was adjusted to *p*H 1–1 5 to precipitate carnitine according to a method by Bregoff *et al*[5] The reineckate precipitates, dissolved in acetone, were decomposed by using accepted methods[3,5,6] and the final solutions were brought to dryness under reduced pressure at 60° C Inorganic salts were removed by repeated methanolic extraction The material precipitated with alkaline reineckate proved to be a mixture of at least two substances with melting points of 192°–196° C and 243°–250° C Chloroaurate treatment[7] yielded two derivatives which melted at 152°–154° C and 257°–263° C In certain instances material with a melting point of 228°–232° C was also obtained which could not be further purified by washing with 1 per cent hydrochloric acid or by crystallization from ethanol The materials precipitated with acid reineckate had melting points of 143° C and 190°–194° C the chloroaurate derivative of these substances melted at 152°–154° C Pure carnitine, carnitine hydrochloride, and carnitine chloroaurate melt at 196°, 142° and 152° C, respectively Pure choline and choline chloroaurate melt at 247° C and 260°–263° C, respectively (Choline chloroaurate crystallized from acid ethanol melts at 230° C)

Further evidence for the presence of carnitine was also obtained from paper chromatography A portion of the hydrolysate was concentrated to near dryness, extracted with methanol to remove salts and the final solution was subjected to chromatography in the butanol/acetic acid/water (4 1·1) system[8] Two components were detected with iodine vapour, which corresponded to carnitine (R_F 0 22) and choline (R_F 0 40)

From these results it would appear that there is some bound carnitine in association with phospholipid fractions of bovine brain In support of this is the fact that some carnitine remains in the phospholipids even after acetone and water extractions Since carnitine resembles choline in chemical structure, it is not unlikely that this former substance is a constituent of phospholipid as was suggested by Mehlman and Wolf[1]

Investigations are now under way to estimate quantitatively the amount of carnitine in phospholipids of brain and to see if phosphatidyl carnitine could not account for at least part of the bound carnitine

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P R PROULX

Department of Biochemistry,
Faculty of Medicine,
University of Ottawa

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Selenium Content of Fresh Eggs from Normal and Dystrophic Chickens

THE biological role of selenium is as yet unknown, but the evidence accumulating from many investigations[1–13] suggests that this element is necessary in trace amounts for normal structure and function of muscle and other tissues In our investigations on the possible relation between selenium content of tissues and muscular dystrophy occurring as a hereditary disease in chickens, it seemed appropriate to determine the amounts of selenium in eggs from normal and dystrophic birds We thank Prof W Landauer of the University of Connecticut at Storrs for his collaboration

After the eggs had been weighed, albumen and yolk separated and freeze-dried, the selenium was determined by the methods previously reported[14,15]

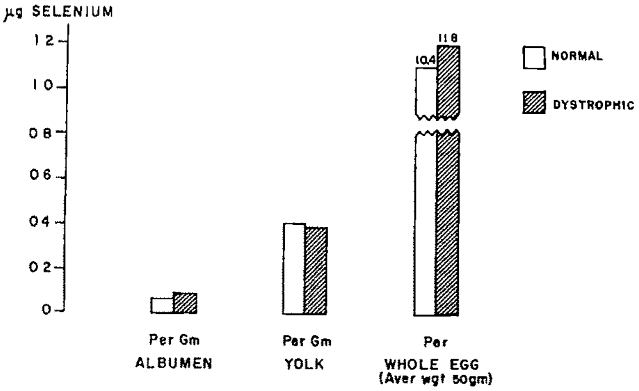

Fig 1 Selenium content of fresh eggs Distribution between yolk and albumen

The results of our investigations are shown in Fig 1 The open columns represent the arithmetic mean of micrograms of selenium per gram of fresh albumen, yoke and whole egg from normal chickens, and the striped bars show the mean values from dystrophic chickens

There was no difference in the selenium content between the eggs from the normal and dystrophic birds , each contained about 11 micrograms of selenium. However, notable and interesting differences in the distribution of the selenium within the egg were found The yolk represents only about a third of the weight of the whole egg, but it contained most of the selenium Similar results were found in all the eggs which were analysed

The presence of appreciable amounts of selenium in the egg, and especially in the yolk, may point to a necessity for selenium in the early development of the embryo Investigations to test this hypothesis are in progress

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H H TAUSSKY
A WASHINGTON
E ZUBILLAGA
A T MILHORAT

Institute for Muscle Disease,
New York

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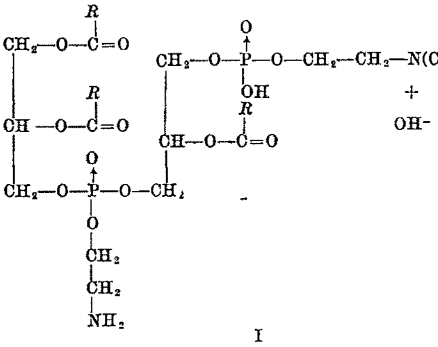
Structure of 'Complex' Phospholipids

RECENTLY, Collins has described the presence of 'complex' phospholipids in animal tissues[1,2] These compounds are shown to be very sensitive towards silicic acid and other mild hydrolytic reagents This characteristic is believed to be the reason why other workers failed to detect the 'complex' phospholipid fraction, since chromatography over silicic acid is universally utilized in the study of phospholipids. Collins defines the complex phospholipids as having a phosphate triester group and structures such as I and II are suggested; that is, the free hydroxy group on the phosphorus of a 'normal' phosphatidyl-choline, -ethanolamine or -serine is esterified with a glycerol moiety. The hydrolysis of these phosphate triesters by chromatography over silicic acid would give rise to 'normal' phosphatidyl-choline, -ethanolamine or -serine and the corresponding lyso derivatives; compounds which are identical to those which are actually obtained.



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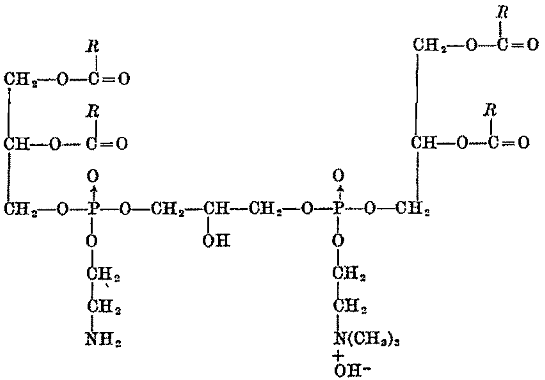

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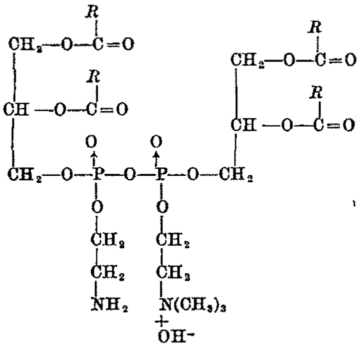

III

For two reasons the evidence described by Collins[1] is, in my opinion, more compatible with a pyrophosphate (acid anhydride) linkage in the molecule, as shown by structure III. First, phosphate triesters are, generally speaking, not unstable compounds[3,4]; pyrophosphates, on the other hand, are easily hydrolysed under moderate conditions[3,5]. Secondly, Collins[1] states that 39 per cent of the rat liver phospholipids consists of complex phospholipids. Hanahan, Dittmer and Warashina[6], who used silicic acid chromatography, found, however, only traces of lysolecithins in rat liver phospholipids. If the complex phospholipids on degradation over silicic acid were to produce lysolecithin this last quantity would be expected to be higher. Hydrolysis of a pyrophosphate structure will liberate only phosphatidyl-choline, -ethanolamine or -serine. The pyrophosphate bond has been shown to exist in proteins[7], and is present in numerous other natural substances (coenzymes). It should also be mentioned that a pyrophosphate structure was originally suggested by Fleury[8] for 'glycerophosphatogen', a substance formed on mild alkaline hydrolysis of lecithin. A rigorous proof of the existence of a pyrophosphate bond in these 'complex' phospholipids could be obtained by enzymatic methods such as those used by Perlmann[7].

A. J. DE KONING

Fishing Industry Research Institute,
University of Cape Town,
Rondebosch

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PHYSIOLOGY

Analysis of *A* and *B* Wave Interaction in the Rabbit Retina

WORKING with modified versions of Ames's[1,2] technique for maintaining a functioning but isolated rabbit retina, we have presented evidence which strongly supports the hypothesis that the prominent negative wave exhibited by this preparation is equivalent to the *A* wave of the ordinary electroretinogram[3]. In addition, we have been able to analyse this response into photopic and scotopic functions which probably correspond to the A_1 and A_2 waves identified by Heck and Rendahl[4] and others using intact animals. On occasion, the isolated retinal preparation will exhibit what appear to be both the *A* and *B* waves. (The conditions under which the *B* wave occurs are now under investigation. However, at this time we do not have enough results to offer in the form of a communication. Furthermore, at present we must rely on the spurious occurrence of the potential and this accounts for the embarrassing 60-cycle artefact.)

This communication presents results gathered on a preparation which did show both potentials when illuminated by a single 10 micro-second flash of white light. By means of a strychnine technique (unpublished data), it is possible to disassociate in time the apparent peaking of the two components involved in the *A* wave. When these components are sufficiently separated it is possible to observe the emergence of the positive potential out of each negative trough. Fig. 1 shows the effect on the described preparation of increasing the illumination. It will be noticed that at the lowest illumination-level only the A_2 or scotopic function is observable, followed by a positive *B* wave. As the intensity increases, the magnitude of the A_2 wave increases and the A_1 wave appears. Further increases in intensity bring about the emergence of a positive potential occurring later than the A_1 wave but prior to the occurrence of the A_2 wave. The last two pictures in the series show a notch in the *B* wave which corresponds in time to the A_2 wave.

It should also be noted from the results in Fig. 1 that as the A_1 wave becomes more effective in producing a positive potential the general shape or envelope of the *B* wave changes. The appearance of the two positive waves is consistent with the observations of Motokawa and Mita[5], Adrian[6] and Henkes[7], which demonstrated that the *B* wave is composed of photopic and scotopic processes. Another thing is immediately apparent: the time of the peak value of the A_2 wave appears to decrease with increases in illumination. However, a reasonably simple analysis shows that this is, more than likely, the result of differential amplitude variation between the two components. Furthermore, the slope or

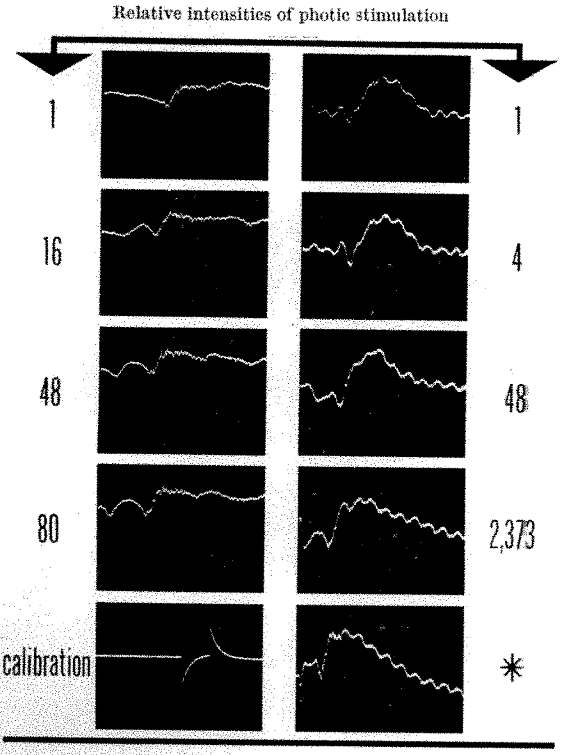

Fig. 1. Effect of increasing the intensity of illumination on the initial negative waves and the emergence of the positive potentials. The results in the left column were obtained by exposing approximately 11 per cent of the centralmost portion of the retina, and the results in the right-hand column were obtained by virtually complete exposure of the retina to photic stimulation. The relative intensities are shown in the figure. Calibration is 100/μV/cm, negative downward. The sweep speed is 10 msec/cm for the data in the left column and 20 msec/cm for the data in the right column. Time constants were set at 0·1 c.p.s. and 10 kc/s and the amplifier was a.c. coupled to the recording oscilloscope

* Preparation moved closer to light source. The relative intensity was not calculated.

rise time of the B wave being superimposed on the A_2 potential would add to the confounding of the apparent time of peaking.

Let us consider that the early part of the electroretinogram is a simple sum of the photopic and scotopic responses. They may have somewhat different onset latencies and widths so that they are disposed in such a manner that the A_1, presumably photopic, peak coincides in time with the rising slope of the A_2, probably scotopic, wave. The A_2 peak coincides with the falling slope of the A_1 wave, which is identical with the front slope of the first B wave component. Also, three further simple assumptions are consistent with recent observations on rabbit and cat retinæ: (1) the shapes of the waves are not substantially altered by increases in the intensity of illumination by white light; (2) their onset latencies change less than the apparent peak times; (3) both waves increase with intensity, but over an intermediate range the A_1 wave emerges out of the noise background and surpasses the A_2 wave amplitude. Therefore, we might say that a most significant dependent variable is the ratio (r) of the amplitude of A_1 to that of A_2.

We are interested in determining when the sum, $f(t)$, of the A_1 and A_2 time functions, $f_1(t)$ and $f_2(t)$, reaches a maximum; which is equivalent to asking when $\frac{df(t)}{dt} = f'(t)$ becomes zero. However, according to our assumptions the waves can be factored into amplitude factors, g and h, which depend on stimulus intensity and time course factors, $f_{11}(t)$ and $f_{22}(t)$, which do not.

$$f(t) = g\,f_{11}(t) + h\,f_{22}(t)$$

$$f'(t) = g\,f_{11}'(t) + h\,f_{22}'(t) = 0$$

at either of the two possible apparent peaks. Or if we consider only practical cases where g and h are both non-zero and $f_{11}'(t)$ and $f_{22}'(t)$ are not simultaneously zero:

$$\frac{1}{h} f'(t) = r f_{11}'(t) + f_{22}'(t) = 0$$

This shows that the time at which $f'(t)$ reaches zero depends on r, therefore on intensity, even though the time shapes of the individual processes do not.

From the last equation, a qualitative description can be made of the variations in the timing of the two peaks with changes in intensity. As the intensity increases both A_1 and A_2 waves should appear to peak earlier. The apparent time of the A_1 wave peak approaches asymptotically the real time of the A_1 process peak. This should serve as a reminder that changes in timing may be more apparent than real, and one must be quite careful when analysing the results to distinguish real latency changes from confounded interacting events.

As already mentioned, the pictures obtained in this series were taken under a rather extraordinary condition which was so manipulated that we were able to observe these events. We would suggest, however, that the temporal relationships of the A_1 and A_2 waves in normal preparations and their summing with each of the subsequent positive potentials may result in the double-peaked activity observed many times in the ordinary B waves[8]. Furthermore, when using chromatic stimulation the interaction would be even more complicated due to the hue-induced changes in shape of at least the A_1 wave represented in the equation as $f_{11}(t)$ (ref. 3).

E. L. Pautler
R. A. Wilson

Life Sciences Research,
Goodyear Aircraft Corporation,
Akron, Ohio.

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'Safety Valve' Effect of Excess Dietary Amino-acids and Protein on Food Intake in Hyperphagic Rats

IMBALANCES in amino-acids and excessively high-protein content of the diet (66 per cent or more) depress food intake and retard growth in rats. It has been suggested that resulting metabolic disturbances affected the mechanism of regulation of food intake. The fact that in some instances administration of insulin counteracts the growth-retarding effect of leucine excess was taken as supporting this view[1]. Going one step further, it has been suggested that blood amino-acid patterns played a crucial part in the mechanism of satiety and the regulation of food intake[2,3]. The experiments presented here were designed to show whether excess or imbalanced amino-acid and protein intake do, in fact, act on the mechanism regulating food intake or whether they limit food intake by acting through other structures. Inasmuch as it has been shown that the ventromedial hypothalamic area regulates food intake, that is, adjusts food intake to energy output[4], apparently by inhibiting constantly activated lateral 'feeding centres'[5] and exercising some measure of control over gastric contractions[6], the animals used were ten female albino rats (Charles River strain) the 'satiety' centres of which had been destroyed by electrocoagulation of the ventromedial area.

The success of the operation had been established by allowing the animals to become obese (their weight had

Table 1. Average Daily Food and Protein Intakes of Hyperphagic Rats fed High Protein Diets

Animal	Final body-wt.	Food intake (g)						Protein intake (g)					
		40%	50%	60%	70%	80%	90%	40%	50%	60%	70%	80%	90%
1	445	23·0	16·4	20·2	16·8	15·6	9·0	9·2	8·2	12·1	11·8	12·5	8·1
2	388	16·2	17·6	15·4	11·0	6·0	9·2	6·5	8·8	9·2	7·7	4·8	8·3
3	351	19·2	14·4	17·2	14·0	12·4	11·8	7·7	7·2	10·3	9·8	9·9	10·6
4	280	20·2	18·0	17·0	*	—	—	8·1	9·0	10·2	*	—	—
5	620	30·0	30·0	25·2	24·6	19·2	16·8	12·0	15·0	15·1	17·2	15·4	15·1
6	580	22·0	19·8	17·8	18·6	17·2	14·0	8·8	9·9	10·7	13·0	13·8	12·6
7	590	18·4	21·8	20·6	12·6	15·0	8·8	7·4	10·9	12·4	8·8	12·0	7·9
8	437	24·6	20·6	18·2	14·4	11·4	10·8	9·8	10·3	10·9	10·1	9·1	9·7
9	334	20·4	15·4	19·2	13·6	8·4	3·4	8·2	7·7	11·5	9·5	6·7	3·1
10	340	18·2	18·6	12·4	16·2	13·2	†	7·3	9·3	7·4	11·3	10·6	†
Average		21·2	19·3	18·3	15·8	13·2	10·5	8·5	9·6	11·0	11·0	10·5	9·4

* Negligible intake—not averaged. † Tumour developed.

Semi-synthetic diets containing 4 per cent corn oil, 0·5 per cent cod liver oil, 4 per cent salt mixture, casein as indicated, difference made up of sucrose vitamins as required (ref. 8).

gone from 275 g to an average of 562 g); the animals had then been reduced to 425 g to bring them back to the 'dynamic' phase of hypothalamic hyperphagia. They were then adapted for a period of four days to a control low-protein, semi-synthetic diet containing 9 per cent casein (*L* 1) supplemented with 0·3 per cent *d,l*- methionine and 0·1 per cent *d,l*-tryptophan. At the fourth day, protein depletion had apparently ceased, and, on the fifth day, five of the animals were put on a diet (*L* 5) in which 5 per cent of the sucrose in *L* 1 had been replaced by *l*-leucine. The remaining animals stayed on *L* 1. After five days, the two groups were switched. Food consumption and weight were recorded daily. Except for one rat, daily food intake on *L* 5 fell to 25–50 per cent of food intake on *L* 1. (Average daily intake 22·9 g/day on *L* 1; 10·3 g/day on *L* 5.) Weight was correspondingly affected. The depression of food intake by a dietary excess of leucine is thus obviously not mediated by the satiety centres.

We next explored the possibility that limitations of the ability to metabolize protein affects food intake through a structure other than the satiety centres. The 10 hyperphagic rats were placed on a series of semi-synthetic diets[7] containing 40–90 per cent casein and daily intake determined. The diets were fed five days each in the following order: 60, 40, 70, 50, 80, chow, 90 per cent. This order was chosen to preclude a progressive adaptation.

Table 1 gives the average daily intake for each animal on each diet, with the average amount of protein ingested daily. It is clear that for most animals there is a range (50–80 per cent of protein) over which the amount of protein ingested is relatively constant. In this range, which incidentally represents protein-levels in considerable excess of what would be found in natural diets, caloric intake is insufficient to maintain body-weight. Larger animals with the greater intakes at the lower protein-levels tend to be better able to maintain the limiting protein intake over a wide range of dietary protein-levels. In the 10–40 per cent protein range, protein-level does not influence intake.

The limitation of food intake in hyperphagic animals fed abnormally high protein diets so that protein intake does not exceed a limiting value is obviously independent of the presence of the ventromedial satiety centres. It differs in principle from the homeostatic control of food intake in that it does not equate energy intake to energy output. It appears to act like a 'safety valve', preventing the excess ingestion of protein rather than like a servomechanism. Limitation of food intake by amino-acid excess or imbalance may be the result of a similar phenomenon. The classic experiments of Kennedy[8] on the effect of heat on hypothalamic hyperphagic rats can be interpreted again as demonstrating a similar 'safety valve' effect for heat and dehydration.

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Ronald M. Krauss
Jean Mayer

Department of Nutrition,
Harvard School of Public Health,
Boston, Mass.

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HÆMATOLOGY

Stimulation of Factor VIII (Antihæmophilic) Activity by Transfused Serum

Patients with factor VIII deficiency and prolonged skin bleeding time (von Willebrand's syndrome) who receive fresh blood or plasma from normal donors or from patients with hæmophilia *A* with very low factor VIII activity show a much higher factor VIII activity in their plasma than could possibly be explained by the amounts given. The maximum factor VIII activity is reached 4–8 h after transfusion and persists longer than in hæmophilia *A* (refs. 1 and 2).

The prolonged skin bleeding time may be temporarily shortened. The component responsible for the factor VIII stimulation has not been distinguished from that associated with the skin bleeding time.

Evidence is presented which suggests that the two factors are different.

A 21-year-old male (D. W.) with a bleeding tendency since infancy has approximately 10 per cent of factor VIII

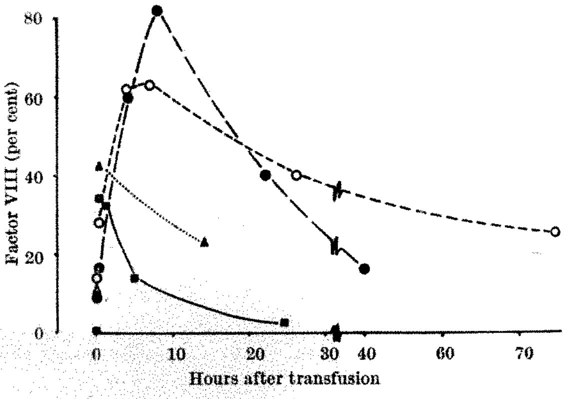

Fig. 1

Table 1

Patient	Transfusion data				Factor VIII activity				Skin bleeding time 10 min after transfusion (min)
	Material	Volume (ml.)	Factor VIII activity (*a*) (ml.)	Duration of transfusion (min)	Before transfusion (%)	Expected activity (*b*) (%)	Observed activity (%)	Time after transfusion (h)	
Von Willebrand's disease (D. W.)	Plasma (*c*)	1,000	1,000	37	14	33	62	4–8	7
	Serum (*d*)	1,100	<11	65	9	10	82	8	>20
	Plasma fraction I-0 (*e*)	180	540	15	10	27	42	14	12
Hæmophilia *A* (R. C.)	Plasma fraction I-0 (*f*)	700	1,800	54	<1	44	35	1	—

(*a*) Expressed as ml. of plasma with 100 per cent normal factor VIII activity. (*b*) Calculated on basis of simple mixing. (*c*) 9 vol. of normal blood added to 1 vol. of a solution containing 0·053 M citric acid and 0·105 M trisodium citrate, plasma obtained by centrifugation and stored at −20° C for 30 days. Thawed and warmed to 37° C immediately before use (Fig. 1, curve ○). (*d*) Blood collected without anticoagulant; kept at room temperature for 24 h, centrifuged, serum collected, stored for 24 h at room temperature and then 24 h at 2° C (curve ●). (*e*) Dry preparation from 1,650 ml. of A.C.D. plasma reconstituted (curve ▲). (*f*) As for (*e*) but from 4,900 ml. of A.C.D. plasma (curve ■).

activity in his plasma and his skin bleeding time is consistently in excess of 20 min, except after blood transfusions. His plasma volume is assumed to be 3 litres. He has been transfused successively with plasma, serum and plasma fraction I–0 (ref. 3). Factor VIII determinations in plasma and skin bleeding time were performed at intervals. The relevant results are shown in Table 1 and Fig. 1 and are compared with that of a patient (R. C.) with severe hæmophilia *A* (less than 1 per cent plasma factor VIII) whose plasma volume is assumed to be 3·5 litres. The results after transfusion observed in R. C. are typical of those seen in hæmophilia *A* in which the half-life of transfused factor VIII is between 5 and 15 h. In the case of D. W. the plasma factor VIII activity rose much higher than expected following transfusion of plasma or serum. In addition the skin bleeding time was shortened after the transfusions of plasma or of the plasma fraction I–0 but the serum transfusion did not shorten the skin bleeding time. Factor VIII activity was assayed by two independent techniques (partial thromboplastin time and thromboplastin generation), using plasma with less than 1 per cent factor VIII from hæmophilia *A* patients. Non-specific effects were excluded. It is believed the results represent true factor VIII-levels.

The observations suggest that normal plasma contains two components which are lacking in patients with von Willebrand's syndrome. The component which stimulates factor VIII activity *in vivo* is present both in citrated plasma (*p*H 7·1) stored at −20° C for 30 days and in serum. Only a low activity is present in lyophilized plasma fraction I–0. The second component which shortens the bleeding time is present in frozen plasma and plasma fraction I–0, but is absent from serum.

Potentiation of factor VIII activity occurs in normal persons and hæmophilic patients with measurable levels of plasma factor VIII immediately after exercise[4] or adrenaline infusion[5], and a rise of factor VIII activity occurs in normal persons 1–3 days after serum infusion[6]. There is no obvious relation between these observations and the reported experiments.

The clinical implications will be reported separately.

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P. Fantl

Baker Medical Research Institute,
Melbourne.

R. J. Sawers

Department of Hæmatology,
Alfred Hospital, Melbourne.

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Correlation between Rheumatic Diseases and Rh Blood Groups

We have recently determined the blood groups of 99 patients with some variety of articular disease (31 with rheumatoid arthritis, 31 with rheumatoid spondylitis, 15 with gout, 9 with disseminated lupus erythematosus, 6 with familial Mediterranean fever, and 7 with various other diseases). Statistical analyses demonstrated no significant heterogeneity among the results obtained for the different diseases. Accordingly, the data were pooled. For comparison we used Wiener's series of 1071 New York Whites[1], as in a previous investigation[2]. There is a significant correlation between the absence of the Rh antigen D and rheumatic disease, as shown in Table 1. No correlation was found between the presence of rheumatic disease and antigens C or E, or with the ABO blood group system. There did, however, seem to be a correlation with the absence of antigen N ($\chi^2_{(2)} = 9{\cdot}59$, $P = 8 \times 10^{-3}$).

Table 1

	Rhesus type		
	D+	D−	Total
Articular disease	71	28	99
Normals (New York City)	902	160	1,071

$\chi^2_{(1)} = 10{\cdot}12$, $P = c.\ 1{\cdot}5 \times 10^{-3}$.

This work was supported by grants (*H*–1076(*C*8)), (*RG*–6939(*C*4)), and *T*1 *AM* 5285(03)) from the U.S. National Institutes of Health, and a research grant (*NSF*–*G*–15082) from the National Science Foundation. One of us (M. H.) is a trainee in human genetics under grant *HTS*–5388 from the National Heart Institute. Another (W. C. B.) is the recipient of a National Institutes of Health research career award (5–*K*6–*GM*–14, 184–02).

Alan S. Cohen
William C. Boyd
Sandra Goldwasser
Edgar S. Cathcart

School of Medicine.
Boston University,

Mary Heisler

Harvard University.

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Changes in the Concentration of Potassium in the Erythrocytes and in Hæmoglobin Type in Merino Sheep under a Severe Anæmic Stress

Most breeds of sheep are polymorphic with respect to the concentration of potassium in their erythrocytes (70–90 m.equiv. K^+ (*HK*) or 10–20 m.equiv. K^+ (*LK*) per litre of red cells[1]) and hæmoglobin type (Hb A, B or AB[2]). High concentrations of K^+ (approximately 110 m.equiv./l.), which fall to normal adult levels 60–100 days after birth, are found in the erythrocytes of all fœtal lambs[3]. In the erythrocytes of normal adult sheep the mean concentration of potassium remains relatively

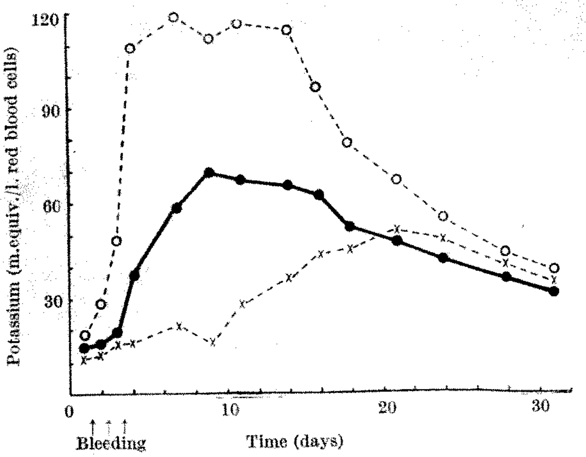

Fig. 1. The mean concentrations of potassium (m.equiv./l. of cells) from the total erythrocyte population (●) and from the top (○) and bottom (×) layers of red cells separated by centrifugation, during an experimental anæmia of a sheep

constant over long periods[4], but during an anæmic episode in *LK* animals the concentration is markedly increased[5].

This rise in the concentration of K^+ has been investigated in 8 *LK* sheep rendered anæmic by experimental loss of blood, and a significant correlation between the percentages of reticulocytes and the mean concentrations of K^+ in erythrocytes has been obtained ($r = 0\cdot8$, $P < 0\cdot001$). As young erythrocytes from man have been shown to be concentrated in the top layers of a centrifuged column of red cells and to have a higher concentration of K^+ than older cells[6], the erythrocytes from the top and bottom fractions of a centrifuged column from *LK* sheep were separated before and during an experimental anæmia. Reticulocyte counts and concentrations of potassium were determined on both fractions from nine *LK* sheep and a typical result for the changes in intracellular K^+ is shown in Fig. 1.

There was a marked rise in the concentration of potassium in the top layer of cells during the course of the anæmia, and this later also showed a very high percentage of reticulocytes as opposed to the bottom layer in which K^+ did not show a similar rise and reticulocyte counts were negative throughout.

The rise in the mean concentration of potassium in erythrocytes during an anæmic episode would therefore appear to be due to the influx of large numbers of young cells with very high concentrations of potassium.

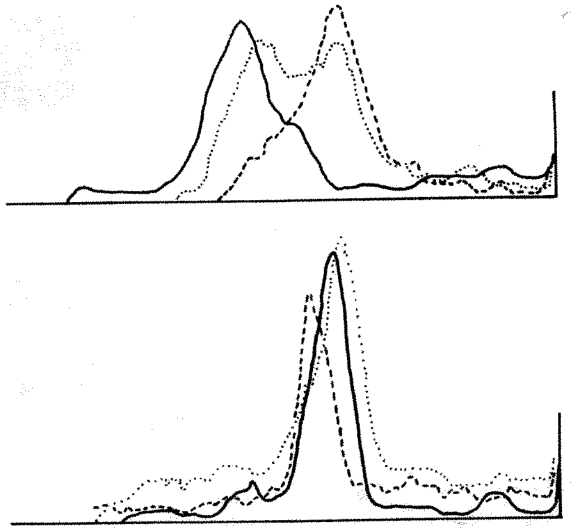

Fig. 2. Scan patterns of hæmoglobin electrophoretograms of unseparated cells (above, hæmoglobin type A; below, hæmoglobin type B) (non-anæmic control period: ———, anæmic period: ....) and separated top cells (– – – –) from sheep with hæmoglobin type A and type B

The electrophoretic patterns of hæmoglobins (*tris*-borate buffer *p*H 9·2) from aliquots of the total erythrocyte population and the top cell layers from hæmoglobin *A* and hæmoglobin *B* sheep were also examined before and during the experimental anæmia. Typical results are shown in Fig. 2. A new hæmoglobin was present in sheep with type A hæmoglobin, but in sheep with type B hæmoglobin the evidence, although suggestive, was not conclusive.

This latter response to anæmia could be due to the production of fœtal hæmoglobin and the association of the new hæmoglobin in the anæmic adult with cells with high concentrations of potassium, similar to those found in fœtal and new-born lambs, would support this. There is reason, however, to believe that it could be a new hæmoglobin, electrophoretically similar to hæmoglobin B and different to fœtal hæmoglobin, which is associated with immature red cells.

A hæmoglobin, distinct from the normal adult type and associated with immature red cells, has been suggested in the rabbit[7] and rat[8].

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M. H. BLUNT
J. V. EVANS

Department of Physiology,
University of New England,
Armidale, New South Wales.

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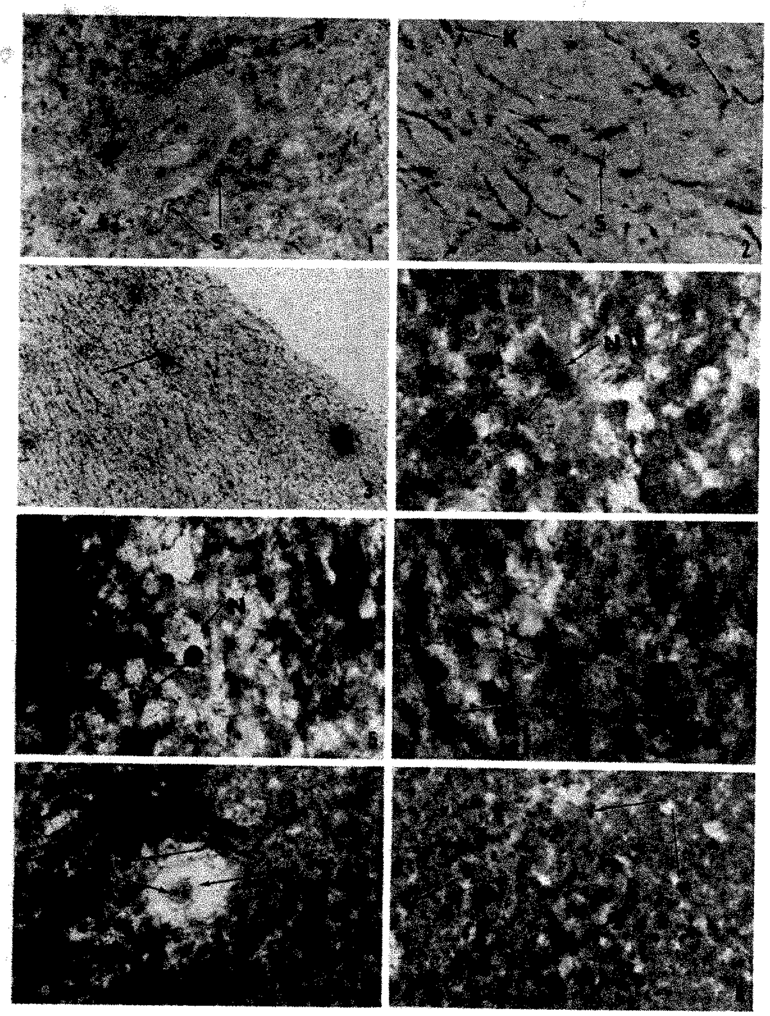
HISTOCHEMISTRY

Alkaline Phosphatases in Brain and Spinal Cord

THERE has been much controversy as to whether alkaline phosphatase is a single enzyme or whether a number of such enzymes are present in tissues. The evidence derived from inhibition and *p*H studies suggests the existence of a plurality of alkaline phosphatases[1-3]. The subject has been studied histochemically by Glick and Fischer[4], Dempsey[5], Friedenwald and Maengwyn-Davies[6] and Bourne[7]. The results of these authors also suggest a multiplicity of alkaline phosphatases. Sandler and Bourne[8] separated out a number of substrate specific phosphatases by starch-gel electrophoresis, thus again indicating the plurality of alkaline phosphatase.

In the present study, fresh frozen sections of spinal cord (15μ) and cerebral cortex (10μ) of rat were examined by the methods of Burstone[9] and Gomori[10] for alkaline phosphatase, the period of incubation being 2 and 3 h, respectively. The control tests were performed by boiling the sections before incubation and also by incubating without the substrate. Paraffin sections (10μ) have also been stained by silver nitrate[11] to demonstrate the nerve endings in the spinal cord.

With Burstone's method the neurones of the spinal cord were negative even after prolonged incubation (Fig. 1). Although the cells and their processes were negative, the synaptic regions, both on the cell surface (Fig. 1) and on the cell processes, showed a positive reaction in the form of granular deposits. The distribution of such reactions corresponded closely to the distribution of endings found by Bodian's method[11]. The blood vessels and the pia-arachnoid gave a positive reaction.



All preparations are of fresh frozen sections

Fig. 1. Alkaline phosphatase reaction (ref. 9). Neurone of the spinal cord. Note positive reaction in the synaptic regions (*S*) on the surface of the cell and around, although the cell itself is negative (× 750)

Fig. 2. Alkaline phosphatase reaction (ref. 9). The plexiform layer of the cerebral cortex. Note positive reaction in the cell processes and synaptic regions (*S*). Note also the positive knob-like synaptic areas (*K*) (× 750)

Fig. 3. Alkaline phosphatase reaction (ref. 9). Plexiform layer of the cerebral cortex. Note positive reaction in the synaptic region (arrow) (× 75)

Fig. 4. Alkaline phosphatase reaction (ref. 10). Neurone of the spinal cord. Note reaction is most intense in the nucleolus (*n*), less in the nucleus (*N*) and still less in the cytoplasm (× 750)

Fig. 5. Alkaline phosphatase reaction (ref. 10). Neurone of the spinal cord. Note intense reaction in nucleolus (*n*), mild reaction in the cytoplasm and almost negative in the nucleus (*N*) (× 750)

Fig. 6. Alkaline phosphatase reaction (ref. 10). Grey matter of spinal cord. Note positive reaction in the nerves (*P*) and their synaptic regions (*S*) (× 750)

Fig. 7. Alkaline phosphatase reaction (ref. 10). Neurone of the cerebral cortex. Note the two rounded positive bodies (*nb*) inside the nucleolus. Note also positive reaction on the cell surface probably indicating sites of synaptic areas (*S*). The nuclear wall (*N*) shows a mild reaction. The blood vessels (*B*) also show a strong reaction (× 750)

Fig. 8. Alkaline phosphatase reaction (ref. 10). Plexiform layer of the cerebral cortex. Note positive reaction in the knob-like synaptic areas (arrows) in that region (× 750)

Burstone's method gave a similar picture in the cerebral cortex. The nerve cells were almost completely negative. In spite of the absence of reaction from the cells, the synaptic regions always exhibited a positive reaction of moderate intensity, both around the cells and along the fibres (Figs. 2 and 3). The latter picture was most conspicuous in the plexiform layer of the cortex (Figs. 2 and 3). The pattern of reaction in such regions was either in the form of fine beads or larger knobs around the cells and along their processes.

A markedly different picture was observed with Gomori's method in the spinal cord. The nucleolus of each neurone always gave an intensely positive reaction; however, the nucleus and cytoplasm varied in different neurones from a faint diffuse reaction to a moderately strong one (Figs. 4 and 5). The processes of the neurones showed a positive reaction. A reaction was demonstrated also in the nerve processes forming a plexiform arrangement around the neurones, in the synaptic regions between one neurone and the processes of another and between the processes of different neurones (Fig. 6). This was also evident in paraffin sections of the spinal cord. Some neurones showed patches of intensely positive reaction in their cytoplasm, which may indicate the presence of the enzyme in lipofuscin pigments.

In the cerebral cortex the enzyme was located by Gomori's method in the nucleolus, nucleus and cytoplasm of the cells. The reaction was most intense in the nucleolus in the form of fine granules distributed unevenly. It was interesting to note the presence of round positive bodies, which varied in number, in the nucleolus, and these always exhibited a stronger reaction than the rest of the nucleolus (Fig. 7). The nucleus and cytoplasm showed positive reactions of varying intensity. The cell processes also showed a positive reaction which was finely granular and mainly located near the synaptic regions around the cells, and also along the fibres. The presence of a positive reaction in such regions was more marked in the plexiform layer of the cortex, which exhibited knob-like, positive bodies distributed unevenly (Fig. 8). Apart from the neurones, the blood vessels and the pia-arachnoid also showed a strong reaction as in the spinal cord.

A histochemical study of the enzymes in different parts of the central nervous system was carried out by Bourne[7] and by Tewari and Bourne[12]. Bourne[7], using Gomori's method for alkaline phosphatase in the brain, suggested that the possible role of phosphatases in synaptic transmission should be considered. The localization of this enzyme in the wall of the blood vessels and at the synaptic regions may be perhaps reconciled by the suggestion that this enzyme may be related to the alteration of permeability and supply of free energy. Bourne[13] also noticed increased hydrolytic activity in senescent cells.

The work recorded here suggests that at least in the spinal cord and cerebral cortex the Burstone reaction for alkaline phosphatase demonstrates a different enzyme from that shown by Gomori's technique. This is supported by the starch-gel observations of Sandler and Bourne[8].

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K. Nandy
G. H. Bourne

Department of Anatomy,
and Yerkes Regional Primate Research Center of Emory University, Atlanta 22, Ga.

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Histochemical Localization of Choline Oxidase in Rat Liver and Kidney

RAT liver is capable of oxidizing hydrolysed acetylcholine[1]. It was shown to be due to oxidation of choline, and betaine aldehyde is the oxidation product[2]. The oxidase system involved was found to consist of a dehydrogenase, choline dehydrogenase, cytochrome *C* and cytochrome oxidase[3]. Though this enzyme system does not need diphosphopyridine nucleotide (DPN), yet it is sensitive to the action of amytal[4]. This oxidase system is also sensitive to antimycin *A*, cyanide and azide, but to a much lesser extent than that of succinic oxidase system[5]. These findings indicate that a respiratory chain identical to that of succinic oxidase may be involved in the hydrogen transfer from choline. According to some authors, flavin adenine dinucleotide (FAD) may be involved in this reaction[6], but by others it plays no part at all[7]. Choline oxidase activity was studied biochemically using different hydrogen acceptors as ferricyanide, methylene blue, cytochrome *C*, 2,6-dichlorophenol indophenol[8] and triphenyl tetrazolium bromide[9]. But phenazine methosulphate was found to be the only reliable hydrogen acceptor for purified choline dehydrogenase[7]. This oxidase may play an important part in the transfer of labile methyl group to methionine[10].

Young and adult rats, 5 guinea pigs, two rabbits and one cat, were bled after light ether anæsthesia. Tissues were immediately frozen in liquid nitrogen and were carried to the cryotome. Some biopsy material of human skin and cirrhotic human liver were also examined. These unfixed tissues were sectioned in a cryostat-microtome at 8–10μ. The sections were collected on coverslips and dried in air for 10 min. Air-dried sections were put directly into the incubation medium. The incubation medium is composed of 3·5 mg of choline chloride (about 0·005 M), 1 mg of nitroblue tetrazolium ('Nitro *BT* Grade III' of Sigma) dissolved in 5 ml. of 0·05 M phosphate buffer at *p*H 6·9. Incubation time varied from 30 min to 1 h. After incubation, sections were treated with neutral formol for 10 min, then washed with distilled water and mounted in Apathy's syrup. In some cases incubated sections were quickly dehydrated and were mounted in Canada balsam. Control reaction was carried out without choline chloride in the incubation medium.

Among rat tissues, only liver and kidney showed an activity, while cerebellum, sub-maxillary salivary gland, thyroid, spleen and adrenal gland showed no activity at all. Rat pancreas was coloured only feebly. Guinea pig liver, as excepted[1], was totally devoid of choline oxidase activity. Neither cat liver nor rabbit liver showed any appreciable activity. Unaffected parenchyma of human liver could scarcely show any activity. Human skin was entirely negative to this reaction. Rat liver showed a more intense activity in the parenchymal cells of the perilobular region and in the parenchymal cells around the blood vessels, while the cells of the centrolobular region show moderate activity (Fig. 1). This activity is revealed by the deposition of violet granules of formazan. But this deposit turns blue when the section is dehydrated. In rat kidney only the cortical portion shows a positive reaction. In the cortex, proximal and distal convoluted tubules are positive to the choline oxidase activity, while glomeruli and loops of Henle are entirely negative (Fig. 2). Medullary rays passing through the cortex are entirely negative. Collecting tubules are devoid of this activity. The reaction is usually more intense in the basal part of the tubular cells than that in the apical part. This is probably due to larger concentration of mitochondria in the basal part of these cells. Walls of the blood vessels are devoid of any activity.

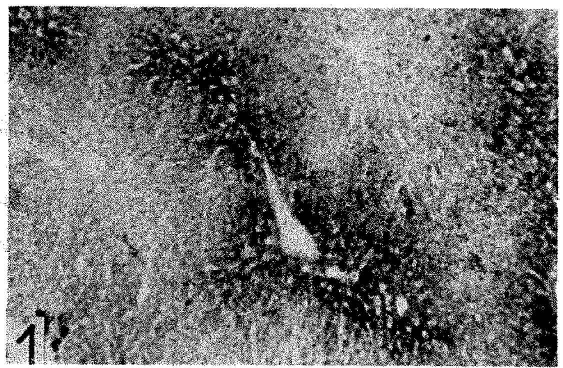

Fig. 1. Choline oxidase activity in rat liver. Activity is intense in perilobular region

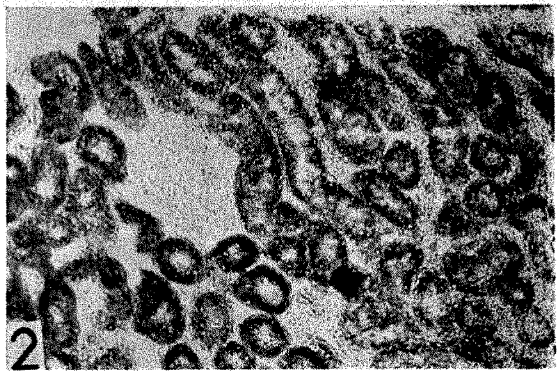

Fig. 2. Activity of choline oxidase in rat kidney cortex. Activity is present in the proximal and distal convoluted tubules

Both chloride and hydrochloride forms of choline can serve as substrate in this reaction. When acetylcholine or butyrylcholine was substituted for choline in the incubation medium, no reaction could be obtained. But substitution by succinylcholine showed a feeble activity. This reaction may be due to quicker hydrolysis of succinylcholine than that of acetyl- or butyryl-choline.

Several experiments were done in order to dissociate the respiratory chain used by the choline oxidase system. Previously neutralized solution of potassium cyanide (0·004 M) was added to the incubation medium; but no appreciable diminution of choline oxidase activity was observed. Nor was cytochrome oxidase inhibited by potassium cyanide at that concentration. Sodium azide also did not inhibit the choline oxidase activity. Added cytochrome *C* (10^{-4} M) did not increase choline oxidase activity. These results, however, are in agreement with biochemical findings[5]. Betaine chlorohydrate, at an equimolar concentration of choline, completely inhibited choline oxidase activity. It is considered that phenazine methosulphate is the only reliable hydrogen acceptor for purified choline dehydrogenase[7]. In an attempt to dissociate the respiratory chain, unfixed sections of tissues were left overnight in phosphate-buffered solution made isotonic with sucrose. No activity could be obtained in these pretreated sections. Since choline dehydrogenase is insoluble in water[6], this inhibition may be due to disruption of the respiratory chain. But addition of phenazine methosulphate did not restore this activity. Some sections were fixed in absolute alcohol at −30° for a period ranging from 1 to 18 h. These sections were deprived of their usual choline oxidase activity; but the addition of phenazine methosulphate in the incubation medium did show an activity in alcohol-fixed sections of rat liver and kidney. It was found that cold alcohol renders choline dehydrogenase insoluble without destroying its activity[10]. It appears that this hydrogen transport through phenazine methosulphate in alcohol-treated sections may be due to the dehydrogenase component alone of this oxidase system. Further tests are being carried out in order to find out whether there is a real dissociation of the choline dehydrogenase from its respiratory chain. Sections fixed in acetone at −30° for a short time retained choline oxidase activity, while overnight fixation totally destroyed it. Even phenazine methosulphate in the incubation medium could not restore this activity. This finding, however, is in contradiction with an anterior histochemical finding of Chang and Hori[11], who found that choline dehydrogenase could be demonstrated after fixation in acetone in dry ice temperature, in the presence of phenazine methosulphate.

Highest activity was obtained between *p*H 6·8 and 7·4. At *p*H 6·0 choline oxidase activity is totally absent, while at *p*H 7·9 this activity still persists. Only phosphate buffer was found to give a satisfactory result. Triethanolamine buffer totally inhibits this reaction. A comparison was made with succinic oxidase activity. Though both succinic and choline oxidase activity were found to be variable yet the reaction of choline oxidase is approximately half as intense as that of succinic oxidase. When choline and succinate are added together in the incubation medium, often an additive effect is observed which may indicate the absence of competition between succinic and choline dehydrogenase for the respiratory chain.

SIBAJYOTI GUHA
RAYMOND WEGMANN

Institut d'Histochimie médicale,
Faculté de Médecine,
45 rue des Saints-Pères, Paris, 6.

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HISTOLOGY

Isolation of Nuclei from Avian Erythrocytes by Partial Electrocytolysis

INVESTIGATION into the presence of hæmoglobin and its possible synthesis within the nucleus of primitive erythroblasts obtained from chick blood islands cultured *in vitro*[1] has indicated the desirability of a micro-method to isolate nuclei from relatively small numbers of cells[1,2].

The mature nucleated red cell of the adult bird or amphibian represents a useful test system. It is simple to obtain a suspension of free cells, and, in addition, the protein hæmoglobin is found in both cytoplasm and nucleus. During an electrocytolytic process, as with any isolation technique, the boundary surface of the nucleus may be modified. Consequently it is important to be able to assess the extent of nuclear protein loss. The nuclear hæmoglobin is easily identifiable, and this protein therefore affords a valuable means of making such assessment.

The method involves the controlled electrolytic breakdown of the cell wall, the electrophoretic removal of cytoplasmic debris and the ultimate isolation of the nucleus.

The cells are suspended in a fluid medium or in an agar gel. The gel has advantages in the control of partial cytolysis and where the sample is small. The latter circumstance imposes the necessity for a restriction of electrophoretic mobility so that cells remain localized for subsequent treatment. Both methods may be combined with direct microscopic observation. It has been found simple and convenient to form the reaction space using a 1 per cent agar gel on a 3 in. × 2 in. glass slide. When cells are suspended in a fluid medium a coverslip over the space restricts the sometimes rapid fluid depletion due to electrolytic diffusion and possible electro-osmosis. A coverslip also facilitates microscopic observation and allows the slide to be orientated at any required angle so as to utilize, if desired, the different sedimentation rates of nuclei and cytoplasmic debris. The components of the cytoplasm and nucleus undergo electrophoresis and may be localized and detected in the gel by suitable methods. In this particular case there is early diffusion of cytoplasmic hæmoglobin into the gel. Later, if the process is carried far enough, nuclear cytolysis occurs and nuclear hæmoglobin is similarly liberated. The hæmoglobin may be detected in the gel by its catalase reaction with *o*-dianisidine[3].

The following media were used: Pannett–Compton saline, Hanks's medium, Tyrode and Ringer. Buffers included Sorensen's phosphate, citrate–hydrochloric acid, and veronal–hydrochloric acid. The range of initial *p*H has extended from 3·0 to 10·0. The variation in *p*H throughout the electrolysis varies with the medium used, the degree of buffering and the initial *p*H. The electrolytic ionization involved, especially in the gel medium, is very complex and is doubtless the effector mechanism bringing about the cytolysis. As yet no serious investigation of the physical-chemistry of the reacting system has been undertaken.

The field intensities used were 20–100 V/cm, the current 5–70 m.amp. and the temperatures 18°–20° C.

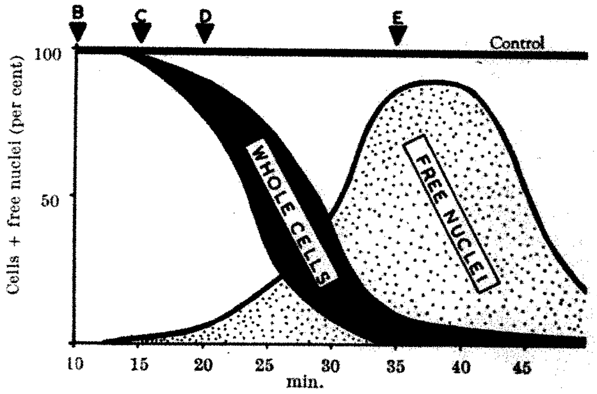

Fig. 1. No. of whole cells (that is, cell wall intact) and free nuclei expressed as a percentage of the total (whole cells + free nuclei) in relation to the time of electrolysis. Control suspension of cells shows no cytolysis; shaded region (percentage of whole cells) indicates degree of variation between experiments. Stippled region (percentage of free nuclei) falls away as nuclei cytolyse. Arrows at points 10 min, 15 min, 20 min, 35 min represent periods when the cells shown in Fig. 2*b*, *c*, *d*, *e* were sampled. Medium: Pannett–Compton sucrose saline buffered to *p*H 5·5 with Sorensen phosphate. Field intensity, 100 V/cm; current, 25 m.amp; temperature, 18° C

Fig. 1 summarizes a reaction in which the cells were suspended in a Pannett–Compton sucrose saline buffered to *p*H 5·5 with Sorensen-phosphate. The curves express the percentage of whole cells (cell wall intact) and the percentage of free nuclei in the combined total (cells plus free nuclei). Cell counts are made in two ways: (*a*) at intervals of time the reaction is stopped by breaking the circuit, electrophoretic movement allowed to cease and counts made in a succession of microscopic fields along the centre line of the reaction space (× 40 Cooke–A.E.I. long working distance semireflecting objective and green filter); (*b*) at similar intervals the reaction is stopped, the slide taken from the apparatus, coverslip removed and the whole gel treated with formol–acetic alcohol vapour. When the circuit is broken electrocytolysis ceases. After drying the slide and removing the agar it is stained in Weigert's hæmatoxylin and light green. Cells and free nuclei are then counted in the same way. As might be expected, method (*b*) yields a higher percentage of free nuclei than method (*a*), but this does not alter the general picture.

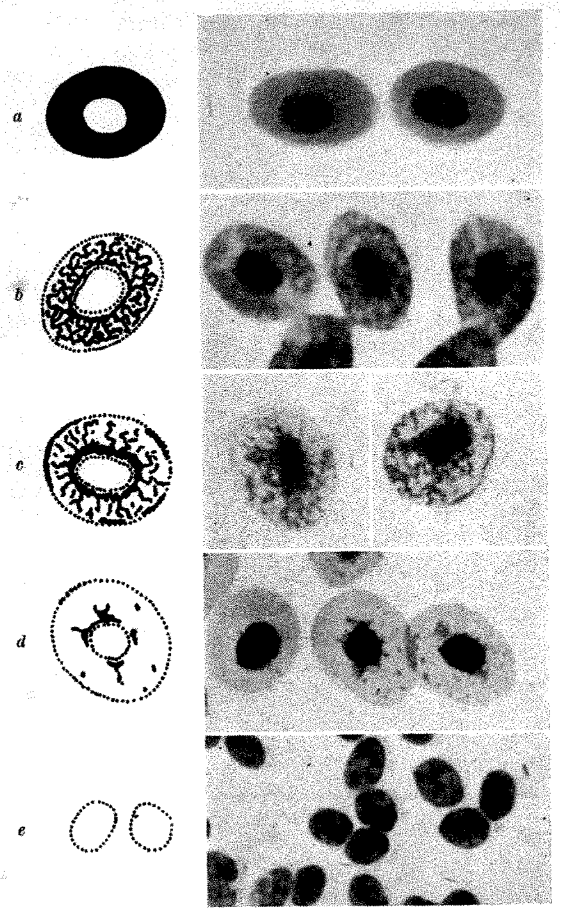

Fig. 2. Avian erythrocytes during partial electrocytolysis stained in Weigert's hæmatoxylin and light green. Cells suspended in Pannett–Compton sucrose saline buffered to pH 5·5. Shaded diagrams on left-hand side indicate the distribution of the light green cytoplasmic stain. *a*, Erythrocytes at 0 min; *b*, 10 min; *c*, 15 min; *d*, 20 min; *e*, 35 min. Field intensity, 100 V/cm; current, 25 m.amp.; temperature, 18° C. Zeiss photomicroscope, magnification on film (× 224)

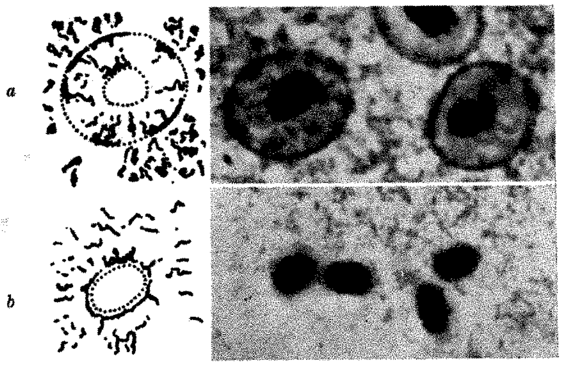

Fig. 3. Cells suspended in 0·6 per cent agar gel made up with Pannett–Compton sucrose saline buffered to pH 5·5. Experimental conditions as in Fig. 2. *a*, 15 min; *b*, 35 min. Note the light green positive material in the gel while cell wall remains intact

The arrows indicate points at which samples were taken and Fig. 2*b–e* demonstrates the morphology of the cells at corresponding times. The cytoplasmic distribution of substances reacting with the light green stain is markedly altered. Fig. 2*d* poses the question whether the substance formerly reacting with the stain is present but no longer stained, or whether it has left the cell. Fig. 3*a* represents the same stage in time as Fig. 2*d* when the reaction occurs in an agar gel. The extracellular material is stained by the light green and is present at a stage prior to the breakdown of the cell wall in all but a very few cells, indicating a passage of cytoplasmic substance through the apparently intact cell membrane. The reaction of the nucleus with hæmatoxylin shows no diminution at this time. Fig. 3*b* corresponds to Fig. 2*e* and demonstrates a persistent but indistinct zone of light green positive material around the nucleus for a short time when the electrocytolysis occurs in a gel.

It is realized that both avian and amphibian erythrocytes have particularly resistant and inactive nuclei that may lend themselves better than other cell nuclei to this process. In accordance with original intention, the technique is being extended to the primitive blood island erythroblast, and to disaggregated embryonic liver cells. It has been found possible to obtain free nuclei from trypsin and versene dissociated chick liver cells. Whether nuclei isolated in this manner are morphologically intact when viewed with the electron microscope, or, what is more important, whether they retain their metabolic activity and potential, is yet to be determined.

B. R. A. O'Brien

School of Anatomy,
University of New South Wales,
Kensington, Sydney.

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Orthogonal Diameters in the Analysis of Muscle Fibre Size and Form

Distributional changes in sizes of fibres have been correlated with pathological processes affecting muscle. Micrometry of muscle fibres in histological cross-section, for example, has been frequently used to follow the course of denervation atrophy[1]. Unfortunately, the complex polygonal form of the fibre and the lack of a standard technique of measurement make it difficult to compare the results of different studies[2].

A single arbitrarily selected diameter has been used by most investigators. The precise method of measurement has rarely been specified, however, and the data give no information on the configuration of the fibres. Cross-sectional fibre areas have occasionally been estimated[3], but planimetric measurements are too cumbersome for routine use and the method ignores variations in fibre form. Similarly, determinations of muscle weight[4] may be misleading and such data have no relevance to the examination of biopsy material.

The analysis of size of muscle fibre is obviously difficult to test by ordinary line and area criteria. More reliable information might therefore be obtained through the use

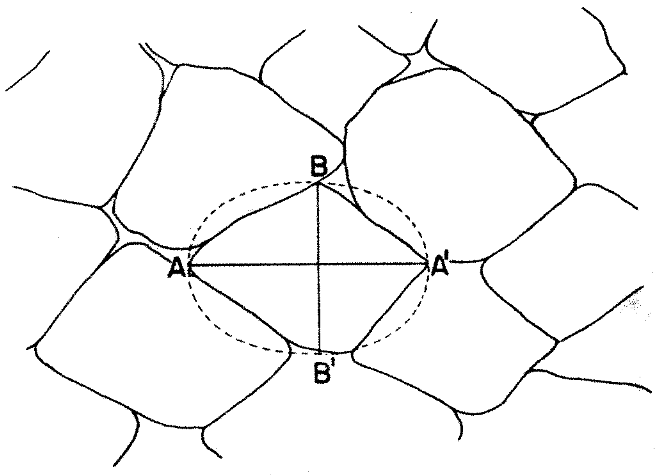

Fig. 1. Camera lucida tracing of transversely sectioned rat gastrocnemius muscle fibres (× *c*. 200). Definition of major and minor axis diameters is illustrated (*A–A'* = major axis, *B–B'* = minor axis). Broken line is drawn to demonstrate the irregular ellipse that can be proscribed around the polygonal muscle fibre

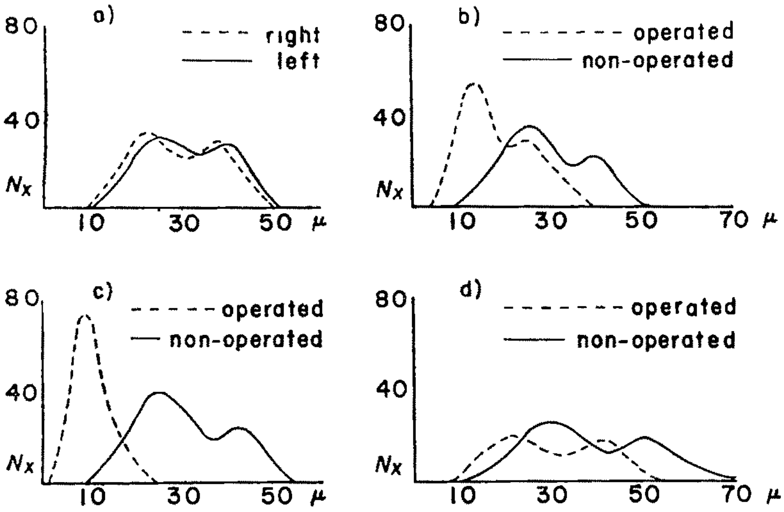

Fig 2 Size-frequency distribution of major and minor axes following denervation (N_x = 150 fibre measurements)
a, Normal non-operated rat (body-weight 120 g), *b*, one week after resection of right sciatic nerve bimodal distribution persists on the operated side, but major and minor axes show distinct shift to the left, *c*, four weeks operated side now shows unimodal distribution of fibre diameters, *d*, ten weeks following denervation recovery is confirmed by the return of a bimodal distribution of fibre diameters (body-weight 250 g)

of size metameters (for example, largest diameter, diameter in a given direction, circumference, etc) and form metameters (ratio of maximum to minimum diameter, ratio of circumference to area, ratio of outside to inside circle radii, etc)[5] After testing a number of possible measurements, it was found that two orthogonal diameters in the muscle fibre gave a reliable index of changing fibre size and shape

The polygonal form of the muscle fibre can be inscribed within a closed oval resembling an ellipse. The area of this form is proportional to its major and minor axes For purposes of measurement, the major axis is defined as the diagonal connecting the most distant angular points in the cross-section of the fibre The longest perpendicular offset from this major axis to the remaining angular points constitutes the minor axis (Fig 1) The biaxial diameter ratio is the ratio of the major axis to the minor axis. Adequate sampling of such measurements from a given muscle should provide a reliable indication of the average large and small diameters (major and minor axes), the size-frequency distribution of these diameters, and the average cross-sectional form of the fibres

This method was evaluated in gastrocnemius muscles of rats that had been subjected to 5-mm resections of the right sciatic nerve 1 day–6 months prior to death Identical muscle from the unoperated limb and from non-operated rats, matched for age, sex and weight, served as controls Muscle samples with a maximum thickness of 5 mm were briefly air dried on filter paper, fixed in Bouin's fixative, and embedded in paraffin with fibres oriented for longitudinal or transverse sections Paraffin sections were prepared with a Spencer rotary microtome set at a section thickness of 4μ An adjacent muscle sample was quenched in 2-methylbutane (isopentane) cooled with dry ice Comparison of unfixed cryostat sections with paraffin sections showed a shrinkage of 10 per cent in the latter. This reduction in size incident to fixation and embedding was uniform throughout the muscle bundle and there was no change in the cross-sectional form of the fibre Micrometry was performed at a magnification of 400 using a binocular microscope equipped with a calibrated eyepiece graticule Areas containing properly oriented cross-sections of muscle fibres in groups of 200 or more were selected from each section Obliquely oriented fibres and the edges of sections were avoided Major and minor axes were measured in each of 150 fibres Measurements were made in consecutive fibres by traversing the histological sections horizontally and vertically with a graduated mechanical stage to ensure uniform sampling

Table 1 Effect of Denervation on Orthogonal Diameters and Biaxial Diameter Ratio of Rat Gastrocnemius Muscle

Time after nerve resection (weeks)	Major axis diameter* (μ)	Minor axis diameter* (μ)	Biaxial diameter ratio
0 (control)	38 6 ± 0 89	28 3 ± 0 69	1 36
1	22 3 ± 0 48	17 1 ± 0 41	1 30
4	14 1 ± 0 82	12 3 ± 0 72	1 14
10	41 7 ± 1 48	27 6 ± 1 34	1 51

* Values expressed as mean ± standard error of the mean

The size-frequency distribution of the normal muscle is shown in Fig 2*a* The ellipsoid character of the muscle fibre is reflected in the bimodal distribution of major and minor axis diameters and the biaxial diameter ratio, which is greater than one (Table 1)

Throughout the early phases of atrophy (Fig 2*b*) the diameters were smaller than normal but a bimodal distribution persisted and the biaxial diameter ratio remained greater than 1, confirming the observation that no significant alteration in cross-sectional form of the fibre had occurred During the state of maximum atrophy (4–7 weeks, Fig 2*c*) the diameters were sharply reduced, the size-frequency distribution was narrowly unimodal, and the biaxial diameter ratio (close to unity) correlated with the tendency of the maximally atrophied fibre to assume a rounded form (Table 1) The phase of recovery (Fig 2*d*) was marked by the reappearance of a bimodal distribution of fibre diameters and a progressive increase in the biaxial diameter ratio

The method of orthogonal diameter measurement can therefore be recommended for the sizing of muscle fibres It gives more reliable data on the size-frequency distribution of fibre diameters and the cross-sectional form of the fibre can be accurately expressed by the biaxial diameter ratio (ratio of major to minor axis) The method is particularly suitable for the investigation of changes in distribution of fibre sizes and variations in fibre form.

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Sun K. Song
Nobuo Shimada
Paul J Anderson

Department of Neuropathology,
Mount Sinai Hospital and Department of Neuropathology,
College of Physicians and Surgeons,
Columbia University, New York.

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RADIOBIOLOGY

Bone-marrow Mitotic Inhibition induced by Local Splenic X-irradiation

An increasing number of clinical and experimental observations suggests that the spleen releases after irradiation an inhibitor of mitosis acting on remote organs and particularly on bone-marrow, but no clear evidence of this hypothetical factor has yet been given[1-3] In order to investigate the mechanism of this distant radiological effect, a selective splenic irradiation was carried out on lead-shielded rabbits, and certain hæmatological consequences of this procedure were studied in the blood and bone-marrow Furthermore, the isolation of an antimitotic factor from the irradiated spleen was attempted

The preliminary experiments performed on 8 pure-strain rabbits show that 48–66 h after 300 r of X-rays (dose rate 113 r /min , distance 30 cm , 180 kV , 15 m amp , filtration by 0 5 mm copper and 2 0 mm aluminium) given exclusively to the surgically exteriorized spleen, the reticulocyte blood-level drops from a mean value of 27 5 per thousand (± 3 71 = *S D* , *S E* , 0 3) to a mean value of 11 3 per thousand (± 2 75 = *S D* , *S E* , 0 5) This reticulocytopenia lasting 24–36 h points to a temporary inhibition of the erythropoiesis , this is confirmed by the fact that a decrease can be observed in bone-marrow activity 20 h after the splenic irradiation The post-irradiation mitotic index measured by a stathmokinetic method was found to be 45 per cent lower than the pre-irradiation mitotic value

A second series of 8 previously splenectomized rabbits was irradiated with the same X-ray dose on the 'splenic area' (that is, stomach and pancreas) These animals did not show any fall in reticulocyte-level or bone-marrow mitotic index This finding suggests that the transient erythropoietic inhibition occurring in the former experiments is not a consequence of some direct radio-lesion of the bone-marrow, but results from the splenic irradiation

A third set of experiments was undertaken to determine at what time after irradiation the spleen releases its hypothetical antimitotic factor For this purpose 12 rabbits were splenectomized at different intervals after irradiation of this organ and tested for erythropoietic changes It was demonstrated that only the animals the spleens of which were left in place at least 2 h after irradiation showed the typical erythropoietic inhibition, while earlier splenectomy prevented the development of this antimitotic effect

The object of the last experimental step was the isolation of an antimitotic factor from the irradiated spleen For this purpose, 12 rabbits were used, 6 as irradiated donors and 6 as test recipients As the previous results suggested that the antimitotic factor is released between 2 and 3 h after splenic irradiation, we attempted to concentrate this factor in the organ In each of the donors, the splenic veins were clamped 2 h after the 300 r. exposure, and 1 h later the blood of the congested spleen was collected with heparin After centrifugation the plasma was injected into a normal rabbit and induced in this unirradiated recipient the same erythropoietic depression previously described for the irradiated animals (bone-marrow mitotic depression and reticulocytopenia) This finding was reproduced fully in 6 experiments On the other hand, blood, collected from non-irradiated but similarly operated spleens, was shown to be devoid of any antimitotic activity This fact emphasizes the essential role of the splenic radiolesion in the production of the bone-marrow depressing factor The chemical nature of this remarkable agent is at present under investigation

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P A Maurice
A Jeanrenaud

Oncology-Hæmatology Unit,
University Hospital,
Geneva

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Establishment and Stability of Chimerism between Two Closely Related Lines of Mice

THE Medical Research Council Radiobiology Unit at Harwell recently developed a strain of *CBA* mice containing the *T*6 marker chromosomes[3,4] They kindly sent a breeding unit to the Chester Beatty Research Institute This communication concerns a short series of experiments involving descendants of these (*CBA*/*T*6*T*6) mice and the Chester Beatty *CBA* The purpose of the experiments was, first, to define the immunological relationship between the strains , secondly, to describe the conditions under which chimerism could be established between them, thirdly, to investigate the extent and stability of this chimeric state

Skin grafting Skin grafts were exchanged between male *CBA*/*T*6*T*6 and *CBA* mice following the method of Billingham and Medawar[2] In addition, skin isografts were made to both strains of mice No intra-strain graft was rejected The mean survival times of the inter-strain grafts are shown in Table 1 The fact that rejection of interstrain grafts between *CBA* and *CBA*/*T*6*T*6 occurred in all cases proves that the two strains are genetically different but, as the rejection times were significantly (*P* = 0 001) prolonged in relation to homograft rejection times involving a known *H*-2 difference, it can be assumed that the Chester Beatty *CBA* and *CBA*/*T*6*T*6 do not differ at the *H*-2 locus The similarity between the survival times of *Ak* and *CBA*/*T*6*T*6 skin grafts on *CBA* mice confirms this It should be noted that Harwell *CBA* mice do not reject *CBA*/*T*6*T*6 skin grafts[4]

Table 1 MEAN MEDIAN SURVIVAL TIMES IN DAYS OF SKIN GRAFTS EXCHANGED BETWEEN VARIOUS STRAINS OF MICE

Donor	*H*-2	Host	*H*-2	Mean median ± S D* survival time
CBA	*k*	*CBA*/*T*6*T*6	?	22 7 ± 4 9
CBA/*T*6*T*6	?	*CBA*	*k*	23 3 ± 2 3
Ak	*k*	*CBA*	*k*	21 7 ± 2 5
*C*57*B*1	*b*	*CBA*	*k*	15 57 ± 1 3

*$n >$ 10 in each case

Injection of new-born mice 10 × 10^6 *CBA*/*T*6*T*6 adult spleen cells were injected intravenously into new-born *CBA* mice All injected mice survived and their body-weight growth curves resembled those of *CBA* mice injected with 10 × 10^6 *CBA* spleen cells No splenomegaly was observed The *CBA* mice injected with *CBA*/*T*6*T*6 spleen cells proved to be tolerant of *CBA*/*T*6*T*6 skin grafts when tested six weeks after the injection. Cytological tests, however, failed to reveal any *CBA*/*T*6*T*6 cells in the spleen, bone marrow or thymuses of the tolerant mice A total of 250 arrested metaphases were examined in four mice

These experiments emphasize the ease with which tolerance between the two strains can be acquired and show that graft-versus-host reactions between the strains, if they occur, are very limited in their effects

Radiation Chimeras Groups of thirty *CBA* mice were irradiated with 300, 500 or 700 r and then injected intravenously with 10 × 10^6 cells of *CBA*/*T*6*T*6 bone marrow Thirty and fifty days later four mice were killed in each group and cells from the bone marrow, spleen and thymus were subjected to cytological analysis following the method of Fox and Zeiss[5] The results of this analysis are given in Table 2

Table 2 NUMBERS OF HOST AND DONOR CELLS SCORED BY CYTOLOGICAL ANALYSIS IN VARIOUS GROUPS OF *CBA*/*T*6*T*6 $\longrightarrow$ *CBA* CHIMÆRAS

	Thirty days						Fifty days					
	Bone marrow		Spleen		Thymus		Bone marrow		Spleen		Thymus	
Dose	Host	Donor	Host	Donor	Host	Donor	Host	Donor	Host	Donor	Host	Donor
300	42	42	2	4	7	31	12	19	6	8	11	3
500	3	67	0	1	3	131	0	8	0	3	0	6
700	0	45	0	41	0	40	0	8	0	1	0	14

After 300 r no mice were found which did not have some dividing host cells at both thirty and fifty days, generally there was a preponderance of donor cells After 500 r three mice out of four at thirty days and none out of four at fifty days showed any host cells In no animal did these host cells account for more than 5 per cent of the total number of cells observed. No host cells were seen after 700 r

In a second experiment 850 r was the radiation dose of choice again to *CBA* mice, but in this case the number of *CBA/T6T6* bone marrow cells injected therapeutically was varied Cytological analysis was carried out on four mice from each experimental group 30, 50 and 100 days later. The results are shown in Table 3 All the cells scored except three turned out to be donor cells, a total of about fifteen hundred cells in thirty-six mice Two of the host cells were found fifty days after irradiation in animals which had been injected with 5 × 10^6 cells The remaining cell was found 100 days after irradiation in the same experimental group

Table 3 NUMBER OF CELLS SUBJECTED TO CYTOLOGICAL ANALYSIS IN VARIOUS GROUPS OF *CBA/T6T6*⟶*CBA* CHIMERAS

No of cells injected	Thirty days			Fifty days			One hundred days		
	Bone marrow	Spleen	Thymus	Bone marrow	Spleen	Thymus	Bone marrow	Spleen	Thymus
5 × 10^6	77	75	80	11	6	34	39	25	96
1 × 10^6	92	80	178	16	5	0	37	21	54
2 5 × 10^5	109	117	20	35	5	0	21	9	181

It had been hoped at the beginning of these experiments that the two strains of mice involved would prove syngeneic with respect to skin transplants This would have facilitated a study of chimerism in as near syngeneic a strain combination as is compatible with the presence of a chromosome marker

It is particularly important to be sure that syngeneic chimeras are, in fact, functionally chimeric with very few or no host cells contributing to hæmatopoiesis, otherwise the use of radiation animals as tests for viability of, say, frozen cells[1] is fraught with interpretative difficulty Fortunately, Ford and Micklem[4] recently found that four syngeneic chimeras out of 16 showed some host cells but that in no case did these cells account for more than 5 per cent of the dividing cells discovered In the present work few host cells were found and altogether it appears clear that minor genetic differences are probably of little importance in impeding the establishment of chimerism after irradiation, and that mice irradiated and then injected with syngeneic or near syngeneic bone marrow cells are for practical purposes chimeric It is, in any event, impossible to deny that host cells may be present, it is only possible to be sure that they do not divide with appreciable frequency and therefore probably do not make a significant contribution to hæmatopoiesis

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A J. S. DAVIES
SHEILA M A DOAK
ELIZABETH LEUCHARS

Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
London, S W 3

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BIOLOGY

Anaerobic Life in a Tropical Crater Lake

THERE are very few records of animals other than Protozoa found living in oxygen-free water for which there seems to be good evidence of prolonged and active anaerobic life[1,2] In many cases there is the possibility of periodic visits to oxygenated water and in others the oxygen demand is lowered by the reduction or even complete cessation of activity Certain planktonic crustacea especially *Cyclops* spp and some insect larvæ and molluscs have, however, been found living actively in the oxygen-free water and mud at the bottom of temperate eutrophic lakes during summer stratification, and the evidence for anærobiosis lasting for a few months is convincing.

In the tropics the temperature régime is such that more or less permanent stratification and deoxygenation of lakes is rather common, Lake Tanganyika being the supreme example There have been, so far as I know, no records of planktonic animals from the anoxic lower water of such lakes and in Lake Tanganyika they are reported to be completely absent[3]

Ruttner[4], who had much experience of lakes both in Europe and in the tropics of Java and Sumatra, expressed the opinion that, owing to the temperature which may be almost 20° C above that at the bottom of temperate lakes, the rate of metabolism in tropical lakes might be high enough to prohibit adaptation to low oxygen-levels.

It is, therefore, of interest to record some observations on a small volcanic crater lake, L Nkugute, in Western Uganda which is about 1 000 m in diameter and 58 m deep and is very well protected from wind disturbance by the crater wall and surrounding hills These circumstances and the tropical temperature régime ensure a permanent stratification, and from about 15 m to the bottom the water is out of circulation and devoid of oxygen, and the lake is consequently very unproductive

By means of a vertically hauled closing plankton-net and later by pumping up and filtering 200-litre samples, the copepod *Thermocyclops schuurmanni*, the rotifers *Horaella brehmi* and *Keratella tropica*, and a ciliate protozoan were taken as close to the bottom as it was possible to collect, that is to say with some 40 m of oxygen-free water above them, and where there was a constant temperature of 22° C

T schuurmanni is particularly interesting because the adult egg-bearing females were confined to the upper oxygenated water, where all stages of development were also to be found, but the lowest samples contained only nauplius larvæ and young copepods

The evidence therefore suggests that eggs are produced only in the upper oxygenated water and that some of them are shed and fall to the bottom where the first stages of development proceed in the absence of oxygen Whether the young copepods then return to the surface to mature and reproduce is not yet known Preliminary experiments indicate that the adults cannot live for more than a few hours without oxygen and one is led to the conclusion that the respiratory metabolism changes radically during development

Redox potentials in the lower water and mud are considerably higher than most of those recorded for temperate eutrophic lakes at the height of summer stratification For example, Mortimer[5] recorded zero potentials (E_h) just above the mud in Esthwaite Water in September The corresponding potential in Lake Nkugute was +180 to +220 mV This is perhaps not unexpected under conditions of permanent stratification where, in the absence of an overturn, readily reducible substances would be more nearly exhausted and only small quantities would be available as particles descending from the unproductive surface water It would seem that lack of oxygen is less of a barrier than a low redox potential usually associated with the accumulation of toxic substances such as hydrogen sulphide, which in the bottom water of Lake Nkugute was in extremely low concentration and only just detectable by smell but which, from all accounts, is abundant below 150 m in Lake Tanganyika (max depth, 1,400 m)

The activities of organisms such as *Thermocyclops schuurmanni* would cause at least a small chemical circulation between upper and lower water under conditions of stable stratification

Further observations and experiments are obviously needed, but preliminary work has shown a similar situation in Lake Bunyoni (about 50 m deep), which is a narrow-flooded branching river valley blocked by a lava flow

I thank Drs J P Harding and J D Green for identifying the copepod and rotifers

L C BEADLE

Department of Zoology,
Makerere University College,
Kampala, Uganda

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A Comparison of the Sensitivity to Storage at 37° C in Tyrode Solution of Immunologically Competent Cells and of Myeloid Cells

IN an earlier investigation[1,2], we have demonstrated that storage for 2 h at 37° C in Tyrode solution causes hæmopoietic cells to lose a considerable part of their capacity to induce, through a graft reaction to the host, secondary syndromes in the irradiated allogenic recipient, whereas their capacity to restore myeloid tissue is not noticeably affected

Two different explanations of this phenomenon are possible: (*a*) either the immunologically competent cells are more sensitive to this type of storage than the immature myeloid cells, (*b*) or the sensitivities of these two types of cells to this form of aggression are the same and the difference of the effects on the graft reaction to the host and the capacity to restore myeloid tissue is due to the fact that the relations of these two phenomena to the numbers of cells that cause them are regulated by different laws

In order to test these two hypotheses it is necessary to carry out a quantitative determination of the respective relations between the frequency of death from graft reaction to the host and the number of lymphoid cells on one hand and between the myeloid restoration and the number of myeloid cells on the other

Then it will be possible to compare the effect of storage for 2 h at 37° C in Tyrode solution on the graft reaction to the host by the number of lymphoid cells affected, and its effect on the myeloid restoration by the number of the myeloid cells affected

Material and methods (*a*) 240 F_1 mice of the strain $DBA/2 \times C_{57}Bl/6$, three months old, were exposed to whole-body irradiation with 500 rad (200 kV, 12 m amp, 50 cm distance, 0 5 aluminium, 0 5 copper) Immediately after the irradiation they received intravenous injections of 10^7 non-preserved $C_{57}Bl/6$ medullary cells The animals were divided non-selectively into 2 groups, *A* and *B*, of 120 animals each

The mice of group *A* were divided over 6 sub-groups of 20 animals each and these groups were given intravenous injections of non-preserved $C_{57}Bl/6$ lymph-node cells, of 4×10^7 cells, $2\ 5 \times 10^7$ cells, 10^7 cells, 5×10^6 cells, 10^6 and 10^5 cells, respectively

The mice of group *B* were also divided over 6 subgroups of 20 animals each, which were treated intravenously with 4×10^7, $2\ 5 \times 10^7$, 10^7, 5×10^6, 10^6 and 10^5 C_{57} $Bl/6$ lymph-node cells that had been stored for 2 h at 37° in Tyrode solution The conditions of the storage were as follows a suspension of 2×10^5 cells/mm³ of Tyrode solution was prepared, 3–4 ml of this suspension was placed in glass tubes of 20 ml. and kept for 2 h at 37° C in continuous rotation

(*b*) 160 F_1 mice of strain $DBA/2 \times C_{57}Bl/6$ were irradiated with a dose of 950 rads and divided at random over 2 sub-groups, *A* and *B*, of 80 animals each Group *A* was subdivided into 4 groups of 20 animals treated intravenously with 10^6, 10^5, 10^4 and 10^3 fresh isogenic medullary cells, respectively

Group *B* was also divided into 4 sub-groups of 20 animals each, which were treated intravenously with 10^6, 10^5, 10^4 and 10^3 isogenic medullary cells, respectively, that had been stored for 2 h at 37° C under the conditions mentioned here

Lymphoid cells and graft reaction to the host The results obtained are listed in Table 1 Calculation by the probits method of the number of fresh lymph-node cells required to cause 50 per cent deaths by the graft reaction to the host gives a number of about 1,070,000 cells in group *A*

This determination is impossible in group *B*, since the number of surviving animals in the most affected subgroup was 16 of 20; it is nevertheless certain that the number of preserved lymph-node cells required to bring about 50 per cent deaths is larger than the largest number of preserved lymph-node cells injected, which amounted to 40,000,000

Table 1 Graft-to-host reaction provoked by fresh C_{57} $Bl/6$ lymph-node cells (group *A*) and by $C_{57}Bl/6$ lymph-node cells stored for 2 h at 37° C (group *B*) in F_1 ($DBA/2 \times C_{57}Bl/6$) hosts irradiated with a dose of 500 rads and treated with 10^7 fresh $C_{57}Bl/6$ medullary cells

Group *A*	No of test animals	Animals surviving after the hundredth day
4×10^7 fresh lymph-node cells	20	0
$2\ 5 \times 10^7$ fresh lymph-node cells	20	0
10^7 fresh lymph-node cells	20	4
5×10^6 fresh lymph-node cells	20	6
10^6 fresh lymph-node cells	20	8
10^5 fresh lymph-node cells	20	14
Group *B*		
4×10^7 preserved lymph-node cells	20	19
$2\ 5 \times 10^7$ preserved lymph-node cells	20	20
10^7 preserved lymph-node cells	20	16
5×10^6 preserved lymph-node cells	20	16
10^6 preserved lymph-node cells	20	18
10^5 preserved lymph-node cells	20	17

Medullary cells and capacity to restore myeloid tissue The results of this are listed in Table 2 The criterion of the myeloid restoration was survival on the thirtieth day Calculation by the probits method of the number of medullary cells necessary to bring about myeloid restoration in 50 per cent of the animals gave about 216,000 ($170,000 < ED_{50} < 275,000$ for $P = 0\ 05$) for the fresh isogenic medullary cells and approximately 214,000 ($153,000 < ED_{50} < 298,000$ for $P = 0\ 05$) for the medullary cells that had been stored for 2 h at 37° C

Table 2 Myeloid restoration by isogenic medullary cells, either fresh (group *A*) or stored for 2 h at 37° C (group *B*) in F_1 ($DBA/2 \times C_{57}Bl/6$) hosts irradiated with a dose of 950 rads

Group *A*	No of test animals	Animals dead before the thirtieth day
10^6 fresh medullary cells	20	7
10^5 fresh medullary cells	20	14
10^4 fresh medullary cells	20	13
10^3 fresh medullary cells	20	20
Group *B*		
10^6 preserved medullary cells	20	7
10^5 preserved medullary cells	20	13
10^4 preserved medullary cells	20	17
10^3 preserved medullary cells	20	18

We may conclude that the results obtained answer the question investigated (*a*) In a hæmopoietic graft, preservation for 2 h at 37° C in Tyrode solution does not notably influence the number of cells required to bring about myeloid restoration in an irradiated recipient; in order to obtain the same biological result, namely, 50 per cent restoration, which is the result for which the determination of the required number of cells can be the most accurate, there must not be more preserved than fresh medullary cells. (*b*) On the other hand, this preservation has a considerable influence on the immunologically competent cells which, when immunized against a semi-allogenic host, are killed through a graft-to-host reaction: 1,000,000 fresh lymph-node cells kill 50 per cent of the animals, but of the cells that have been stored for 2 h at 37° C in Tyrode solution 40,000,000 are not enough to bring about the same effect; accordingly it may be concluded that this preservation affects more than 97·5 per cent of those cells that are capable of evoking a graft-to-host reaction.

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J. L. Amiel
G. Mathé
Cancerological and Radiological Research Centre,
Claude Bernard Association,
Hôpital St. Louis, Paris.

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Frontal Foramina and Tripodes of the Characin *Crenuchus*

Gery[1] has recently described a pair of foramina in the frontal bones of *Crenuchus spilurus* Günther. These foramina underlie the posterior margin of a pad of fatty tissue which covers the anterior part of the skull roof. Gery found by dissection that they contained structures which seemed to be connected to the brain. His specimens were not well enough preserved for histological examination.

I possess two well-preserved specimens of the same species. I have cut serial transverse and horizontal sections of their heads, and stained them with Mallory's triple stain. Each frontal foramen is filled by a plug of tightly packed, tangled collagen fibres, penetrated by a few small blood vessels (Fig. 1). The collagen fibres of the plug are continuous with those of the fatty pad, of the periosteum of the frontals, and of the interesting structure shown in Fig. 2. This structure lies posterior to the plug, within the cranial cavity. It includes blood vessels, collagen fibres, and a group of elongate vesicles, partially filled by a lightly staining homogenous substance. It is served by a substantial branch of the superior ophthalmic nerve.

It seems probable that this is a sense organ. The vesicles (reminiscent of ampullæ of Lorenzini, though they have no external openings) and the innervation by the superior ophthalmic nerve suggest derivation from lateral line organs. If it is an exteroceptor, the stimuli to which it is sensitive must be able to affect it through the collagenous pad.

Gery[1] also discussed the tripodes of *Crenuchus*. He stated that they are not of the type found in other characins. I have cut horizontal sections of the Weberian apparatus of one of my specimens of *Crenuchus*, and am unable to confirm this. The anterior edges of the tripodes (Fig. 3) are formed by bony lamellæ which are fused to the third centrum but not, apparently, to the more posterior bony parts of the tripodes. This structure is identical with that of the tripodes of *Astyanax* (typical of Characinoidei) and contrasts strongly with that of *Carassius* (typical of Cyprinoidei) which have been illustrated elsewhere[2]. In Cyprinoidei there are no separate bony lamellæ, and the tripodes are attached to the centrum by the cartilage which fills them.

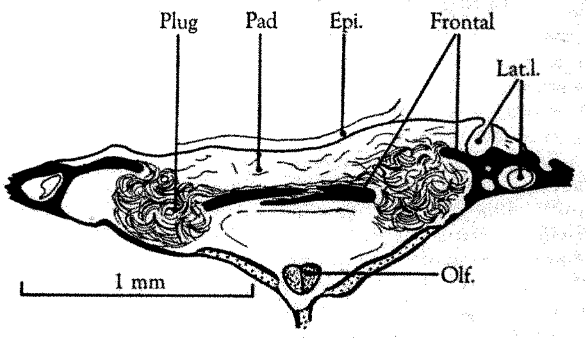

Fig. 1. Camera lucida drawing of part of a transverse section of *Crenuchus* showing the frontal foramina, collagenous plugs and fatty pad. *Epi.*, epidermis (damaged); *Lat. l.*, lateral line canal and pore; *Olf.*, olfactory tract

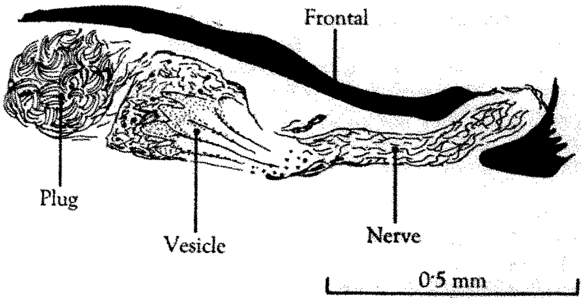

Fig. 2. Camera lucida drawing of part of a horizontal section of *Crenuchus* showing a collagenous plug, the vesiculate structure associated with it, and the nerve. The top of the figure is lateral and the left anterior

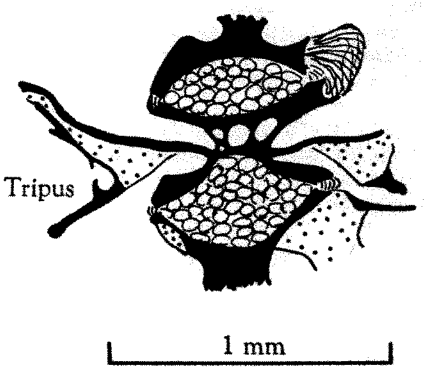

Fig. 3. Camera lucida drawing of a horizontal section through the third centrum and tripodes of *Crenuchus*. The top of the figure is anterior. Bone is shown black and cartilage is stippled

Gery[1] also suggests that the tripodes of Erythrininae may lack the bony lamella. The tripodes can, however, be seen to be attached to the centrum by a bony lamella, and seem to be of entirely normal characin type, in my sections of *Erythrinus erythrinus* (Bloch and Schneider).

Weitzman's[3] definition of the Characinoidei in terms of the structure of the tripodes, to which Gery[1] refers, is faulty in that, of the Cypriniformes, it excludes only the Cyprinoidei. The tripodes of Gymnotoidei[2] and Siluroidei[4,5] are attached to the centrum by bony lamellæ. In Siluroidei, however, the lamella is fused to the main body of the tripus.

The specimens described here were collected by the Cambridge Expedition to British Guiana, 1960, of which I was a member.

R. McN. Alexander
Department of Zoology,
University College of North Wales,
Bangor.

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Temperatures in Heather Burning

Recently the subject of heather burning has come under more critical review. Of particular interest is the measurement of the temperatures recorded in fires and the effect of this heat on the establishment of new growth and the long-term effects on the fertility of the soil.

Whittaker[1] recorded temperatures occurring in heather burning by means of 'Thermocolours', heat-sensitive paints, and in later experiments[2], organic compounds of specific melting points. Her results show that the maximum temperatures experienced in heather burning are of the order of 840° C and that the temperatures most frequently recorded are between 300° C and 500° C at ground-level. It is suggested[1] that these temperatures are maintained for periods of less than 2·5 min.

The time-temperature complex, that is, the time during which fixed temperatures are maintained, is important from three different aspects. The regeneration of new growth both from seedlings and from the base of the old stem depends on this. In addition, work in this laboratory has shown that the losses of nitrogen in smoke depend on the period for which temperatures are maintained; and has demonstrated the varying solubility of nutrients in plant ash derived from heather leaves burnt under various temperature conditions.

Whittaker suggested the use of thermocouples as an accurate method for the measurement of fire temperatures. The major difficulty with this type of measurement is the recording of data under field conditions with no source of electricity to power bulky recorders and amplifying systems. Mervyn Instruments, Ltd., have recently produced a portable transistorized recorder. This makes possible the recording of temperatures over any given range with the appropriate thermocouples.

Recordings were made over the range 0°–1,000° C. The recording equipment consisted of 5 thermocouple units connected to a manual switch, which in turn was connected to the recording instrument. Each thermocouple unit was made up from 3 ft. 'Insuglass Chromel Alumel' connected to compensating wire by copper points buried in the ground. The overall distance from the thermocouple point to the recorder was 40 ft.

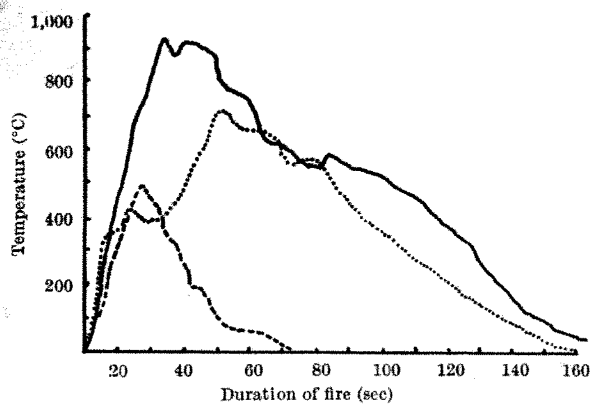

Fig. 1. Three chart records for vegetation of different ages. ——, 35 yr. old; · · · ·, 20 yr. old; – – –, 10 yr. old

The results presented here are the result of work carried out in April 1963 on fires in Glen Clova, Angus. Fig. 1 shows the typical recording for heather of different ages, showing clearly the general increase in temperature of fires with increase in age of vegetation. All the readings are for temperatures recorded in the leafy parts of the plants. The maximum temperature recorded was 940° C and the average maximum from 35 fires was 670° C. These temperatures are higher than those recorded by Whittaker[1] under similar conditions. As the conditions last spring were particularly wet, it may well be that these temperatures may be exceeded when burning old heather.

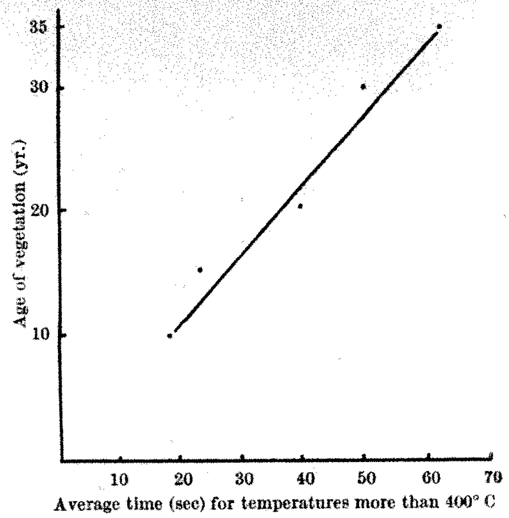

Fig. 2

At temperatures above 400° C there are significant losses of nitrogen in smoke, and thus it is important to estimate the time for which temperatures more than 400° C are maintained. Fig. 2 shows the average time for which temperatures more than 400° C are maintained in the fires studied and its dependence on the age of the vegetation. Whittaker[2] has used similar time periods for the study of heat tolerance of heather seeds. These results have confirmed Whittaker's predictions.

J. B. Kenworthy

Department of Botany,
University of St. Andrews.

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A Mould Inhibitor in Soybeans

Seeds of *Glycine max* Merr. (soybean) contain several biologically active materials, including saponins and trypsin inhibitors[1]. However, in a recent review of the antimicrobial activity of vascular plants[2], no mention is made of the occurrence of antimicrobial compounds in soybeans. We are reporting the presence of a factor(s) that inhibits the mould growth of *Rhizopus* sp. (provisionally identified as *R. oligosporus* Saito), which is utilized in the fermentation of soybeans to make the Indonesian food tempeh[3].

In making tempeh[4], soybeans are soaked in water for several hours, and then this water is discarded at the time the soybean hulls are removed. The dehulled beans are then boiled for 30 min in sufficient fresh water to cover the beans during the entire boiling process. This boiled water extract is discarded prior to inoculation and fermentation with *Rhizopus*. Under the fermentation conditions, the mould grows rapidly and in less than 24 h completely covers the soybeans. The soybeans are held together by the hyphæ of the mould and little sporulation occurs except at the edges of the moulded soybean mass if the fermentation is done in Petri dishes. In our investigations of tempeh, we found that an excellent product could be produced by using full-fat soybean grits. However, when the foregoing procedure of discarding the soaking and cooking water was used, it was discovered that considerable soybean material was discarded with the soaking and cooking water. For example, 1,200 g of dry, full-fat soybean grits were placed in beakers, covered with tap water and allowed to take up water, washed in 1,000 ml. of tap water, again covered with tap water and boiled for 30 min and drained. After these uninoculated grits were freeze-dried, the dried mass weighed 845 g, a loss of 355 g. The loss in weight was due to the presence of small suspended particles as well as dissolved material in the liquor that was discarded.

To reduce the processing loss, we modified the procedure for making tempeh, by two methods: the first method was to retain the soaking water and then add sufficient water so that the beans could be boiled for 30 min. At the end of this time, practically all the water had evaporated, leaving the soybeans moist and sterile. The second method was to soak the beans in sufficient water so that the grits were thoroughly moistened but practically no excess water was left. These moist grits were then steamed for 30 min for sterilization. In each method, no water was discarded in order to reduce the processing loss. When soybeans were prepared for fermentation by either of the above methods, the product was quite inferior to normal tempeh. For example, the following experiment was typical of a number we performed. Full-fat grits made from Hawkeye soybeans were given the following treatments and then each product was divided equally into inoculated and uninoculated parts.

Treatment 1. Tap water was added at the rate of 175 ml./125 g of grits, which were allowed to stand overnight. Next, they were autoclaved for 30 min at 5-lb. pressure, then cooled, and half the sample was inoculated with spores of strain N.R.R.L. 2710 *R. oligosporus* and packed in Petri dishes. The remaining uninoculated half of the sample was stored in a refrigerator.

Treatment 2. Tap water was added at the rate of 300 ml./125 g of grits, which were allowed to stand overnight. They were boiled to evaporate excess water but were left still moist. The sample was divided and handled as in treatment 1.

Treatment 3. Grits were soaked overnight in sufficient tap water to leave a small amount of water above the grits at the end of the soaking period. The excess water was discarded, new water was added, the grits were boiled for 30 min, and the water was again discarded. The grits were cooled and part inoculated as in treatment 1. This method was essentially the Indonesian one, except grits were used instead of whole soybeans.

Treatment 4. Grits were washed with tap water but not soaked, then fresh tap water was added in excess, the grits were boiled for 30 min and this water was discarded. The grits were then handled as in treatment 1.

Each part that had been inoculated was allowed to ferment at 31° C for approximately 24 h.

The finished fermented tempeh made from treatments 1 and 2 showed reduced growth and poor fermentation, had a disagreeable odour, was greyish-white, had reduced development of mycelium and had sporangia scattered through the cake. On the other hand, the fermented products of treatments 3 and 4 were white cakes with abundant mycelium, sporulation restricted to the edges and a pleasant odour. When the fermented soybeans were sliced and cooked in vegetable oil, the tempeh from 1 and 2 had a dark reddish-brown colour, poor flavour and odour described by some as like that of burnt rancid bacon. Those from treatments 3 and 4 were normal tempeh with a bright light colour and excellent flavour. The four samples of uninoculated grits were also cooked, and those from treatments 1 and 2 were darker in colour than those from 3 and 4. However, the flavour of the unfermented grits of 1 and 2 were not obviously disagreeable; whether or not grits were soaked overnight made very little difference in making good tempeh. Washing also removed some of the colour from the beans. However, if the water from soaking and cooking was not discarded, poor tempeh resulted.

One may conclude that a factor or factors present in the soybean grits needs to be washed out to get a good fermentation. The inhibitory factor must be heat-stable and water-soluble. The inhibition of mould development offered the possibility of using *R. oligosporus* as an assay organism for the isolation of the factor which might be important not only in tempeh but also in soybean milk, soybean flour and other soybean products with respect to their food value. Whether the factor has an effect on higher organisms is not known. When whole soybeans instead of grits were prepared and when no water was discarded in the process, a poor fermentation resulted, as described here. The actual amount of substrate metabolized indicated a considerable reduction over that metabolized in making normal tempeh. This factor is present not only in the cultivated variety Hawkeye, but also in 15 other varieties examined from the United States and Japan in varying amounts. Of the varieties tested, Norchief appeared to have more of the factor present than any of the others tested, but made excellent tempeh when the factor was removed.

In making tempeh, the cooked soybeans are spread out to cool and to dry the surface of the beans before inoculation with *Rhizopus*. This permits some bacterial contamination to occur from the air. The possibility exists, therefore, that growth-promoting substances for bacteria might remain in the soybeans if the water is not discarded, thereby promoting bacterial growth which in turn restricts the growth of *Rhizopus*. Accordingly, tempeh was prepared deliberately to give poor tempeh, and at the time of inoculation varying levels of tetracycline from 5 to 25 μg/g were added to the beans. At the end of the fermentation, all the tempeh was uniformly poor. When the tempeh was examined for bacteria by streaking out some of the material on nutrient agar, no bacteria could be detected in the tempeh in which 20 and 25 μg/g of tetracycline had been added. Their absence would seem to eliminate the possibility of bacteria interfering with the development of the mould.

C. W. HESSELTINE
R. DE CAMARGO
J. J. RACKIS

Northern Utilization Research and Development Division,
Agricultural Research Service,
U.S. Department of Agriculture,
Peoria, Illinois.

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A Glass Micro-bead System for the Investigation of Soil Micro-organisms

INVESTIGATION of the microbial flora of soils is greatly complicated by the physical complexity of soil as a medium. There are, of course, many biochemical and respirometric studies of the activity of micro-organisms in soil which treat it as though it is a homogeneous tissue. In fact, however, the system lacks continuity and consists of innumerable semi-independent micro-habitats. Soil is a porous medium with large internal surface. The distribution, retention or movement of water in soil depends on capillary and surface forces, and is of great moment in determining the distribution of micro-organisms and the characteristics of the micro-habitats that can be colonized if substrate is present. Most natural soils are texturally heterogeneous with the result that the behaviour of micro-organisms in them is the summation of many different situations.

In attempts to obtain a clearer understanding of factors affecting the distribution of bacteria and fungi in soils we have made use of a growth medium comprising glass micro-beads of uniform size. With such a system it is possible either to calculate, or to determine directly, such properties as internal surface, pore size, porosity, moisture tension relationships and water distribution, as some soil physicists have shown[1,2]. If soluble nutrients are supplied in the water, microbial growth and activity can be followed by a diversity of appropriate procedures.

Although micro-beads are available in a wide range of sizes (Microbeads, Inc., Jackson, Mississippi), we have carried out orienting experiments with two sizes only—37μ diameter and 149μ diameter (referred to in this communication as small and large beads respectively). Prior to their use in microbiological work the beads, as supplied by the manufacturer, require rigorous cleaning with acid followed by rinsing and decantation with distilled water to remove surface alkali. Washing must be continued until the *p*H of water in equilibrium with the beads is close to that of the distilled water used. Table 1 gives observations of some physical properties of the two sizes of micro-beads used. It will be noted that there is a four-fold difference in internal surface between the large and the small beads, and that at 100-cm tension the small beads retain 40 per cent more water than the large.

For microbiological studies a constant aeration procedure has been used in which each micro-bead system, contained in 8-oz. glass tumblers, is ventilated with moist, sterile air free from carbon dioxide at a controlled rate of flow of 9 l./h. The bead systems are initially wetted with nutrient liquid into which is incorporated the inoculum of the organism or organisms being examined. Measurement of the amount of carbon dioxide evolved by the microbial population of the bead medium is made by passing the effluent air stream into 0·75 N sodium hydroxide. Carbonate formed is then precipitated by addition of 25 per cent barium chloride and excess base determined with standard hydrochloric acid in an automatic titrator.

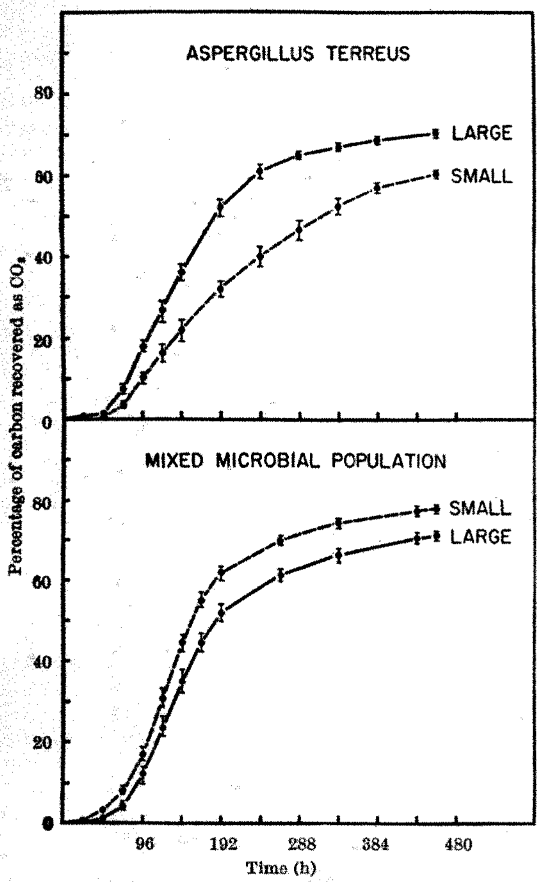

Fig. 1. Percentage of glucose carbon recovered as carbon dioxide from glass micro-bead media inoculated with (upper curves) *Aspergillus terreus* or (lower curves) a mixed soil microbial population. Systems consisted of 100 g of small beads (37μ mean diameter) or large beads (149μ mean diameter) plus 2 g glucose at 100 cm moisture tension. Each point is the mean of five replicates; limits of variation shown by vertical lines

Table 1. PHYSICAL PROPERTIES OF TWO SIZES OF MICRO-BEADS

	Large beads	Small beads
Mean diameter (μ)	149	37
Total surface (open pack) (cm²/g)	140	564
Bulk density (g/c.c.)	1·52	1·45
Particle density (g/c.c.)	2·50	2·50
Pore space (percentage of total volume)	39	42
Moisture retention at 100 cm tension (per cent, oven dry weight)	17·5	24·5
Theoretical values assuming perfect spheres		
Bulk density (g/c.c.)	Close pack	1·85
	Open pack	1·35
Pore space (percentage of total volume)	Close pack	26·0
	Open pack	47·6

At present we are following the rate of glucose degradation by various micro-organisms and mixed populations at the same moisture tension in systems comprised of small or large beads. Developmental changes in the numbers or distribution of the organisms are followed by sampling, plating and by direct and indirect examination of the bead mass. In this work 100-g samples of micro-beads have been initially wetted with nutrient containing 2 per cent glucose, with determinations of evolution of carbon dioxide and other changes at intervals. Fig. 1 gives information on the percentage of glucose carbon recovered as carbon dioxide over a 450-h period from bead systems inoculated with *Aspergillus terreus* compared to systems inoculated with a mixed microbial population of unknown constitution, derived from a soil suspension. The behaviour of replicate units in such experiments has been remarkably uniform. In experiments so far, we have observed that the micro-bead size by affecting the microbial environment clearly influences microbial distribution and activity.

Calculations of microbial activity on a known substrate (using evolution of carbon dioxide as the index of activity) can be related to other observations, and the effects of physical changes in the environment can be followed.

It appears that the use of micro-bead media has considerable potential for studying the growth and activity of soil micro-organisms, allowing, as it does, the construction of closely defined solid–liquid–gas systems, akin to soils, into which a wide range of energy and nutrient sources can be incorporated. The energy source may be soluble and therefore continuous, or insoluble and discontinuous. Micro-organisms may be examined in pure cultures or in known combinations. Water relationships and internal surface may be varied by the selection of appropriately sized beads or the incorporation of a homogeneous clay. Changes in gas diffusion rates accompanying colonization of the water film can be followed experimentally in such systems. The micro-bead media may also be used as a rooting material for seedlings of higher plants and thus applied in studies on root-microbe interactions or rhizosphere ecology.

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J. F. PARR
D. PARKINSON *
A. G. NORMAN

Department of Botany,
University of Michigan,
Ann Arbor.

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* Permanent address: Hartley Botanical Laboratories, University of Liverpool.

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Branch, Stem and Collar Rot of Apple caused by *Phytophthora* Species of the '*cactorum* Group'

BARK rot of apple trees, caused by *Phytophthora cactorum* (Leb. and Cohn) Schroeter, has been reported from most of the world's major apple-growing areas. Outbreaks of this soil-borne disease are often epidemic in character and may result in the complete bark-girdling and death of many trees.

Both *P. cactorum* and the closely related *P. syringae* Kleb. have been shown to be pathogenic to apple bark[1-3]; but a recent report from Holland[4] is the only published record of crown or collar rot of apple caused by *P. syringae*. This fungus has been isolated from naturally infected trees in Essex, Kent and Suffolk, and it now appears possible that *P. syringae* bark rot may be more widespread in England than that caused by *P. cactorum*, which in Europe and New Zealand primarily affects the variety Cox's Orange Pippin. *P. syringae* has been isolated from mature trees of Cox's Orange Pippin, Worcester Pearmain, Laxton's Fortune and Superb, and Miller's Seedling, and from one-year-old trees of Cox, Worcester and Tydeman's Early Worcester. *P. syringae* thus appears to be capable of attacking a wider range of varieties under orchard conditions than *P. cactorum*.

Disease outbreaks in 20–30-year old trees tend, like those caused by *P. cactorum*, to be widespread in the first instance. Trees are rarely completely girdled before external symptoms are visible; bark lesions frequently extend up the trunk in narrow tongues seldom spreading more than one-third around it.

Inoculation investigations indicate that susceptibility to both species varies markedly with the stage of host development, and that *P. syringae* can cause extensive damage at low temperatures in February and March when inoculations with *P. cactorum* have frequently failed. Resistance to both pathogens is lowest during and for some weeks following budburst. Extension of established *P. cactorum* lesions may continue in the subsequent summer and winter, whereas *P. syringae* lesions are often arrested in the summer; subsequent bark splitting and callus formation around the lesion may stop further extension and trees then recover without treatment.

Both species are difficult to isolate from rotted bark, especially *P. syringae* which grows and produces sporing structures very slowly. In the most successful isolation method, 5-mm cubes of bark from the margins of active lesions were irrigated in running tap water for 24–48 h and then incubated at laboratory temperature in a shallow layer of still water for a further 12 h. Under a wide-field binocular microscope *P. cactorum* was detected by the presence of the characteristic, coarse, irregular hyphæ, frequently bearing sporangia. The hyphæ of *P. syringae* were less readily seen for they protruded only a short distance from the bark blocks and rarely formed sporangia. Suspect hyphæ were inserted into apple fruits on which firm, brown, somewhat irregular lesions developed after 5–10 days and isolations from the margins of these on to cornmeal agar generally yielded pure cultures of the pathogen. Incubation temperatures should not exceed 20° C. Most isolates of *P. cactorum* readily produce sporangia and oospores, but *P. syringae* does so very sparsely and may fail to form oospores except in old cultures. Many *P. syringae* isolates form characteristic complex chains of hyphal swellings. All isolates of *P. syringae* have failed to grow at 25° C, the optimum temperature for *P. cactorum*.

Investigations of *Phytophthora* spp. in orchard soils have revealed marked differences in their seasonal activity, and also that the third member of the '*cactorum* group', *P. citricola* Sawada (syn. *P. cactorum* var. *applanata* Chester), is widespread and abundant. This species causes black root rot of hops[5], and as certain isolates have been shown by the authors and Smith[6] to be very mildly pathogenic to apple bark, all species of the '*cactorum* group' may be implicated to varying degrees in collar and crown rot diseases of apple. The Malling and Malling–Merton apple rootstocks have been tested[2] for resistance to *P. cactorum*; tests with *P. syringae* are in progress together with comparative studies of the resistance of apple scion varieties to both pathogens.

G. W. F. SEWELL
J. F. WILSON

East Malling Research Station,
Maidstone,
Kent.

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Apple Scald Induction by Anaerobiosis

SCALD is a physiological disorder which seriously affects the appearance and utility of the apple fruit. It initially appears during, or following, cold storage as a brown discoloration of the epidermis of the green-coloured portion of the fruit. With advancement, it affects the anthocyanin-bearing epidermis, the hypodermis and the adjacent cortex. Extensive investigations over the past 60 years have provided several theories[1-7]; but relatively little knowledge pertaining to the cause of scald. Several means of control have been devised[2,8,9]. As early as 1903[1], it was observed that scald formation was influenced by the availability of oxygen to the fruit. Our results now provide a basis for defining the ætiology of apple scald in terms of basic cause and effect components, anaerobiosis and aerobiosis.

Fruits of the McIntosh and Red Rome varieties of apple (*Malus sylvestris*, Miller) stored for 3 months at 0°–2° C were used for this investigation. Scald-free apples were placed in chambers at 20° C through which either air or nitrogen gas was supplied for various durations at a rate to provide two atmosphere changes per hour. The fruits were examined for scald at the termination of nitrogen

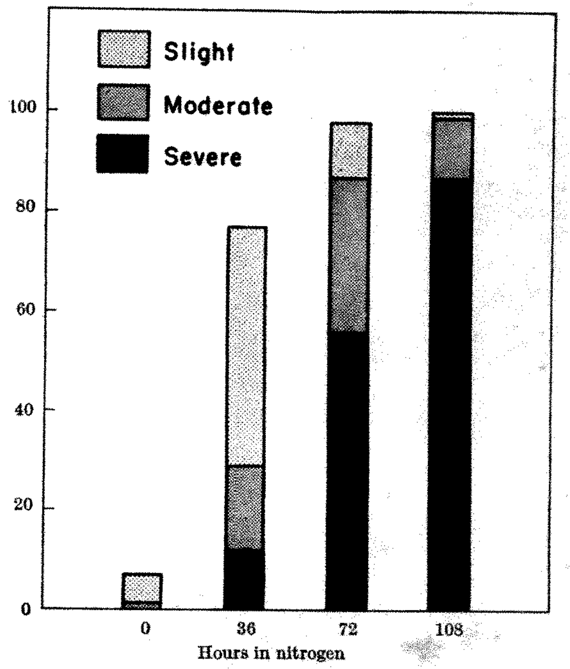

Fig. 1. Percentage and severity of scald on McIntosh apples following anaerobiosis treatments after 7 days in air at 20° C

treatments and subsequent periods in air at the same temperature. McIntosh fruits exposed to nitrogen for 36, 72 or 108 h were free of scald on removal, but afterwards developed scald on exposure to air (Fig. 1). The incidence and severity of scald were directly proportional to the duration of anaerobiosis. The rate of scald development in air following nitrogen induction was considerably lower at 0° C than at 20° C. This dependence on temperature clearly links the developmental phase of this disorder with aerobic respiration. Thus, scald was induced anaerobically and developed aerobically.

To relate physiologically the symptoms of induced and naturally occurring scald, Red Rome apples, which had been treated prior to storage with diphenylamine (DPA), a scald inhibitor[8], were compared with a comparable non-DPA-treated sample. One-half of each fruit sample received a nitrogen atmosphere for 108 h. The other half was maintained in air for the same period. All fruits were evaluated for scald after an additional 36 h in air. Diphenylamine significantly inhibited or retarded scald development on apples exposed to 0–108 h of nitrogen (Table 1). The scald-inhibiting properties of DPA counteracted or reduced the inductive influence of anaerobiosis on scald development aerobically. Therefore, it is likely that naturally occurring scald and anaerobically induced scald are the same disorder.

Table 1. INCIDENCE OF SCALDED RED ROME APPLES 36 H AFTER 108 H OF AEROBIC OR ANAEROBIC ATMOSPHERES AS AFFECTED BY PRE-TREATMENT WITH 2,000 P.P.M. DIPHENYLAMINE (DPA)

Atmosphere	Mean per cent scalded fruits*	
	Non-treated	Treated
Aerobic (air)	9·4*a*	0*a*
Anaerobic (N_2)	34·4*b*	7·3*a*

* Means followed by dissimilar subscripts differ at odds of 19 : 1.

Apparently, apple scald is composed of two physiologically distinct phases, one of anaerobic induction and one of aerobic development. This pattern is compatible with most theories on development and control of this disorder. The mechanism of scald induction and development is under investigation.

D. R. DILLEY
R. R. DEDOLPH
D. C. MACLEAN
D. H. DEWEY

Department of Horticulture,
Michigan State University,
East Lansing, Michigan.

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Reciprocal Effects of Insect and Plant-growth Substances

INJECTION of homogenates of active prothoracic glands into fourth instar locust larvæ, a day after moulting, causes them to moult again prematurely. The instar is shortened by about 12–18 h (Fig. 1). The active agent appears to be an ecdysone (which we have named ecdysone-λ). Injection of those fractions of extracts of whole locusts that contain ecdysone-λ have a similar effect. The plant growth hormone gibberellic acid likewise shortens the instar when injected at the appropriate time into larvæ of both *Locusta migratoria migratorioides* (R. and F.) and *Schistocerca gregaria* (Forskål) (Fig. 1).

Blood taken from locusts about to moult and ecdysone from *Bombyx* (ecdysone-α) have specific effects on the nervous system of locusts that results in reduced locomotory activity[1]. Fresh homogenates of active prothoracic glands of locusts, solutions of gibberellic acid and locust extracts containing ecdysone-λ all have a similar effect.

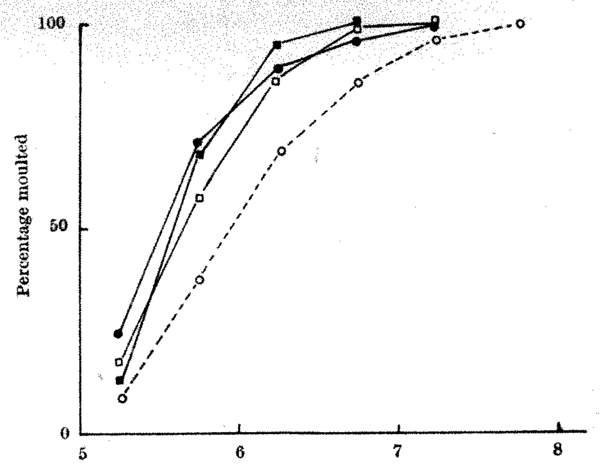

Fig. 1. Cumulative percentage of locusts moulting in successive periods of 12 h after injection of: ○ -- ○, solvent alone; ■, fresh homogenate of prothoracic gland, one gland per animal; □, whole-locust extract; ●, gibberellic acid, 1 μg per animal. 366 fourth instar *Locusta migratoria* were used and injected 18–30 h after the previous moult

Growth of the internodes of dwarf pea plants is greatly increased by the addition of gibberellic acid. Fractions of the locust extracts have been tested on dwarf pea seedlings, var. 'Meteor', in a standard bioassay for gibberellin activity[2]. Those fractions containing large amounts of ecdysone-λ exert a significant stimulation of growth (Fig. 2), corresponding to approximately 10 per cent of that exerted by gibberellin-A_3.

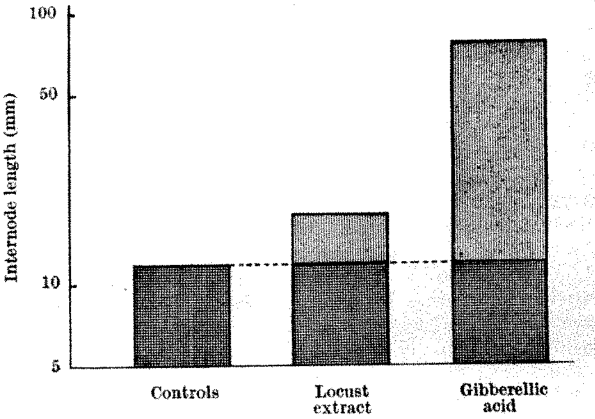

Fig. 2. The mean internode length in dwarf peas var. 'Meteor' treated with solvent alone ('controls'), with the same locust extract as that used in the the experiment illustrated in Fig. 1, and with gibberellin-A_3. The dose of the latter material was chosen to produce optimal growth. 108 plants were used; 3 internodes per plant

Thus the insect growth-substance ecdysone-λ and the plant growth-substance gibberellic acid have similar effects on both plants and locusts.

D. B. CARLISLE
D. J. OSBORNE
P. E. ELLIS
J. E. MOORHOUSE

Anti-Locust Research Centre,
1, Princes Gate,
London, S.W.7,
and
Department of Agriculture,
Oxford University.

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MICROBIOLOGY

Differences in the Mode of Action of Strepzyme and *Helix pomatia* Enzyme Preparations on *Trichotecium roseum* Spores

For the past months, we have been examining the lytic agents affecting yeast and mould cell-walls. Micro-organisms capable of lysing the cell-walls of these organisms were isolated from soil samples. The digestion of the cell-wall material of mould spores, by means of a partially purified enzyme produced by the *Streptomyces GM*, has been described already in a previous communication[1]. Some of us, using the method of digestion by the strepzyme or the gastric juice of the snail *Helix pomatia*, have published several reports on their investigations with protoplasts prepared from various moulds[2-5]. The results obtained clearly demonstrated that protoplasts of *Trichotecium roseum* could be produced by using the lytic enzymes under appropriate experimental conditions.

This communication describes the production of protoplasts from *T. roseum* spores and the differences in the mode of action of the lytic systems used during this investigation. As test organism a young sporulated culture of *T. roseum* was used, growing it at 28° C for 4 days in a medium of the following composition: glucose 10 g; asparagine 1 g; yeast extract 0·5 g; K_2HPO_4 0·5 g; $MgSO_4.7H_2O$ 0·5 g; $Fe_2(SO_4)_3$ 0·01 g; tap water 100 ml. and distilled water 900 ml.

Spores were recovered from the media by careful shaking with sterile distilled water and sedimentation in a low-speed centrifuge. 0·5 ml. of washed suspensions of spores, adjusted at about 10,000 spores per ml., was incubated with 0·5 ml. of the concentrated lytic enzyme solutions (strepzyme or a gut juice preparation from *Helix pomatia* obtained from L'Industrie Biologique Française) in the presence of 0·1 M phosphate buffer *p*H 6·8 or 0·1 M phosphate–citrate buffer *p*H 5·8, both containing 0·8 M mannitol as a stabilizing agent. The digestion of the cell wall was allowed to take place with gentle shaking at 25° C for 16 h for the liberation of the protoplasts.

Examination under phase-contrast microscope shows that when the gut juice preparation from *Helix pomatia* is used the first visible effect of the enzyme is that the spores become partially detached from the cell wall. The rupture of the cell wall starts at the polar end of the cell that was in contact with the conidiophore and then the other one. The protoplasts can be seen to emerge through the aperture attaining a spherical shape (Fig. 1). The protoplast nature of these structures can be demonstrated simply by dilution of the medium with water when the protoplasts will burst.

On the other hand, when a *T. roseum* spore undergoes the digestive treatment using the strepzyme it was seen that, as previously described, the lytic attack on the cell-wall and the cross-wall is very uniform, giving rise to the formation of two protoplasts which rise together and at the same time from the previous double-dotted cell spore.

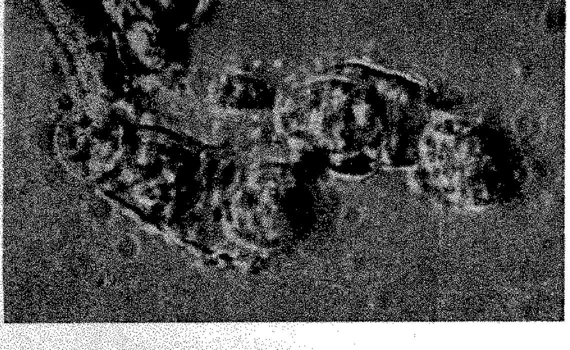

Fig. 1. Shows protoplast formation, after the digestion with the enzyme preparation from *Helix pomatia*. The protoplasts leave the cell-wall at both ends of the double-dotted cell spore of *T. roseum*

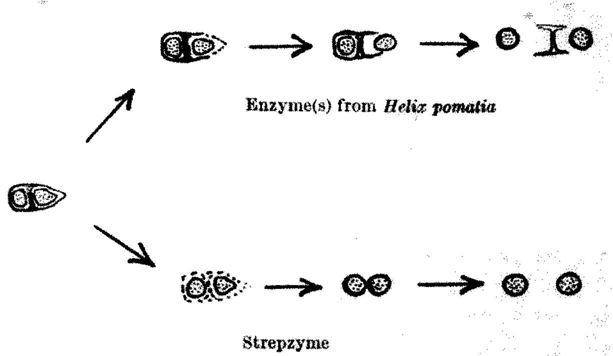

Fig. 2

Fig. 2 shows, diagrammatically, the two different stages in the release of protoplasts from the double-dotted cell spores of *T. roseum*.

These preliminary experiments have shown that the strepzyme produces protoplasts from *T. roseum* spores completely deprived of residual cell-walls which represents a great advantage on the enzyme preparation from *Helix pomatia*.

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Isabel Garcia Acha
J. R. Villanueva

Instituto "Jaime Ferrán" de Microbiologia,
Velazquez 138,
Madrid-6, Spain.

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A Rapid Method for Determining Phytase Activity of Soil Micro-organisms

During an examination of the breakdown of organophosphorus compounds by soil micro-organisms it was found necessary to determine phytase activity in a large number of isolates. The following modification of a recently published technique[1] for the detection of glutamic acid decarboxylase in bacteria proved suitable for this purpose.

Organisms to be examined were grown as streak cultures on glucose–nutrient agar plates incubated at 23°. When sufficient growth had been obtained it was treated with toluene vapour, as described by Stewart[1], and transferred by means of a platinum loop to small tared filter paper disks (7/16 in. diam. Whatman No. 1 filter paper) in 30-mg (wet weight) amounts. A torsion balance was used for rapid weighing in this operation. Organisms (for example, Actinomycetes), the growth of which is difficult to remove from agar, were grown on 'Cellophane' laid on the solidified agar surface. This facilitated removal of the growth and prevented its contamination with agar. After weighing, the disks plus organism were inverted on to glass coverslips and gentle pressure was applied to ensure the formation of a uniform layer of organism between disk and coverslip. Sodium phytate solution (0·01 ml. containing 20 μg phosphorus) was added from an 'AGLA' micrometer syringe (Burroughs Wellcome, Ltd.) to the disks, which were then incubated at 23° for 3 h in a moist atmosphere. Control disks were prepared in exactly the same way except that the sodium phytate was added at the end of the incubation period. In general the *p*H of both sets of disks fell within the range of

4·5–5·5. To stop further activity after incubation, one drop of 10 per cent trichloroacetic acid was added to both sets of disks, which were then extracted in a little water, centrifuged and the supernatants analysed for inorganic phosphate by a modification[2] of the method of Dickman and Bray, using an EEL colorimeter with red filter *OR*1. Table 1 gives the results obtained for a number of organisms.

Table 1. PHYTASE ACTIVITY OF MICRO-ORGANISMS

Organism	Inorganic phosphorus in control disk (μg)	Inorganic phosphorus in experimental disk (μg)	Percentage hydrolysis of sodium phytate
Aerobacter aerogenes	5·0	15·0	50
Rhodotorula sp.	1·25	4·75	17·5
Streptomyces sp.	7·00	10·5	17·5
Bacillus sp.	15·0	25·0	50
Unidentified short Gram-negative rod	8·0	28·0	100
Unidentified Gram-negative pleomorphic rod	9·75	17·25	37·5

The reproducibility of the method was checked using a strain of *Aerobacter aerogenes* and the following results, expressed as μg phosphorus released, were obtained for five replicates disks: 10·0, 9·5, 9·80, 10·0, 10·5.

This method has shown that 30–50 per cent of the organisms in soil and on plant roots possess an enzyme capable of hydrolysing sodium phytate with consequent release of inorganic phosphate. Further details of this work will be published elsewhere.

We thank Dr. F. W. Norris of the University of Birmingham for supplying the pure recrystallized sodium phytate used in this work.

M. P. GREAVES
G. ANDERSON
D. M. WEBLEY

Macaulay Institute for Soil Research,
Craigiebuckler,
Aberdeen.

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Facilitation of the Removal of the Total Surface Growth of Bacteria from Solid Media

To make a suspension of bacterial colonies by removing them from the surface of culture media, using a wire loop, is often difficult. In using a wire loop for this purpose the growth generally slides over the wire of the loop, and the complete removal of all surface growth is thus rendered both difficult and time-consuming.

It has been found that if a wire loop is first charged with a film of liquid before attempting the removal of surface growth and then run across the bacterial colonies growing on the plate, the film of liquid allows the colonies to collect in the loop.

In practice the technique used is to sterilize the loop (2·5 mm diam.), cool in the suspending liquid, remove the loop being careful not to break the liquid film, touch off the excess liquid, run the side of the loop across the plate with the loop at right angles to the surface of the agar, continue running the loop across the bacteria until completely full, shake off into the suspending liquid and start the cycle again.

By using this technique the whole growth from a standard Petri dish can be removed in ten such operations. The use of this procedure reduces the risk of contamination associated with the more usual techniques utilized for this purpose.

P. G. D. NAYLOR

Clinical Bacteriology Research,
Pfizer Ltd.,
Sandwich, Kent.

ENTOMOLOGY

Oviposition on Dry Surfaces by *Anopheles* (*Cellia*) *gambiae* Giles

UNTIL now, it has been held that, before any species of mosquito will oviposit, water must be present as surface moisture or as a collection[1]. Some forty-two years ago, however, Wesenberg-Lund[2] recorded *Aedes* (*Ochlerotatus*) *communis* (De Geer) ovipositing among superficially dry leaves on a dried lake bed and under leaves in the laboratory, and concluded that this mosquito probably lays its eggs on dry earth. The observations recorded here have shown definitely that *Anopheles* (*Cellia*) *gambiae* Giles will oviposit on absolutely dry surfaces (at least in the laboratory), if the gravid female ready to lay its eggs is denied access to water. The eggs so oviposited, however, fail to turn black and are not viable. A survey of the literature locally available has revealed no definite previous record of oviposition on dry surfaces by mosquitoes.

Fully gravid *A. gambiae* females were put in 8 in. × 8 in. × 8 in. and 11 in. × 11 in. × 10 in. cages, in lots of 50 and 100–200 per cage, respectively. Each cage had a sheet of dry white blotting paper covering the entire floor area. No water was provided for oviposition. The mosquitoes were maintained at 25° C and 85 per cent relative humidity, and were subjected to 12 h light and 12 h dark in each 24 h.

After a day or two, several of the females in a cage were observed to have flat abdomens (that is, in Sella's stage I[3]). At first, this was puzzling, since, at a casual glance, the familiar black eggs of mosquitoes could not be seen on the blotting paper. It was only later that thorough examination with a magnifying glass revealed numerous off-white eggs on the blotting paper, indicating that oviposition was taking place in the absence of water.

It was assumed that since mosquito eggs on a dry surface are subjected to maximum exposure to oxygen, this should facilitate tanning and melanin formation. Yet eggs oviposited on dry surfaces failed to darken. It was then thought that perhaps a gravid mosquito denied access to water, when it is ready to oviposit, might undergo some physiological change affecting the eggs; so that when and wherever these are laid, darkening would be inhibited anyway. This hypothesis was tested using the arrangement shown in Fig. 1. Those eggs which were trapped into the funnel and succeeded in reaching the water below, through a very tiny hole at the apex of the filter-paper cone, became black and were viable; those which fell on to the dry blotting paper on the floor or remained on the dry surface of the filter-paper cone retained their pale coloration and were not viable. This indicates that eggs oviposited on dry surfaces are basically normal. With regard to coloration, it might well be that eggs laid on a dry surface would lose water from the chorion so rapidly that this would inhibit darkening (Prof. V. B. Wigglesworth, personal communication), and it is known that if the eggs of anopheline mosquitoes are removed from contact with water immediately after being laid, they retain their pale coloration[4].

Whether oviposition on dry surfaces occurs under natural conditions is at present unknown; but the presence, during the dry season, of *A. gambiae* females with well-developed eggs and ready to oviposit as soon as a certain degree of mean relative humidity is reached has been reported by a number of workers[5]. A comprehensive study of the age composition and oviposition habits of dry-season populations of this mosquito might prove to be of more than academic interest; for if it can be shown that oviposition on dry surfaces does occur in the field (with the result that further blood-taking is not held up), this would contribute immensely to our understanding

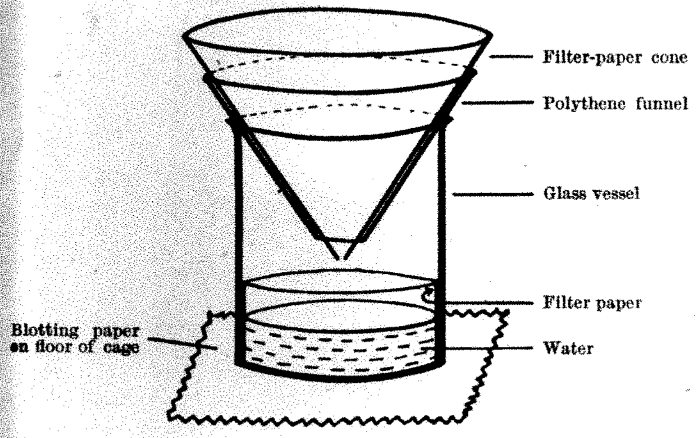

Fig. 1. Method of trapping into water eggs laid on a dry surface

of the continuity of endemic malaria in the savanna regions of Africa.

I thank Prof. V. B. Wigglesworth for his advice.

L. K. H. Goma

E.A. Virus Research Institute,
P.O. Box 49,
Entebbe,
Uganda.

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Parthenogenesis in the Cattle Tick, *Boophilus microplus*

Although bisexual reproduction is usual among ticks, there have been reports of parthenogenesis in various species including *Amblyomma agamum*[1], *Rhipicephalus bursa*[2], *A. dissimile*[3], *Hyalomma anatolicum*[4], *Ornithodorus moubata*[5], and *Haemaphysalis bispinosa*[6]; but there appears to be no previous record of parthenogenesis in *Boophilus microplus*. In fact, it was considered by many workers on the cattle tick in Australia that *B. microplus* females were unable to complete engorgement in the absence of males, and it was reported[7] that female cattle ticks isolated from males in small areas on a sheep by means of repellent barriers did not engorge completely. Furthermore, Riek[8] observed that unfertilized female cattle ticks remained attached to mice for considerable periods without engorging. In both instances the subsequent introduction of males led to rapid engorgement of the females.

It has now been shown that in the Yeerongpilly (Queensland) strain of *B. microplus*, 62 per cent of 185 females, freshly moulted from individually tubed, replete nymphs, engorged and detached from a steer in the complete absence of males. Fifty-five per cent of these unmated engorged females produced some viable eggs, the hatch of individual egg masses ranging from 0·1 to 48 per cent (mean of 2·2 per cent) when stored at 80° F and 80–90 per cent relative humidity. By comparison, it is usual under these conditions of temperature and humidity for more than 95 per cent of engorged females detaching from artificially infested cattle to lay large numbers of viable eggs, most egg masses having a high percentage hatch (Hitchcock[9] recorded a hatch of 84 per cent at 79° F and 85 per cent relative humidity). In another experiment, unmated females took significantly longer to engorge, and the mean engorged weight was significantly less than for mated females raised on the same steer. Unfertilized eggs had a longer minimum developmental period than fertilized eggs.

Various malformations appeared in parthenogenetic embryos and larvæ, but 2 per cent of parthenogenetic larvæ attached to a steer and reached maturity. All the adults detected, after careful searching of the 1-in.-diam. areas of skin exposed to ticks under the confining box, were female, suggesting a form of thelytoky. Seventy per cent of these fatherless females engorged to medium size or larger, 45 per cent gave eggs and 22 per cent produced progeny. Thus, about 0·25 per cent of first generation parthenogenetic larvæ were capable of reaching maturity and then of giving rise to a second generation without males. Only 2 second-generation fatherless females were obtained (no males), but one of these gave rise to third-generation parthenogenetic larvæ.

It is unlikely that a parthenogenetic laboratory strain of *B. microplus* will be established from this stock. However, the other exotic cattle tick in Australia (*H. bispinosa*) has been shown by Bremner[6] to reproduce by obligate parthenogenesis in one Queensland strain, males being rare and nonfunctional, although in Japan[10] a strictly bisexual race as well as a parthenogenetic race of this latter species is known to exist.

It is not certain whether this phenomenon demonstrated for *B. microplus* can be regarded as true facultative parthenogenesis or whether it is accidental parthenogenesis which, according to Bergerard[11], does not establish itself in Nature. Further work is also required to disclose the type of cytological mechanism involved, to see whether parthenogenesis occurs in other strains of the cattle tick and to determine if it has any significance so far as control and eradication programmes are concerned.

B. F. Stone

C.S.I.R.O.,
Division of Entomology,
Yeerongpilly,
Queensland.

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A Non-colonizing Aphid Vector of Potato Virus Diseases

It is now well established that migrating aphids alight in, and feed on, crops which they are unable to colonize[1], and Broadbent[2] has pointed out that such aphids may be responsible for the spread of virus diseases within those crops. An aphid which appears to fill this role in the case of two potato stylet-borne viruses, potato virus *Y* (PVY) and potato virus *A* (PVA), in certain districts of Northern Ireland, is *Brachycaudus helichrysi* (Kltb), the leaf-curling plum aphid.

The possibility that *B. helichrysi* might be responsible for the spread of these two diseases which occasionally occurs in Northern Ireland[3,4] was first considered in June 1961; at that time winged forms of the aphid were found feeding on about 40 per cent of the potato plants (3–6 in. high) in a field located near the site of an earlier outbreak of PVY.

In a small preliminary experiment to test the vector ability of the aphid, winged migrants, taken from infested damson (*Prunus* sp.) foliage, were allowed to feed on the sprouts of PVY infected tubers before being transferred to ten virus-free sprouting tubers. The test plants, together with controls, were grown under insect-free conditions and two of them developed symptoms of PVY; the infections were confirmed by sap inoculation to tobacco and by growing the progeny.

In 1962 a more detailed investigation was undertaken involving both PVY (20 tests on sprouting tubers of the variety Arran Pilot), and PVA (19 tests using the variety Ulster Torch). Winged aphids were collected from infested blackthorn (*P. spinosa.* L.) foliage and 'fasted' (1–24 h) before being fed briefly on virus-infected sprouting tubers. Following the acquisition feed the aphids (1–4 per test) were immediately transferred to the sprouts of healthy tubers, where they were left to feed, generally for two days, before being recovered and preserved as a check on their identity. The test tubers were all 'virus tested nuclear stock' material but, to provide additional evidence of their health, as many as possible were halved before use (PVY, 17 and PVA, 10) and the other half of each was used as a control. With each virus three positive transmissions were effected and of these only two, both PVA, lacked individual control halves. The presence or absence of virus infection in all test and control plants was determined by sap inoculations to tobacco, both from the parent foliage and from the sprouts of the tuber progeny.

The ability of the aphid to transmit these diseases under simulated field conditions was also demonstrated in an experiment using outdoor cages. Ten virus-tested nuclear stock tubers, variety Arran Pilot, were cut lengthwise and the halves of each planted in two separate cages; two tubers infected with PVY were also planted in the centre of each cage. During the period in which the plants were emerging through the soil, twigs of damson infested with *B. helichrysi* were introduced into one of the cages, but no aphids were added to the other cage, which was left to serve as a control. A similar experimental layout, but using tubers of the variety Ulster Torch, was utilized to test the aphid with PVA. Virus transmission was assessed by sap inoculation to tobacco; in the test cages four Arran Pilot plants were found to be infected with PVY and five Ulster Torch plants with PVA: no infections were discovered among the control plants.

The relative vector efficiency of *B. helichrysi* has yet to be determined, but as Kennedy[1] points out "the key considerations affecting the virus-spreading efficiency of a given aphid species are its ability to transmit the virus and the abundance and activity of its winged forms". The ability of this aphid to transmit PVY and PVA has been demonstrated, and in June of this year further evidence was obtained of the large numbers of spring migrants which settle into potato crops when these are situated near the aphid's primary hosts. Two such crops were kept under observation during the early susceptible[5] stage of growth and, at the height of the aphid's migration, vagrant alatæ were found feeding on 70–80 per cent of the plants, then no more than 9 in. high. The highest number of aphids recorded on a single plant was five and the average per infested plant examined was two.

From the evidence presented it is suggested that in years when the spring migration of *B. helichrysi* is heavy, and coincides with the emergence of the potato crop, this aphid can bring about a significant spread of either PVY or PVA should sources of infection be present. (Present experiments[6] have shown that the aphid will transmit yet another important stylet-borne virus of potato—the tobacco veinal necrosis virus, PVY^N.)

A. R. Edwards

Agricultural Entomology Division,
Ministry of Agriculture,
Northern Ireland,
The Queen's University of Belfast.

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CYTOLOGY

Chemical Nature of the System controlling Gene Activities in Insect Cells

Salivary glands of *Chironomus thummi* explanted into hæmolymph or saline revert their pattern of gene activities —visible in the giant chromosomes as puffs—to a more juvenile stage[1,2]. It has turned out that this process—called 'rejuvenation'—is due to an influx of Na$^+$ ions into the nucleus. By stepwise changing the Na$^+$/K$^+$

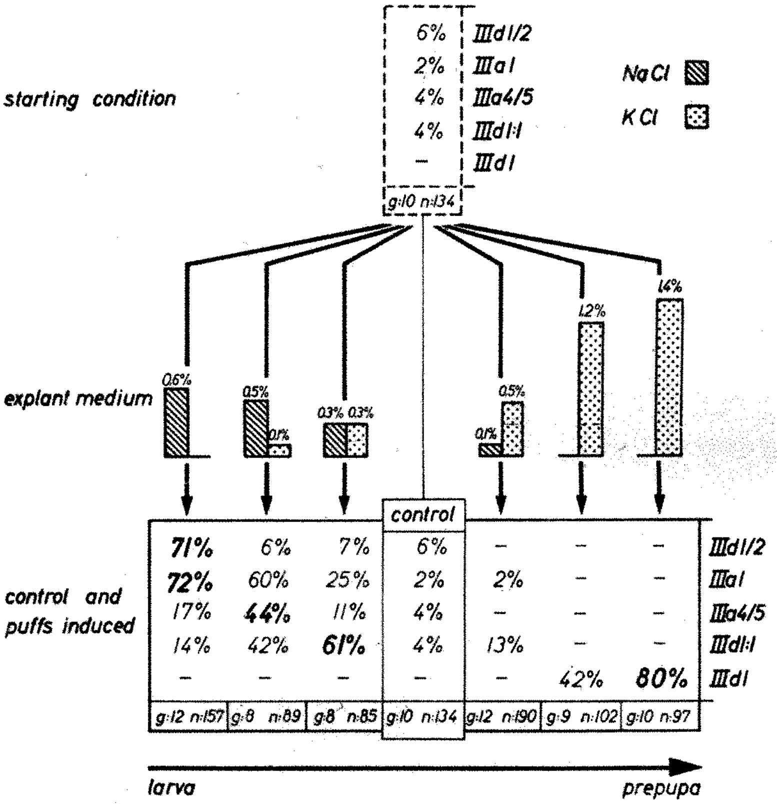

Fig. 1. Induction of puffs in chromosome 3 of *Chironomus thummi* by explantation of salivary glands into media possessing different ratios of sodium chloride and potassium chloride. All media contained 0·021 per cent calcium chloride in addition to the alkali chlorides. 10 control glands (2 arbitrarily chosen sister glands from each group of glands exposed to a different medium) serve to give the puffing pattern of the glands at beginning of experiment. Exposure of glands to explant medium, 2 h; *g*, No. of glands explants; *n*, No. of nuclei with fully analysable 3 chromosome. Figures in body of the Table give percentage of nuclei containing the respective puff. Bold-faced, highest percentage in which a puff is present. Symbols at right: regions (designation after Keyl (ref. 3)) in which the respective puffs appeared. (Illustrations of all puffs in Kroeger (ref. 2) except III *d* 1 : 1, a puff situated between III *d* 1 and III *d* 1/2)

balance in the medium it is possible to activate a graded series of genetic loci, every Na^+ : K^+ ratio inciting different groups of loci to form puffs (Fig. 1). The series of puffing patterns induced by stepwise replacing Na^+ by K^+ is identical with the sequence of puffing patterns which in salivary glands marks the transition from the adult larva to the middle pre-pupa.

In previous experiments[1,2] it was shown that the insect hormone ecdysone, and possibly also juvenile hormone, do not act directly on the genetic loci of a cell but instead exert their effect on a system in the nuclear sap which in turn controls the activity of at least a large fraction of the genetic loci in the chromosomes. It has been concluded that this control system can be present at different levels, each level inciting some loci to become active, others to become inactive. Since the stepwise replacement of Na^+ by K^+ produces the effect of those 'different levels', it seems now that the control system actually consists of the Na^+/K^+ balance in the nuclear sap; both hormones would then act by regulating the ratio between Na^+ and K^+, ecdysone increasing the K^+ concentration, juvenile hormone maintaining a high Na^+ level.

The existence of mechanisms for the extrusion of Na^+ in almost all somatic cells suggests that activation and inactivation of genetic loci by a progressive shift in the Na^+/K^+ balance in the nuclear sap may be a rather universal mechanism.

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H. Kroeger

Zoologisches Institut,
Eidgenössische Technische Hochschule,
Zürich.

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Differential Ribonucleic Acid Synthesis of *X* and Autosomes during Meiosis

SEVERAL investigations[1-3] of chromosomal RNA synthesis during mitosis have established that such synthesis, maximal in interphase, continues during mitotic prophase. It decreases progressively as chromosome contraction advances, none occurring during the fully contracted stages of metaphase and anaphase. RNA synthesis recommences with chromosome despiralization at late telophase.

Only two accounts of RNA synthesis throughout meiosis have been published[4,5]. In one, inorganic phosphorus-32, which is incorporated into DNA, RNA and protein, was used, while in the other, ^{14}C-glycine, ^{14}C-orotic acid and ^{3}H-cytidine were used. The relatively RNA specific precursor, uridine, was not utilized, and the results presented are not entirely unambiguous. RNA synthesis was shown to continue into early meiotic prophase but with some precursors a drop was reported at the time of DNA synthesis and at the zygotene pairing stages.

I have sought more information on this question by following the course of RNA biosynthesis autoradiographically through all stages of male meiosis in the locust *Schistocerca gregaria*, using ^{3}H-uridine. All autosomes actively synthesize RNA throughout the whole of first meiotic prophase. Leptotene and zygotene nuclei are evenly labelled after short exposures to the isotope. After longer exposures the two small nucleoli are noticeably more heavily labelled than the remainder of the nucleus. During pachytene and diplotene the truly lampbrush nature of the fuzzy bivalents is evidenced by their continued synthetic activity. Synthesis decreases progressively during diakinesis, as chromosome coiling becomes more and more complete, and has ceased by the time that nuclear membrane breakdown occurs. No RNA precursor is incorporated into any autosome during first metaphase or anaphase. During second prophase RNA is again synthesized by the uncoiled autosomes. Incorporation decreases as chromosome coiling becomes more advanced and it does not occur at the fully contracted stages of second metaphase and anaphase.

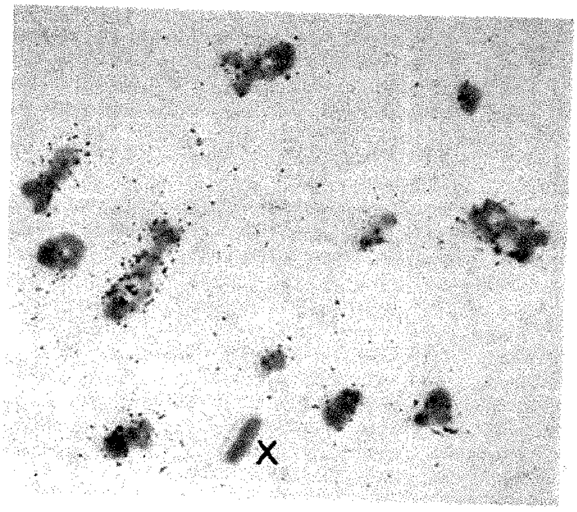

Fig. 1. Diakinesis in *Schistocerca gregaria* showing the distribution of newly synthesized RNA, labelled with ^{3}H-uridine. All autosomal bivalents are labelled, but the *X* univalent is not

Throughout most of meiosis the single *X*-chromosome is allocyclic relative to the autosomes. It is tightly coiled and heavily staining during both first prophase and, in those cells in which it is found, second prophase. In this condition it is synthetically inactive. No RNA precursor is incorporated and the *X*-chromosome is unlabelled throughout its entire length (Fig. 1).

S. A. Henderson

Department of Genetics,
University of Cambridge.

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Effect of *p*H and Temperature on Cell Re-aggregation

THE successful re-aggregation of cells has been used widely as a test for their adhesiveness[1,2]. Moscona[1] observed that the re-aggregation of chick embryonic cells does not occur at temperatures at or below 15° C. Steinberg[2] found that re-aggregated cells did not de-adhere at low temperatures and re-interpreted Moscona's results by suggesting that the initial adhesions of cells alone were prevented below such temperatures. Both authors interpreted their results to indicate that the adhesion of cells in re-aggregation involves a temperature-dependent mechanism. Similarly, Steinberg[3] argued that since amphibian embryonic cells could not re-aggregate at *p*H 4·0, carboxyl ionization and calcium-linked binding between cell surfaces are probably involved in cell adhesion. It remains possible that the failure of re-aggregation in these systems was due to the particular conditions of re-aggregation used, and that low temperature or *p*H values do not of themselves generally impede the adhesiveness of normal viable cells. Successful re-aggregation of cells at low temperatures or *p*H values will demonstrate that low temperature and *p*H do not in themselves necessarily affect cell adhesion. The following tests show that this suggestion is probably correct and that other conclusions can be drawn about the modes of cell adhesion.

Cells from 5-day embryonic chicks and gastrula stages of the amphibian, *Xenopus laevis*, were used. In examining the effects of temperature chick limb-bud or liver cells were used, being disaggregated by treatment for 5 min with 0·002 M *p*H 8·0 solution of ethylenediamine tetraacetate made up in Hanks's calcium- and magnesium-free saline, followed by washing and mechanical disruption in Hanks's calcium- and magnesium-free saline. The cells were centrifuged at 50*g* for 1 min to sediment all but single cells, and from the supernatant single cell suspensions about 1·5 million ml. were prepared in the culture media. Single cells accounted for > 98 per cent of cells in all suspensions. Four culture media were used; the first medium (1) was composed of horse serum (Burroughs-Wellcome No. 2), Medium 199 (Glaxo Laboratories) and Hanks's saline in equal proportions. This and all other media were sterilized and freed from dust by filtration ('Millipore', *HA* filters). The other media used in testing the effect of temperature on re-aggregation were as follows: (2) serum-free medium, equal parts of Hanks's saline and Medium 199; (3) medium 2 with 1/3 per cent v/v horse serum; (4) medium 2 with 3 per cent v/v horse serum. After suspension in one or other of the media (diluted with about 15 per cent Hanks's calcium- and magnesium-free saline) the cells were shaken in a constant temperature bath at 92 i.p.m. for 20–36 h. At 36° C re-aggregation, as judged by the appearance of macroscopic coherent re-aggregates and by the reduction of the population of single cells in the medium to about 0·08 million/ml., was extensive in all media. At 6° C or 3° C only one visible re-aggregate was found in 6 flasks using medium 1, though a certain degree of adhesion occurred since the single cell density was reduced to between 60 and 40 per cent of its initial value, numerous cell pairs, triplets, etc., being formed. In the serum-free media extensive visible coherent re-aggregation occurred and the population of single cells was reduced to about 0·08 million/ml. A similar situation was found in medium 3 but more single cells were found (mean 0·2 million/ml.) and rather fewer and smaller visible aggregates formed. With medium 4 a few visible re-aggregates formed; the single cell population remained high (mean 0·6 million/ml.).

The occurrence of re-aggregation at *p*H 4·0 was tested with both chick and amphibian cells. For chick cells prepared as above, medium 1 and the serum-free medium 2 were buffered at *p*H 4·0 with 0·004 M maleate or acetate-hydrochloric acid buffers or at *p*H 6·7 with added maleate or acetate (0·004 M). *p*H values were checked before and after re-aggregation. At *p*H 4·0 very slight precipitation of serum components occurs. Cells were shaken at 36° C for 36 h: no macroscopically visible re-aggregation of cells was observed in serum containing medium buffered at *p*H 4·0, though the proportion of single cells fell to the same level as in suspensions in which visible re-aggregation occurred. Re-aggregation at *p*H 6·7 was visible and extensive in both serum-containing and serum-free media. At *p*H 4·0 in serum-free media re-aggregation was extremely rapid, visible coherent re-aggregates appearing in 1 h and the population of single cells was reduced below detectable levels. Gastrula cells of the amphibian, *Xenopus laevis*, were disaggregated by a chelating agent technique[4]. The cells were re-aggregated in Holtfreter medium buffered at *p*H 7·2 or 4·2 with acetate or maleate buffers (0·004 M). In still unshaken medium at *p*H 7·2 re-aggregation is extensive whereas at *p*H 4·2 it apparently does not occur. However, it is known that cell movement is essential for amphibian re-aggregation and it was found that cell movement is inhibited at *p*H 4·2 (ref. 5). Providing cell movement artificially, by stirring or shaking the cells gently, produces aggregation at *p*H 4·2.

Warburg manometry of chick cells re-aggregated at 3° C in serum-free media showed that on rewarming the aggregates to 37° C they have the same high respiratory rate as cells re-aggregated at 37° C. This indicates that the chick cells are alive after re-aggregation at 3° C. Thus these results show that there is clear evidence that the re-aggregation of live cells is possible at low temperatures, and in consequence it cannot be argued, as Moscona did, that a temperature-dependent mechanism is necessarily involved in cell adhesion, or, as Steinberg maintained, that the first stage of adhesion in re-aggregation is temperature-dependent. Although examination of sectioned material suggests that re-aggregates formed at *p*H 4·2 are composed of viable normal cells this question of viability is of little importance since the arguments concerning cell adhesion expressed by Steinberg[3] were based on similarly treated cells. These results suggest that serum contains some factor or factors interfering with re-aggregation at about *p*H 4·0 and at low temperatures. The failure of Steinberg to obtain amphibian re-aggregation at about *p*H 4·0 can be explained as being due to the inhibition of cell movement, so that potentially adhesive cells were unable to make sufficient contact for large masses of cells to form. The fact that cells can form adhesions at about *p*H 4·0, where carboxyl ionization on their surfaces is suppressed[6], or at low temperatures, can be explained by lyophobic colloid theory of cell adhesion[5].

A. S. G. CURTIS

Department of Zoology,
University College, London.

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GENETICS

Natural Selection on an Isoallele

IN *Drosophila pseudoobscura* a genetic factor has previously been described[1], the only observable effect of which is to cause a small change in the banding pattern of the third polytene chromosome. This factor behaves in crosses as a simple recessive gene, and has been named 'salivary' (*sal*). Because of the recondite nature of its effect, *sal* may be considered an isoallele of a gene for normal banding pattern.

The population cage (*W*–11) on which *sal* was discovered contained the gene sequences Arrowhead (*AR*) and Chiricahua (*CH*). In this population only *CH* homokaryotypes exhibited the altered banding pattern. It may therefore be inferred that *sal* was located only on third chromosomes containing the *CH* gene sequence. That *AR* chromosomes do not in themselves inhibit the expression of *sal* will be shown here.

In population *W*–11 the *CH* gene sequence was anomalously fitter than usual[2]. In order to determine whether this situation was related to the presence of *sal*, three additional populations were set up in the manner described elsewhere[2] and maintained at 25° C. One population (*V*–12) contained normal *CH*, one (*V*–13) contained *AR*, and one (*V*–14) contained Standard (*ST*), each together with *sal*-bearing *CH* chromosomes. All the initial flies were F_1's of crosses between the stocks used, ensuring equal initial frequencies of the two genotypes in each case. Generation time under the conditions of this experiment has been estimated[2] at 29 days. Three hundred chromosomes were examined in each egg sample.

The observed frequencies of *CH* chromosomes are presented in Table 1 and Fig. 1, together with results of three comparable previous populations[3,4], each of which contained *AR* and normal *CH*. It has been found by others[5] that populations containing either *ST* and normal *CH* or *AR* and normal *CH* attain equilibrium frequencies of normal *CH* similar to each other. It is apparent from

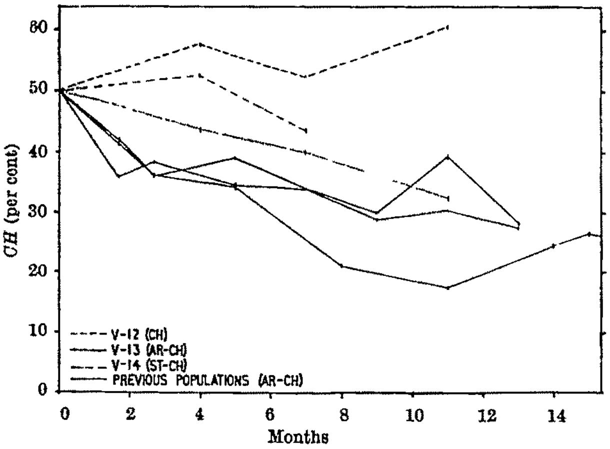

Fig 1 Frequencies of *CH* chromosomes in the populations For population *V*–12 the frequencies are the calculated frequencies of the *sal* gene

our results that *CH* chromosomes containing *sal* (and the alleles in its vicinity) are at least as fit (*V*–13) as, and are in some cases (*V*–12 and *V*–14) clearly fitter than, normal *CH* chromosomes In the absence of an attained equilibrium, no estimate is made of the relative fitnesses Population *V*–14 was terminated as a result of contamination with *D melanogaster*, and the others by accidental exposure to an insecticide

Table 1 Observed Percentages of *CH* Chromosomes in Population Cages

Month	*V*–13 (*AR-CH*)	*V*–14 (*ST-CH*)
0	50 0	50 0
4	43 7	52 7
7	40 0	43 7
11	32 3	—

Because *sal* is recessive, its frequency in a homokaryotypic population could be estimated only from the frequency of homozygotes The frequencies of the various *AR*, *ST*, and *CH* karyotypes in our populations followed the Hardy–Weinberg expectation, and it is assumed that the frequencies of the *sal* genotypes did also The observed frequencies of *sal* homozygotes in *V*–12, *V*–13, and *V*–14 are presented in Table 2, and the calculated frequencies of *sal* in *V*–12 are given in Fig. 1 Population *V*–12, containing only the *CH* gene sequence, confirms that *CH* chromosomes bearing *sal* are fitter than those lacking it

Table 2 Observed Frequencies of Homozygotes for *sal* in Samples from Population Cages The Numerator in Each Case represents Homozygotes for *sal*, the Denominator, all Larvæ of the Designated Karyotype

	V–12	*V*–13			*V*–14		
Month	*CH/CH*	*AR/AR*	*AR/CH*	*CH/CH*	*ST/ST*	*ST/CH*	*CH/CH*
4	$\frac{50}{150}$	$\frac{0}{52}$	$\frac{1}{65}$	$\frac{31}{33}$	$\frac{1}{35}$	$\frac{3}{72}$	$\frac{41}{43}$
7	$\frac{41}{150}$	$\frac{0}{61}$	$\frac{0}{58}$	$\frac{31}{31}$	$\frac{0}{52}$	$\frac{2}{65}$	$\frac{31}{33}$
11	$\frac{55}{150}$	$\frac{0}{68}$	$\frac{0}{67}$	$\frac{15}{15}$	—	—	—

There are a few instances (Table 2) of *CH* homokaryotypes lacking the phenotype of *sal* and of heterokaryotypes (and even of one *ST* homokaryotype) having this phenotype The most likely explanation of these unusual combinations appears to be crossing-over Incomplete penetrance of *sal* is ruled out by the presence (in equal frequency) of both kinds of expected recombinants and by tests of the homozygous *sal* stock A frequency of crossing over of 0 002 (0 2 recombination units) between the *CH* inversion complex and *sal* would (in the absence of greatly changed selection on recombinants) account for the observed frequencies of separation between them, within the limits of sampling error Results of crossing over in inversion heterozygotes of the third chromosome of *D pseudoobscura* have been published by others[6,7] The position of none of the mutants used in those experiments is known with sufficient precision[8] to be able to determine whether or not the frequency of 0 002 is a value of recombination expected between the distal end of the *CH* inversion complex and any more distal mutant, in a heterozygote with *AR* or *ST*. However, such a frequency is at least easily consistent with the available data

As usual in competition experiments with single genes, it is unknown whether the observed difference in fitness between *CH* chromosomes containing *sal* and those lacking it is due to a direct effect of *sal* itself (and if so whether it is a result of the effect on chromosomal banding) or to closely linked genes

These experiments were conducted in the laboratory of Prof Th Dobzhansky at Columbia University. During this time, one of us (L V) was the recipient of a Boese postdoctoral fellowship from Columbia University

Leigh Van Valen *

Zoology Department,
University College, London

Louis Levine

Biology Department,
City College of the City University of New York

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* Present address Department of Vertebrate Paleontology, American Museum of Natural History, New York

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Selection in Natural Populations: Human Fingerprints

In order to estimate the normal intensities of natural selection on quantitative characters, characters must be chosen for reasons other than the likelihood of natural selection operating on them[1] This communication deals with one suitable character

The data consist of total ridge-counts[2,3] of about 250 English families Counts for at least one parent and one child were available for each family used; usually the entire family was available The sample is not a random sample of any particular community, but is more or less representative of England as a whole The restriction to families ensures that the same bias is present in each generation, the possible effects of migration are thereby also excluded.

The use of comparisons between a set of parents and the set of their offspring as a means of investigating natural selection is to my knowledge new and requires comment It is not equivalent to determining the change, if any, between successive generations, because the generations were sampled at different ages Its main advantages are the relative ease of collecting results for man and the elimination of a possible bias due to differential migration; it has, however, relatively poor power For a less highly heritable character than ridge-counts, and especially for the variance of such a character, a difference between age-groups of the same generation, sampled at different times, may be present with only a much smaller difference between generations, even if reverse selection on fertility is absent For the variance this may or may not be true in the present case A change in environmental effects between generations would be confounded with a result of selection, but the presence of a total phenotypic change (including any change in environmental effects) can be tested for by comparing unrelated individuals a generation apart Strong differential mortality in this interval would, however, also produce a difference in the distributions of phenotypes.

Ignoring environmental effects and considering less highly heritable characters than fingerprints, the children are essentially a random sample of the population from which their parents were drawn. The two selective barriers which must be passed in this direction are survival for the length of one generation and the production of one or more viable children. Differential fertility, prezygotic selection, and prenatal and juvenile mortality are the selective barriers actually passed from the sample of parents to that of children. For a character as highly heritable as ridge-counts[4,5] only the second direction of comparison is valid. The statistics are the same for both directions.

The individuals were divided into four classes: fathers, sons, mothers, and daughters. No individual was included in more than one class, and the occasional twins were counted as two individuals. Means and variances of total ridge-count were computed for each class, and are given in Table 1. It is evident that no significant difference is present between the generations for either sex. The statistic Q, useful in comparing estimates of selection for several characters, is the ratio of the observed difference between means to the difference expected 50 per cent of the time by chance for the same sample size[1].

Table 1. STATISTICS FOR TOTAL RIDGE-COUNT (THE STATISTIC Q IS EXPLAINED IN THE TEXT)

	N	s^2	$\bar{X}$	F	Q	$C.V.$
Fathers	231	2,899	139·7 ± 3·5	1·10	1·83	38·5
Sons	283	2,645	145·5 ± 3·1			35·4
Mothers	249	2,749	127·3 ± 3·3	0·92	0·168	41·2
Daughters	276	3,001	126·7 ± 3·3			43·2

The high coefficients of variation are notable, particularly for a non-vestigial character. This is probably not due entirely to the character being composed of separately inherited divisions, because the three conventional classes of fingerprints (arch, loop, and whorl) more or less grade into each other and the total distribution of ridge-counts is bell-shaped[6]. It could be a result of the absence of any selection affecting this highly heritable trait, or it could be a result of heterosis, epistasis, or destabilizing selection, too weak to be detected by the present sample size.

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LEIGH VAN VALEN *

Department of Zoology,
University College, London.

* Present address: Department of Vertebrate Paleontology, American Museum of Natural History, New York.

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Allelism of Silver, Gold, and Imperfect Albinism in the Fowl

WERRET *et al.*[1] described a series of matings, starting with a non-silver male resulting from a cross of a Brown Leghorn sire by a Light Sussex dam, which suggested that the gene for silver (S) had mutated to that for semi-albinism, designated by them as s^{al}. As the mutant gene also proved to be recessive to that for gold (s), together they formed a series of three alleles at one locus on the sex chromosome. Mueller and Hutt[2] first described imperfect or semi-albinism (al), and afterwards Hutt[3] reported on the linkage relationships of his al to other genes on that chromosome. Although Hutt had no specific tests of linkage of al with S, both these genes were tested with others located nearby. Identical linkages (0·7 per cent) were found with the gene for dwarf (dw), but for the gene for rapid feathering (k), where there were more gametes tested and the map distance was considerably less, k was closer (1·6 per cent) to al than to S (2·8 per cent), a situation that did not suggest possible allelism. Hutt therefore placed al between S and k, a little closer (1·2 per cent) to the former.

Because of the apparent closeness of the two loci, the observations of Werret *et al.* could also have been explained by assuming a small deletion rather than a point mutation. It was also possible that they were dealing with a different mutation from that studied by Hutt, which is known to have occurred several times[4]. Either possibility could account for the poor growth rate and defective vision described by Werret *et al.* Neither condition was characteristic of the albinos studied by Hutt, nor is it of those at present on hand here.

(*a*) *A test to determine presence of S or s in albinotic females.* If the gene for albinism (s^{al}) be a third allele at the S–s locus, the hemizygous albinotic female can possess neither S nor s. A small group of albinotic females, derived from a different source[5], were therefore crossed to a Rhode Island Red sire. Some of the dams were heterozygous for I or E, or both genes. However, among the resultant chicks which could be classified for basic down colour (those with columbian plumage pattern, ee), none was silver male. This proved only that the albinotic dams did not carry S, but it did not prove that they did or did not carry s. These same albinotic dams were then mated to a Light Brahma male (ee) in which both sex chromosomes carried S and presumably Al. The male offspring that were silver (S ?) and showed columbian pattern (ee) were then backcrossed to Rhode Island Red females (s- and ee). The resulting progeny were all classifiable as silver or gold, or as albinos (females only).

Among 2,492 progeny from the six F_1 sires tested, the ratio of silver (S) to non-silver (s or albino) was 1,248 to 1,244. Among the 1,272 female chicks there were 657 non-albino silvers to 615 albinos, most of the latter showed a reddish tinge in their down. There were no gold females! If the original albinotic dams had carried gold (s) in addition to al, the F_1 sires would have been heterozygous for both genes ($S\ Al/s\ al$) and there could have been a crossover to yield a gold non-albinotic pullet ($s\ Al$ / – –). With the suggested map distance of 1·2 units between the two genes[3] there should have been at least 15 cross-overs. Half would have been silver albinos which might not be distinguishable from gold albinos. The others would have been gold females and there would be no doubt about classification. The fact that none of these was found indicates that the gene for albinism is an allele of S and s.

(*b*) *Evidence of normal crossing-over in the sex chromosome of sires heterozygous for albinism.* As the albinotic dams were rapid feathering (k –) while the Light Brahma sire was slow feathering (KK), the F_1 males were therefore dihybrids, Kk, Ss^{al}. The Rhode Island Red dams used to produce the back-cross progeny were gold and rapid feathering ($s\ k$ / – –). Thus the presence in the F_1 males of the genes for rate of feathering in the coupling phase with those for down colour made it possible to determine from their progeny the amount of crossing-over between the S and K loci. Classifications of all chicks were as follows:

Parental types			Cross-over types		
SK ♂♂	—	574	*Sk* ♂♂	—	17
sk ♂♂	—	610	*sK* ♂♂	—	19
SK ♀♀	—	651	*Sk* ♀♀	—	6
alk ♀♀	—	601	*alK* ♀♀	—	14
		2,436			56

Assuming that we are dealing with only two loci, the rate of crossing-over was 2·25 per cent, which agrees very well with Hutt's[3] 2·8 per cent and Jaap's[6] 1·3 per cent. The 14 slow-feathering albinotic pullets are proof that crossing-over occurred between genes at these two loci. It seems quite unlikely that if *al* lies between *S* and *k* all the cross-overs would have occurred between *al* and *k* and none between *s* and *al*. If *al* occupies a locus separate from that of *s*, but closer to it than Hutt's data would suggest, then the existence of two separate loci might not be established even with the analysis of more than 600 suitable gametes.

The original albinotic females obtained by Mueller and Hutt and by Cole both showed ghost barring and were probably sired by Barred Plymouth Rock males. In each case the sex chromosome, coming from a Barred Plymouth Rock, would have included the gene *S* closely linked to *al*, if it were not in fact an allele of *al*. However, in the case of the more recent mutation, all albinos tested (8) proved not to carry *S*. While these were 2 or 3 generations removed from the original albinotic dam, it seems highly improbable that *all* would have been derived by crossing-over and show now a very close linkage of *s* to *al*.

On the basis of our present evidence we must conclude that Werret *et al.* were correct in their interpretation that *S*, *s*, and s^{al} form a series of three alleles on the sex chromosome of the fowl.

R. K. Cole
T. K. Jeffers

Department of Poultry Husbandry,
New York State College of Agriculture,
Cornell University,
Ithaca, New York

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PSYCHOLOGY

An Ante-natal Determinant of Intelligence

Reliable investigations of intelligence suggest that at least 75 per cent of individual variations in ability are determined by inheritance[1]. The distribution of intelligence appears to be best described by a Pearson type IV curve[2] determined mainly by polygenic factors.

It would appear now that much of the remaining variance of intelligence can be attributed to factors operating during the embryonic development of the individual. One such factor appears to be that of season of birth as a reflexion of climatic conditions during pregnancy.

There have been in the past a number of investigations reporting small but consistent associations between psychological performance and seasonal changes, including season of birth[3]. Such investigations often involve more average ranges of ability than those to be described here. It seems unlikely, therefore, that the latter results are only applicable to the basically low level of this sample.

I have examined[4] a group of 188 adult subnormal patients with intelligence quotients ranging from 40 to 69 (the third and fourth standard deviation ranges below the mean). It was found that birth in summer and autumn was significantly advantageous to subsequent intelligence performance compared with birth in winter and spring.

Further examination revealed that this association between season of birth and subsequent intellectual performance was probably a reflexion of the trend of climatic temperature during the whole of pregnancy. Thus, relatively high intelligence quotients were significantly associated with the number of embryonic months possessing a mean monthly temperature above that month's mean temperature over the years. Table 1 summarizes the intelligence quotient and climatic temperature data for this sample.

Table 1. Seasonal Temperature and Intelligence Quotient

	Summer	Autumn	Winter	Spring
No. of subjects	46	45	47	50
Mean intelligence quotient	57.1	54.6	51.7	53.1
Mean °F	56.5	55.5	40.9	42.3
Mean diurnal variation °F	14.9	12.6	8.3	10.8

The following example highlights the results obtained. Out of the total sample of 188, 10 subjects had an embryonic development with 8 or 9 months all above the respective mean monthly temperature. Of these 10 subjects, 8 fell in the 55–69 intelligence quotient range (third standard deviation below the mean) and only 2 in the 40–54 intelligence quotient range (fourth standard deviation below the mean).

From Table 1 it can be seen that between summer and winter births there is an average difference in intelligence quotients of more than 5 points. It is possible that if appropriate weighting be given to (1) variations in climate from year to year, (2) individual differences in exposure and susceptibility, then the effect of climate during pregnancy on subsequent intelligence might be more than 5 points of the intelligence quotient.

With chromosomal abnormalities, a somewhat similar result has been recently reported[5]. This investigation revealed a significant relationship between climatic temperature at the approximate time of conception and the incidence of mongol births to younger mothers.

From a survey of a number of investigations[6], a relationship has been claimed between season of birth and the incidence of psychosis (especially schizophrenia). However, in a relevant examination[7], I have produced evidence to suggest this might be an artefact of the noted association between intelligence and the season of birth. Nevertheless, one author[8] has found indications that factors during pregnancy, apart from climatic change, might be a determinant of future deviant behaviour.

It is not known how climatic temperature precisely affects the embryo. Further work is required on the precise nature of the temperature effect. This is presumably a matter of relative changes rather than absolute levels, but investigations in parts of the world with differing modes of temperature variation to the British Isles would be of great interest. For example, it can be seen from Table 1 that the amount of diurnal temperature variation is positively associated with the mean temperature-level. It is conceivable that the association between climatic temperature and intelligence is a result of diurnal changes rather than seasonal ones. There are, in fact, parts of the globe where the diurnal range is greater in winter. Such variations of climate clearly offer ready-made experimental designs for investigations into human performance and their determinants. The work recorded here offers one application of such a conceptual framework.

J. E. Orme

Middlewood Hospital,
Sheffield

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Number of Items presented and recalled as Determinants of Short-term Recall

The percentage of items correctly recalled from a sequence of items decreases as the number of items in the sequence increases[1,2]. Two factors normally vary directly with increasing sequence length: number of items presented and number of items to be recalled. When a twelve-item sequence was presented, Anderson[3] found that increasing the number of items to be recalled from four to eight to twelve increased the rate of error. Further investigation is necessary to determine if this is true substantially above the memory span. The importance of the number of items presented, independent of the number of items to be recalled, has never been adequately investigated. The following experiments on short-term recall of sequences beyond the memory span were designed to determine the independent effects of both these factors.

Using a Wollensak (model *T*-1515-4) tape recorder, 24 subjects were given sequences of digits presented approximately at 1/sec. Subjects had a 25-sec interval between the end of one sequence and the beginning of the next sequence in which to record the digits in order in the appropriate boxes. They were encouraged to guess, but they were not allowed to write down more items than the sequence contained. Subjects were run in two conditions. In condition *P*16-*R*12, 16 digits were presented, but only 12 were to be recalled. The first, fifth, ninth, and thirteenth items were already typed in the appropriate boxes. There were no repeats among the items that were typed-in. In condition *P*12-*R*12, 12 items were presented, and all 12 were to be recalled. Each sequence in condition *P*12-*R*12 was obtained by using the 12 digits to be recalled in a corresponding sequence in condition *P*16-*R*12. A one-second pause in presentation was used in place of each of the four deleted items. Thus, both conditions had the same number of items to be recalled and the same total time of presentation for the 12 items to be recalled. Sequences were randomly selected subject to the constraints that there were no runs of any digit, no forward or backward numerical sequences longer than two, and in *P*16-*R*12 the first, fifth, ninth, and thirteenth items were different from each other. All subjects received 24 sequences in one condition followed by 24 sequences in the other condition, counter-balanced for order. Two alternative sets of both length-16 and length-12 were used so that no subject recalled the same sequences of 12 digits in both conditions.

The data were analysed for ordered recall, item recall and position recall for items. A subject's report of an item is correct by an ordered recall criterion if, and only if, the correct item is recalled in the correct position. A subject's report of an item is correct by an item recall criterion if, and only if, it appears anywhere in his report of the sequence in question. When a digit is repeated in a sequence, a subject must report all occurrences of the item to obtain the maximum item recall score. Position recall, independent of item recall, is obtained by subtracting the item recall errors from the ordered recall errors, reducing the total number of possible errors by the same amount when computing the error rate for position recall.

Table 1 Error Rates for *P*12–*R*12 and *P*16–*R*12 (per cent)

Condition	Trials	Ordered recall	Item recall	Position recall
*P*12–*R*12	1–12	26·71	12·70	16·04
	13–24	19·07	10·10	9·98
	Total	22·88	11·40	12·97
*P*16–*R*12	1–12	43·63	22·77	27·01
	13–24	41·63	20·63	26·47
	Total	42·63	21·70	26·73

Table 1 reports the error rates for ordered recall, item recall. and position recall for the first 12, second 12, and total 24 sequences in each condition. The number of errors in condition *P*16-*R*12 was approximately twice as large as the number of errors in condition *P*12-*R*12 for ordered recall, item recall, and position recall. All differences between the two conditions were significant at well beyond the 0·001 level using a χ^2 test. Comparing the first 12 sequences with the second 12 sequences in each condition, there were significant practice effects in ordered recall, item recall, and position recall for condition *P*12-*R*12 ($P < 0·001$, χ^2 test), but in condition *P*16-*R*12 only the practice effect in item recall was significant ($P < 0·05$, χ^2 test). Items in condition *P*16-*R*12 were analysed into two categories: items identical to any of the items typed-in and items different from the items typed-in in any given sequence. The error rates for ordered recall, item recall, and position recall were computed separately for each of the two item categories in both *P*16-*R*12 and *P*12-*R*12. As expected, in *P*12-*R*12 there were comparable practice effects in both categories of items. However, in *P*16-*R*12, the only significant practice effect was in item recall of items identical to any of the typed-in items. When the number of items to be recalled is held constant, beyond the memory span, it appears that increasing the number of items presented increases the number of errors substantially and diminishes improvement with practice.

Anderson[3] established significant differences in short-term memory when four, eight, or twelve items were to be recalled out of twelve items presented. The following experiment was performed to determine if there is a difference in error rate between recall of 12 and 16 items when 16 items are presented. 24 additional subjects were run in condition *P*16-*R*16 in which 16 items were presented and all 16 were to be recalled. Half the subjects in condition *P*16-*R*16 were given 24 practice sequences before being given condition *P*16-*R*16 in order to make *P*16-*R*12 equivalent in amount of practice to *P*16-*R*12 in the previous experiment. Subjects were instructed to divide the sequence into four groups since division into four groups was achieved by the pauses in *P*12-*R*12 and the typed-in items in *P*16-*R*12. This experiment was identical to the previous experiment in all other respects.

In *P*16-*R*16 the ordered recall rate of error for all 16 items was 44·20 per cent, the ordered recall rate of error for the 12 items identical to the 12 recalled in *P*16-*R*12 was 45·13 per cent. Neither of these rates of error was significantly different from the rate of error in *P*16-*R*16. In *P*16-*R*16 the item recall rate of error for all 16 items was 26·04 per cent; for the comparable 12 items it was 22·61 per cent. The former figure was significantly greater than the item recall error in *P*16-*R*12 ($P < 0·05$, χ^2 test); the latter figure was not. In *P*16-*R*16 the position recall error rate for all 16 items was 24·55 per cent; for the comparable 12 items it was 29·10 per cent. Neither figure was significantly different from the position recall error rate in *P*16-*R*12. No significant improvement occurred with practice in *P*16-*R*16. Increasing the number of items to be recalled from 12 to 16 increased the rate of error, but not very much.

Serial position curves for ordered recall, item recall, and position recall were obtained by dividing the number of errors of each type at each position by the total number of errors of each type. In all three conditions, primacy and recency effects were obtained, with the point of maximum difficulty being the position immediately prior to the middle of the sequence. Superimposed on this overall serial position effect were four smaller serial position effects corresponding to the four groups into which subjects divided the long sequences in each condition. This grouping was the result of the pauses in presentation in *P*12-*R*12, the typed-in items in *P*16-*R*12, and instructions in *P*16-*R*16.

The first item to be recalled (second item presented) in *P*16-*R*12 had an error rate more comparable with the second item to be recalled (second item presented) than to the first item to be recalled (first item presented) in both *P*12-*R*12 and *P*16-*R*16. It appears that the overall primacy effect in *P*16-*R*12 was influenced more by presentation position than by recall position and was therefore greatly reduced. The primacy and recency effects within each group were consistently greater in *P*12-*R*12 than in *P*16-*R*12, presumably resulting from the additional item presented before and after each group in *P*16-*R*12.

Increasing the number of items presented from 12 to 16 doubles the error rate, increasing the number of items to be recalled from 12 to 16 has a much smaller effect. Practice effects the overall serial position curve and within-group serial position curves are affected more by the number of items presented than by the number of items to be recalled, when the number of items presented and recalled is well beyond the memory span as it is in the experiments reported here These findings suggest the general conclusion that the number of items presented is a more important determinant of short-term memory above the memory span than the number of items to be recalled

The decrement in short-term recall produced by increasing the number of items presented and the relatively small improvement with practice suggests that human beings are not able to ignore an item that need not be remembered when it is embedded in a sequence of items that are to be remembered Perhaps the item before the item that need not be remembered is automatically associated to the item that need not be remembered, and this unnecessary association competes with the necessary association between the two items surrounding the item that need not be remembered Alternatively, subjects may not even attempt to learn 12 item sequences in *P*16-*R*12 and may rehearse and recall these sequences the same as ordinary 16 item sequences In either case it appears that the human short-term memory system is unable to ignore the extra items

The relatively small effect of increasing the number of items to be recalled from 12 to 16 contrasts sharply with Anderson's findings for increases from four to eight to twelve[3] It appears that the number of items to be recalled is a much more important variable below or in the vicinity of the memory span than it is above the memory span Subjective reports suggest that there are two stages in short-term recall first, a rapid ordered recall output of those items of which one is surest and, then, a slower free recall output of those items which one thinks were probably in the sequence The ordered recall mechanism is assumed to be more accurate than the free recall mechanism If there are these two semi-independent mechanisms for short-term recall, then the findings concerning the importance of the number of items to be recalled suggest that the ordered recall mechanism is much more vulnerable to interference from the recall of prior items than is the free recall mechanism

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LINDA K SELZER
WAYNE A WICKELGREN

Psychology Section,
Massachusetts Institute of Technology,
Cambridge, Mass

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Neural Effects and the Psychophysical Law

THE claim that the true psychophysical law is a power function[1] instead of a logarithmic[2] law has led to a number of misunderstandings about the real significance of Stevens's point of view

Treisman[3] has tried to show that the so-called 'stimulus function' conceived to be both a power function and a logarithmic one His model involves the stimulus function as a relation between the stimulus-level and the level of central neural effects (C), the magnitude of which depends on the stimulus intensity, $C=f(I)$ To scale the sensory magnitude in a 'direct' ratio-scaling procedure the subject is asked to report whether a variable stimulus is greater or less than twice the standard Such an instruction to the subject involves a criterion to be met by the sensory magnitudes, with the scaling procedure leading to fixed ratios between the adjusted and standard stimulus intensities

On this basis, Treisman shows that two criteria for the central neural effects (C) may arise that is, his formulæ (3) $C''/C' = d = 2$ and (5) $C'' - C' = j = \log 2$ Depending on the choice of the 'stimulus function' these criteria can be derived, the first criterion is brought about by a power function and the second one by a logarithmic relation

At this point in his model, Treisman jumps from the subjective magnitudes to the values of the hypothetical central neural effects (C) Whether this is correct is questionable

In other words the criteria can be described as follows The hypothetic central neural effects (C) have to be adjusted until their values show up a ratio of a factor 2 The second criterion asks for central neural effects which show up a difference in magnitude by the amount of log 2 The first criterion seems rather sound in so far as the instruction is also expressed in terms of making the variable stimulus twice the standard Thus, in using this criterion one may hope that the central neural effects (C) are related to sensory magnitudes in at least a proportional way The second criterion, however, demands a constant absolute difference between the central neural effects to be in line with the instruction of doubling the sensory magnitude Since the difference asked for amounts to log 2 in doubling the sensation, it seems as if the central neural effects (C) are logarithmically related to the sensory magnitudes If so, then jumping from sensory magnitudes to the central neural effects is an unwarranted proposition

The second criterion suggested by Treisman holds that the subject is able to compare central neural effects on an absolute scale and to derive conclusions from this comparison with respect to the magnitudes of the sensation If true, the ratio scaling procedure leads to a proportional relation between the neural effect and the logarithm of the stimulus intensity However, this result does not warrant the logarithmical character of the psychophysical function The psychophysical law tries to relate sensory magnitude and stimulus intensity

Thus, the relation between the neural effects and the sensory magnitudes should be taken into account. By the second criterion a proportionality between the neural effects (C) and the logarithm of the sensory magnitudes (S) is assumed, whereas the condition $j = \log 2$ requires an equality $C = \log S$ Insertion of this equality into the 'stimulus function' (formula (4)) $C = h \log I$ leads to a power function relating sensory magnitude and stimulus intensity

In conclusion, the model suggested by Treisman is a valuable one towards solving the problems of matching abilities and ratio production in relation to neural events His 'stimulus function' is a useful concept to operate with if one is interested in information processing in the nervous system However, the 'stimulus function' cannot be taken as representative of the psychophysical relation between sensory magnitude and stimulus intensity

H W HOREMAN

Instituut voor Perceptie Onderzoek,
Insulindelaan 2, Eindhoven,
Netherlands

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THE question of the proper empirical basis of the 'psychophysical law' has caused much controversy It has been held that 'indirect' methods of scaling 'sensation', such as cumulating *j n d* 's, lead to log functions, and 'direct' procedures to power functions, and argument has usually been directed to showing that one type of procedure is better than the other, and therefore that one function but not the other, gives the 'correct' measure of sensory magnitude. However, it can be argued that this aim is misconceived[1]. In either type of procedure the subject performs a sensori-motor task under instruction, and what we record, on conventional physical scales, are the stimulus intensities (I) administered, and the responses (R) elicited. These are the data and if we are to have something further to measure we need some model of the intervening processes. Perhaps the simplest model is that which assumes that when a stimulus is presented to the subject it produces a central effect, C, lawfully related to the stimulus intensity, I, and that C functions as a measure of the stimulus, being used to determine the selection of the response, R. C is then related to I by a 'stimulus function', $C = f(I)$, and a corresponding response function or lawful procedure relates it to R. Evidence on the relation of I to R, alone, is insufficient simultaneously to specify both functions; only if an arbitrary or conventional choice of one function is made can the data serve to determine the other. Since numbers do not inhere in events or objects, but are assigned by us in accordance with some rule, our initial specification of one of the functions is equivalent to the choice of a rule for scaling C. If we decide that when a response criterion is reached we will consider that a constant increment (j) has been added to C, the stimulus function found will be logarithmic, if we assume that there has been a constant proportional increase (d) in C, a power function will be given; and this is true whether 'direct' or 'indirect' procedures are used, either law can be derived from either set of data, given the appropriate initial assumptions. Further, it was suggested that C should be taken as a measure we attach to an inferred neural effect of the stimulus.

To these arguments Horeman objects that 'sensory magnitudes' cannot be identified with central neural effects, when the subject is instructed to 'double the apparent sensation' (or some equivalent form of words) he applies this instruction to his sensory magnitudes (S) so that a doubling of S corresponds to an increment of log 2 on the scale $C = h \log I$, and C is then logarithmically related to, not identical with, S. The 'psychophysical law' applies to S, not C, and has the form of Stevens's power function[2].

The difficulties in adding an extra dimension, S, to which the instructions apply literally, and on which the subject operates, can perhaps best be illustrated by a 'thought-experiment'. We suppose we have to investigate not a human subject but an 'automatic light-selector', consisting of a photometer linked to a computer with a programming device attached. Lights of varying brightness are supplied to this apparatus, and from them it selects series of lights in accordance with a programme which is fed into the programming device in English, and translated by it into instructions in machine language which control the working of the computer. When we examine the lights selected we find that they fall into the series 10, 100, 1,000 millilamberts. The question now arises: Does the photometer record millilamberts and the computer select lights separated by a constant ratio of 10, or are log millilamberts recorded and lights selected at a constant interval of 1 log unit? To discover the answer we might open the apparatus and inspect the photometer dial, but we find that it has no scale inscribed. We could, if we wish, now calibrate the photometer, writing against the deflexion produced by the 10-millilambert light '10', or '1', and against that for the next light '100', or '2' and so on. Whichever we do the light-selector will work as well as before, and in no different a way, and we shall have solved our problem, arbitrarily, by the choice of a rule for inscribing numbers on the photometer dial, which rule we choose will determine our system of description, but both systems are equally valid if used consistently.

But now various objections might be raised. Thus it might be argued: "The programme controlling the computer reads: Starting with a light of 10 millilamberts select lights whose brightnesses increase by a ratio of 10'—this indicates that the millilambert scale is being used." But this is a fact about the English programme only: the programming device would give the same machine language translation to the instruction "Starting with a light of 1 log millilambert select brightnesses at intervals of one log unit". In either case the computer would work in the same way to select the same lights. Thus the conventions which make it more likely that the English programme will use millilamberts than log millilamberts, and will ask for a light to be doubled rather than increased by 0.3 log units, are not evidence about the nature of a unique scale used by the photometer. Nevertheless, it might still be argued "the instructions call for brightness to increase by a constant ratio, the selector performs the task set it, therefore we can assume a dimension to which the instructions are applied, and it gives a measure of brightness in millilamberts". If this argument leads the objector to calibrate the photometer to agree with the form of instruction that happens to have been given it will not be any the less true that this is an arbitrary choice, an expression not of empirical compulsion but of sentiment or convenience. If it leads him to conclude that the dimension used is not the essentially ambiguous photometer reading but some unobservable parallel system, this suffers the embarrassment of being highly unparsimonious: it is apparent that the computer and photometer are there, operating, and quite able to account for all selections we observe. If it leads him to expand the sequence $I \to C \to R$ into $I \to C \to S \to R$, then S is either parallel to (with the difficulties above) or part of the selector, in either case we are no better off than before: we must now write three functions, and to do so the available information, given by the relation of R to I, must be spread more thinly. Two functions must arbitrarily be assumed before the data will specify the third, and it is clear that a large number of alternatives will be possible.

The light-selector is at best an analogy, and perhaps a false one, for the human subject in a scaling experiment, but it may demonstrate how far commonly acceptable scientific modes of argument will take us in a case of this sort, and illustrate that unless we are to suppose that the use of the word 'sensation' justifies us in using novel principles of reasoning, there is no good basis for postulating an extra dimension, S, additional to C. It should be noted that the arguments about the nature of the stimulus and response functions hold whatever identification is given to C. The suggestion that C should be taken as a measure of a neural effect rests on our knowledge that the neural apparatus is there, and that we have no reason to doubt that it is adequate to mediate all sensory effects on motor activity.

The argument given here does not imply that there can be no unambiguous criterion for choosing a scale for C—one can be evolved[3]—but that the conventional treatments of scaling data do not provide this. It does not imply that no useful account can be given of the concepts subsumed under 'sensation', but that, despite their name and aim, sensory scaling experiments do not do this. It is not meant to detract from the value of the large body of very able experimentation that has been devoted to these problems by Stevens and others[2], only to develop their interpretation. It is necessarily condensed, but some points are dealt with more fully elsewhere[4].

M. TREISMAN

Institute of Experimental Psychology,
University of Oxford

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FORTHCOMING EVENTS

Saturday, December 28

Royal Institution (at 21 Albemarle Street, London, W 1), at 3 p m — Prof Ronald King 'Energy" (First of the One Hundred and Thirty-fourth Course of six lectures adapted to a Juvenile Auditory Further lectures on December 31, January 2, 4, 7 and 9)

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Bacteriologist, an Assistant Chemist, and an Assistant Economist—The Secretary, Edinburgh School of Agriculture, West Mains Road, Edinburgh (December 28)

Bacteriologist (with an honours degree or an equivalent qualification and preferably postgraduate experience in microbiology or the biological sciences) in the Central Laboratory of the Milk Marketing Board for Northern Ireland, Belfast, to have special responsibility for the bacteriological aspects of the Board's work, and to assist in the running of the Board's laboratory services generally—The General Manager, Milk Marketing Board for Northern Ireland, 456 Antrim Road, Belfast 15, Northern Ireland (December 31)

Demonstrator in the Department of Botany—The Registrar, University College of Wales, Aberystwyth (December 31)

Junior Lecturer (preferably interested in cytological or physiological aspects of vertebrate reproduction) in Zoology—The Secretary, Medical School of St Bartholomew's Hospital, West Smithfield, London, E C 1 (January 4)

Senior Lecturer in Forensic Medicine at the University of Singapore —The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 4)

Chair of Sociology at the University of Ghana—The Registrar, University of Ghana, P O Box 25, Legon, Accra, or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (January 6).

Lecturer in Civil Engineering—The Registrar, The University, Sheffield (January 6)

Lecturer (with a recognized degree in agriculture, or in pure science with agricultural or horticultural experience, and preferably a sound knowledge of tropical crops, with some experience in plant breeding and selection) in the Department of Crop Husbandry and Horticulture, University of Ghana—The Registrar, University of Ghana, P O Box 25, Legon, Accra, or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (January 10)

Senior Lecturer and a Lecturer in Mathematics at the University College of Townsville, University of Queensland—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, January 10)

Senior Lecturer or Lecturer (with a veterinary science degree or an honours degree in agricultural science with special reference to animal husbandry, and preferably a knowledge in ruminant nutrition and pasture problems) in Animal Husbandry at the Royal College, Nairobi—The Secretary, Inter-University Council for Higner Education Overseas, 29 Woburn Square, London, W C 1 (January 10)

University Lecturer in Pure Mathematics—The Secretary of the Appointments Committee of the Faculty of Mathematics, Arts School, Bene't Street, Cambridge (January 11)

Senior Lecturer in Physical Chemistry at the University of Western Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 15)

Lecturer in Physical Chemistry at the University of Sydney, Australia —The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 16).

Lecturer (with experience and interest in some aspects of plant physiology or biochemistry, preferably at the cell level) in Botany—The Secretary of University Court, The University, Glasgow (January 16)

Central Electricity Generating Board Fellow in Engineering Science, to engage in full-time research on a topic related to the scientific interests of the C E G B in the University Engineering Laboratory, to assist in supervising research students in that Laboratory, and to undertake a limited amount of teaching of undergraduates in the College—The Principal, Hertford College, Oxford (January 17)

Lecturer in Pharmaceutical Chemistry (Physical Chemistry) at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 17)

Lecturer (with an honours degree in some branch of chemistry and if possible the degree of Doctor of Philosophy) in Inorganic Chemistry at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, January 17)

Lecturer or an Assistant Lecturer in Pure Mathematics—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (January 18).

Lecturer (graduate in science or agriculture) in Agricultural Chemistry at the University of Sydney, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (Australia and London, January 24)

Chair of Civil Engineering—The Registrar, The University, Sheffield (January 27)

Chair of Plant Physiology at Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W C 1 (January 27)

Librarian (graduate with professional qualifications), to be in charge of a new college library—The Tutor, New Hall, Silver Street, Cambridge (January 27)

Assistant Lecturer in the Mechanics of Fluids in the Faculty of Science—The Registrar, The University, Manchester 13 (January 31)

Chair of Chemistry, Chair of Physics, Chair of Botany, and Chair of Mathematics at the University College, Dar es Salaam, University of East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 31)

Chair of Natural Philosophy—The Secretary, The University, Aberdeen (January 31)

Chair of Pure Mathematics, Chair of Psychology and Headship of the Department, Chair of Building Technology, and Chair of Sociology—The Registrar, The University, Nottingham (January 31)

Lecturer in Educational Psychology—The Registrar, The University, Manchester 13 (January 31)

Lecturer or Assistant Lecturer in Philosophy at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 31)

Lecturers or Assistant Lecturers in Chemistry—The Secretary University of Lancaster, Bailrigg House, Lancaster (January 31)

Lecturer (Grade II) in the Department of Inorganic Chemistry—The Registrar, The University, Senate House, Bristol 2 (February 14)

Professor of Physiology—The Secretary, University College, Galway, Republic of Ireland (February 20)

Foundation Chair of Mathematics (either Pure or Applied) at Wollongong University College—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (February 28)

Assistant Master to teach Mathematics at all levels—The Head Master, Wrekin College, Wellington, Shropshire

Master to teach Physics—The Master, Wellington College, Crowthorne, Berkshire

Research Assistant (graduate with an interest and experience in microbiology, mycology and bacteriology) in the Department of Biological Sciences, to undertake work on the taxonomy, ecology and serology of Actinomycetes—The Registrar, Bradford Institute of Technology, Bradford 7

Research Assistant in the Chemistry Department, for work under Prof R M Barrer on the surface and colloid chemistry of apatite and of materials potentially of interest as dental restoratives—Prof R M Barrer, F R S, Chemistry Department, Imperial College, London, S W 7

Research Assistant (with a good degree in zoology or microbiology, and preferably some experience in electron microscopy) in the Electron Microscope Laboratory—Dr R G Bird, London School of Hygiene and Tropical Medicine, Keppel Street, London, W C 1

Senior Research Fellow (with several years postgraduate experience) in Biochemistry in the Department of Pharmacology—The Secretary Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W C 1

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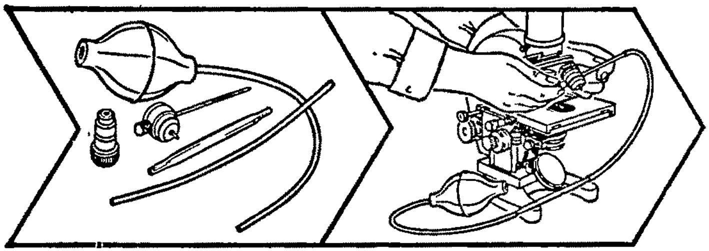

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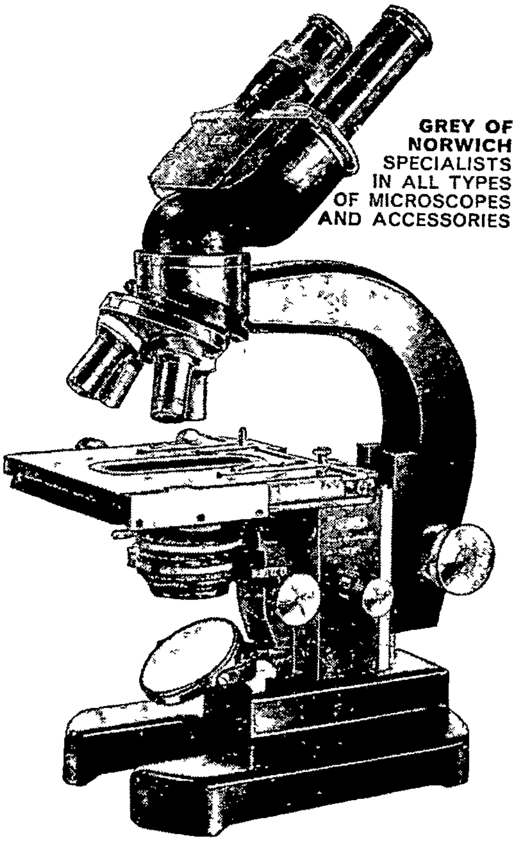
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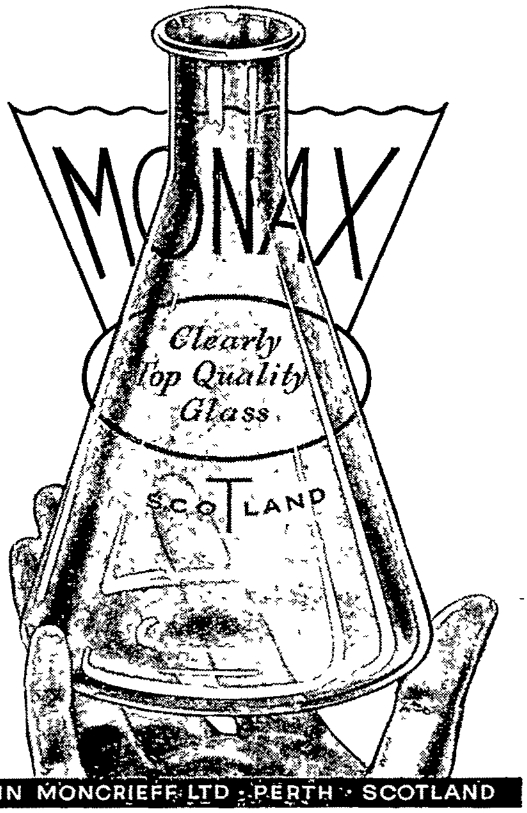

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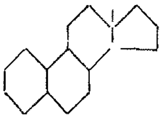

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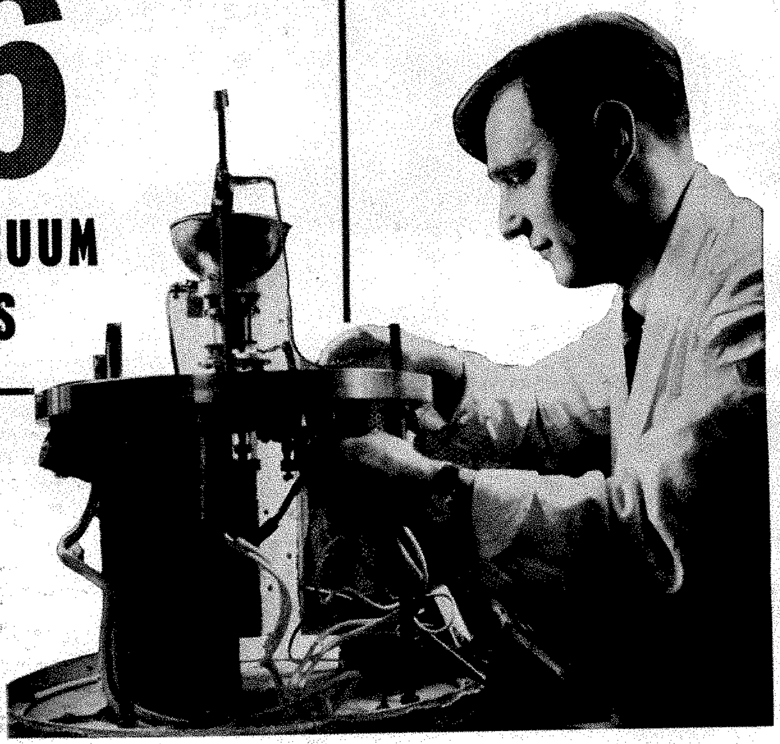

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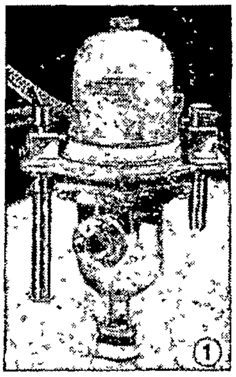
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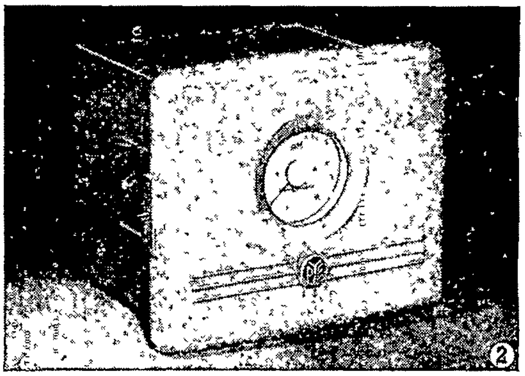
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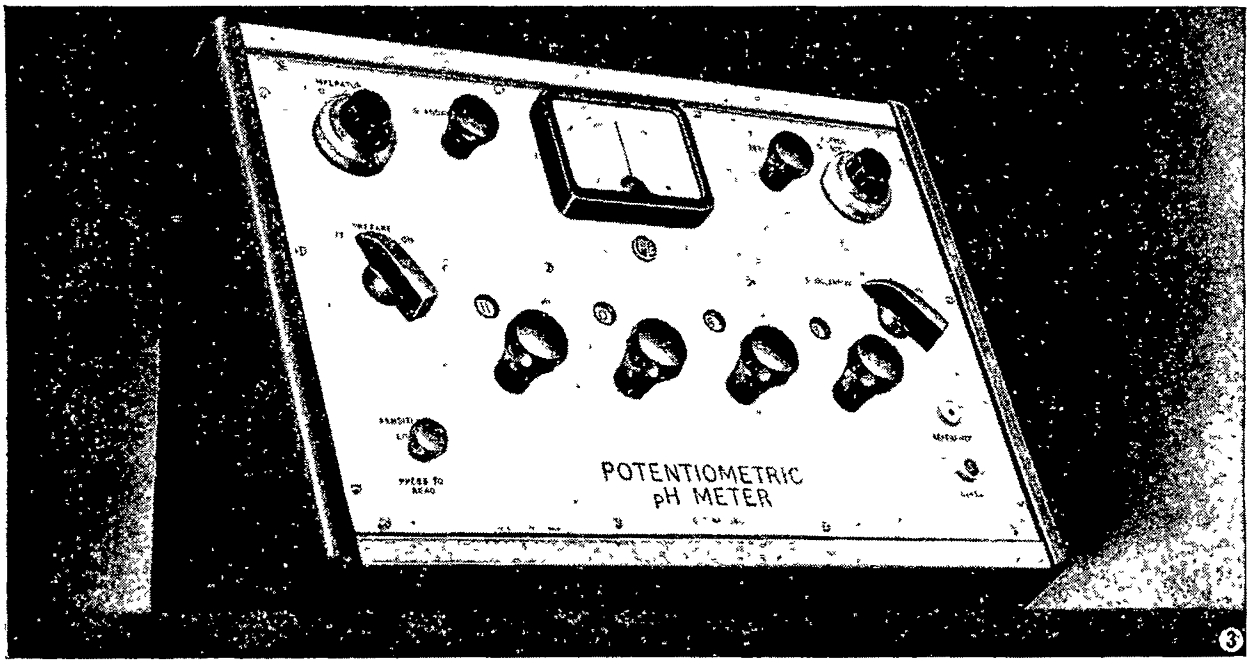

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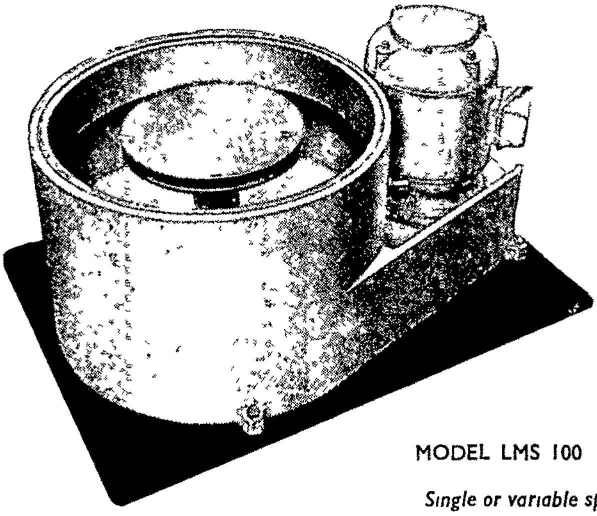

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ADMINISTRATION OF EDUCATION AND CIVIL SCIENCE IN BRITAIN

WHILE the main proposal of the Trend Report* for a fundamental reorganization of the Department of Scientific and Industrial Research is logical and has much to commend it in practice, when the Committee examines the position and functions of the Minister for Science, its proposals are more open to question. Those proposals are intended to discharge the second and third parts of the Committee's terms of reference: to consider the arrangements for determining with appropriate advice the relative importance in the national interest of the claims on the Exchequer for promoting civil scientific research in the various fields and the changes that may be needed in the arrangements for financing the agencies concerned and accounting for their expenditure. The Committee seems to have been content, however, to accept a view of the functions of the Lord President of the Council in regard to intelligence and research put forward in the Haldane Report without any searching enquiry as to the magnitude of subsequent changes in the relations between science and Government, or an expressed coherent view as to the part science and technology play in the State to-day. It admits, indeed, that the division of functions should be kept under regular review and, in particular, that the whole administrative structure should be scrutinized regularly and frequently by an authoritative body. Another generation and a half should not be allowed to pass without such a review, but it seems a little naive to suggest that an internal body such as the new Advisory Council could contribute significantly in this respect.

The structure proposed in the Trend Report is based on the principle of a separate autonomous agency for the conduct of research and the pattern of such agencies is to be rearranged to cover the whole field of civil scientific activity with effective supervision by the Minister for Science. Neither he nor his advisers, however, should seek to influence the scientific judgment of the research agencies acting within their respective terms of reference and within the resources made available to them. The tradition that the Minister for Science does not intervene in the exercise by the Research Councils of scientific judgement within their own fields is fully endorsed.

On the other hand, the Trend Committee believes that the Minister should be given wider powers to enable him to play a more positive and effective part than hitherto in promoting scientific research and development. This includes, first, the encouragement of conditions in which research can flourish and its results be put rapidly to use. It is here that the recommendations of the Trend Committee impinge on those of the Robbins Committee†, and the Trend Committee considers that there would be obvious advantages in vesting responsibility for higher education and responsibility for civil scientific policy in one and the same Minister.

Leaving that point for the moment, the Committee recommends that the Minister for Science should assume, and discharge to Parliament, the responsibility for assessing the financial requirements of the agencies, for settling with the Treasury the funds to be made available for civil research and development and for allocating these funds among the agencies. In the view that it urges in this Report, the Trend Committee regards it as essential that the Vote arrangements should enable the Minister to exercise real and effective supervision over the whole field, but they should not be such as to involve the individual agencies in a greater degree of accounting responsibility than is compatible with the freedom of action implicit in the concept of a grant-in-aid. The Minister should also assume general responsibility for keeping the whole field of scientific research and development under continuous review, to ensure that deficiencies are made good, that unnecessary duplication is avoided and that the broad deployment of effort over the whole field is in accordance with the national interest.

While here the Minister would have the assistance of the reconstituted Advisory Council, what is proposed represents a fundamental break from the negative attitude which has hitherto characterized the Minister for Science. A more positive attitude is also advocated in the recommendation that would make the Minister responsible for the Government's relations with learned bodies in the field of scientific research, particularly the Council of the Royal Society, and also for the Government's international relations in scientific matters, including the appointment and control of scientific attachés. This last-mentioned aspect is a more appropriate and logical arrangement than that which has prevailed in the past, but, taken together the proposals represent a very considerable increase in responsibilities for the Minister for Science. Even if he is divested of all extraneous responsibilities it is legitimate to ask whether it is sound to concentrate virtually all the responsibilities for science in a single Ministry.

This question is not examined by the Trend Committee nor does that body consider the strengthening of the provisions for scientific advice and research within the various Government departments. Obviously, such departments as the Post Office and the Ministry of Defence cannot function without effective means for handling scientific matters internally. The appointment of a director of Research and Development in the Ministry of Public Building and Works and the Trend Committee's own observations regarding the Ministry of Transport and the Road Research Laboratory show that the Committee was fully aware that there may be other departments which would benefit by arrangements to facilitate more effective contact with science It would seem logical that the Minister for Science and his Advisory Council should have advisory responsibilities in science to the Cabinet in general as well as to the individual departments

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* *Committee of Enquiry into the Organization of Civil Science*. Pp. 59. (Cmnd. 2171.) (London: H.M.S.O., 1963.) 4s. net. (See also *Nature*, 200, 1029, 1963.)

† *Committee on Higher Education. Higher Education*. Report of the Committee appointed by the Prime Minister under the Chairmanship of Lord Robbins. Pp. xvi+335. (Cmnd. 2154.) (London: H.M.S.O., 1963.) 15s. net. (See also *Nature*, 200, 925, 1963.)

Is it then practical to link responsibility for higher education with responsibility for civil science? The Robbins Committee proposed to place the Research Councils under a Minister for Arts and Science, along with the University Grants Committee and other autonomous State-supported activities administered on similar principles The Robbins Committee was agreed, in particular, that the colleges of advanced technology should be granted the status of technological universities—a recommendation that the Government has already accepted—and that, accordingly, these institutions should be administered on the grants committee principle Since the Robbins Committee had been informed in evidence that the Treasury would not feel it appropriate to assume responsibility for additional institutions, a change in ministerial responsibility is obviously involved, unless the two sets of institutions are to be the responsibility of different Ministers, with obvious detriment to the hope of unified policy and the disappearance of differentiation

The Robbins Committee, for various reasons given in the Report, did not view with favour giving responsibility for the University Grants Committee to a non-departmental Minister, nor was the majority in favour of placing all responsibility for education on one Minister It considered that the area of responsibility would be too wide and that the methods and problems of higher education differed so considerably from the methods and problems of education in the schools as to call for recognition of their differences in the articulation of the organization of Government The Robbins Committee did not share the fears that many university teachers have expressed as to the effect of placing the University Grants Committee under the Minister of Education, but it attached much weight to the concern of the main institutions of higher education with the preservation and advancement of knowledge It is this organic connexion of the universities with other forms of organized research that led it to suggest that they should be linked with the Research Councils and the Arts Council in the allocation of ministerial responsibility

The Ministry for Arts and Science, creation of which is recommended by the majority, would not, however, be responsible for the work of the non-autonomous sector of further education in the technical and other colleges of further education However, advanced work in these institutions would be related to that in the autonomous institutions through the creation of a new Council for National Academic Awards, with the power to award degrees For Scottish institutions, the Committee recommends a special relationship between the new Minister and the Secretary of State for Scotland. The prime responsibility for co-ordination over the whole field of education would rest with the three Ministers and their departments, advised on specific questions by national advisory bodies, but the Robbins Committee also recommends the establishment of a small Consultative Council to which Ministers can remit questions concerned with higher education as a whole as well as its relations with the schools

It is obvious from the argument of the Robbins Report that a real stumbling block to a single Minister of Education is the special position of Scottish institutions, and, though this is not mentioned, the sensitivity or hypersensitivity of some sections of opinion in Scotland. That manifestly must be taken into account, however irrational some of its manifestations may appear, if a workable scheme is to be devised, nor is it disregarded by Mr H C Shearman in the "Note of Reservation" in which he sets forth his reasons for preferring a single Minister of Education with one or two Ministers of State to assist him This Minister would take over the present sphere of responsibility of the Minister of Education and that of the Chancellor of the Exchequer for the University Grants Committee and possibly some of the other functions suggested by the majority of the Robbins Committee (Mr. Shearman does not specify research), but he would be at the head of a new department for which a forward-looking outlook might be expected He would presumably adopt the administration styles appropriate to the different parts of the wide field which he would be called on to serve, and devolution should be the keynote of his Ministry

Here Mr Shearman strikes the right note and one that should characterize any form of Ministerial responsibility which may be finally adopted in respect of either education or scientific research The Ministry—or Ministries—should be forward-looking and in function and structure appropriate to the purpose to be served Furthermore, it will be noted that one reason why Mr Shearman prefers to see a single Ministry of Education is that he wishes to see the universities providing the leadership and inspiration that they are uniquely qualified to give The chosen arrangements must provide for this leadership as well as foster the sense of common purpose and continuity which all members of the Robbins Committee alike desiderate, and here Mr Shearman in particular refers to the importance of associating the resources, experience and goodwill of the local education authorities

The arrangement preferred by Mr Shearman is very similar to that advocated in a Bow Group pamphlet by R Smith and R Eddison, *Talent for Tomorrow* (*Nature*, 200, 1162, 1963), whereas his colleagues favour rather the type of structure proposed by a Fabian Group in a pamphlet, *The Structure of Higher Education* (*Nature*, 194, 802, 1962), which was submitted in evidence to the Robbins Committee, but which the Bow Group explicitly rejects The need for greater integration in the administration of research leads the Bow Group instead to favour a single ministry responsible for both education and research The Bow Group suggests accordingly a Minister of Education and Research, advised by an Honorary Council on Higher Education and supported by separate Councils for Technological and Commercial Education and for Professional Education, including teacher training The Minister would also be provided with common services in the form of a statistical office, an educational building office and an educational research centre However, while the University Grants Committee would be responsible directly to the Minister, he would have under him two Ministers of State, one for schools and further education covering the present branches of the Ministry of Education for England and Wales, and the other for science and research, to whom the existing Research Councils would be responsible

Such an arrangement could take care of the proposals of the Trend Committee reporting the Research Councils and might involve fewer difficulties than the precise arrangement proposed by the Robbins Committee As regards the size of the Ministry, it would not appear *prima facie* to be larger than the new Ministry of Defence Its efficiency would depend very largely on the effectiveness of the arrangements made for devolution of authority wherever possible as well as for greater liaison between institutions of various kinds from the local level upwards If the Bow Group can argue with reason that, from the point of view of national needs, the greatest single problem in scientific and technological research to-day is that of liaison between the universities and the newer centres of research, such as the Atomic Energy Authority and other Government establishments, the research associations and industrial research laboratories, it is not only in research that improved communications are required

The vital point is that the administrative arrangement should be chosen to serve the specific purposes needed and not the convenience or prestige of a particular political clique or established interest, and this should be demonstrably seen to obtain Its effectiveness will of course depend also on the character and ability of the men chosen to fill the designated posts, but beyond this it will depend also on the understanding and support of their Cabinet colleagues Effective national policy in higher education and in science is not solely a matter of appropriate administrative arrangements or of the staffing of one or even two departments It depends on adjustments and understanding at many levels from the Cabinet downwards on the ability of Cabinet ministers in general to recognize the significance of the arguments which a Minister of Education or for Science brings forward, and also on the capacity of the Departments themselves to appreciate the bearing of science and technology on their own work No Ministry for Science is likely to prove fully effective unless in many of the Departments there are scientific advisers of high standing and supported by considerable sections able to make full use of new knowledge and new methods Nor should it be forgotten that the shaping of the Government organization to meet the tasks of to-day means also equipping it at all levels to utilize the new tools of management which the computor and other technical advances have placed in its hands Even if a prime responsibility in this sphere continues to rest with the Operations and Methods Division of the Treasury, it only demonstrates that the Treasury itself will need to be in close touch with the new knowledge thrown up continuously under the Minister for Science

There are indeed some signs that this is already being done The establishment in October of a Centre for Administrative Studies within the Civil Service is one sign of new thinking So is the creation of the new post of Secretary of State for Industry, Trade and Regional Development and linking with it the Presidency of the Board of Trade, but much more will be required if the Government structure throughout is to be geared to meet the challenge of what Prof Colin Cherry most aptly termed the "incipient revolution" (*Nature*, 200, 308, 1963) The leader of the Opposition, Mr Harold Wilson, has also shown himself well aware of the challenge, and if neither the Government nor the Opposition has as yet indicated at all precisely how it proposes to meet the challenge on administrative requirements, there is little that need keep political parties apart. It is a valid test of the sincerity of both political parties in their professed support for science and for education whether or not they are prepared to drop party political arguments sufficiently to ensure that, during the short period of life that remains to the present Parliament, the necessary administrative measures are put through to provide a structure which is adequate to serve the needs of the nation in respect of science and of education in the critical years ahead

Nor is the form of the structure required tied too closely to some of the decisions which are most difficult or may require further consideration It does not depend, for example, on decisions regarding the future of the research associations and the stimulation of co-operative industrial research Nor does it depend on the precise location and status of the teacher training colleges, or again on the location of the research stations of the Department of Scientific and Industrial Research, which certainly merits fuller consideration than the Trend Committee proposes for all but two of them On balance, a single Ministry for Science and Education appears to be proposed on the grounds of flexibility as well as avoiding certain difficulties which the Trend Committee and the Robbins Committee alike admit It might also help to minimize the proliferation of committees, under the University Grants Committee proposed by the Robbins Committee, but which has been the subject of some adverse comment It should certainly favour the placing of the colleges of advanced technology on the same footing as the universities, though here a more generous policy than that which the Bow Group contemplates is essential and more in line with that proposed by the Robbins Committee Within the one Ministry, moreover, it should be easier to work out a satisfactory policy for converting the teachers' training colleges into Colleges of Education while securing the full support and understanding of the universities and the local education authorities alike Adequate devolution, understanding and co-operation at all levels are vital, and it is at least possible that this would be facilitated by association of responsibility for science and for education within the one ministry Industry, at least, is unlikely to forget, as a Ministry of Higher Education might, that education is whole, and that the primary and secondary levels are fully as important as the higher levels

If these, then, are the essential needs that the new administrative arrangements must serve it must not be forgotten that there is also an essential task of public education that has to proceed while the detailed arrangements are being worked out and the legislative steps taken This is indispensable if the new structure is to be used effectively and if public support is to be forthcoming for the vast expenditure that is involved, whatever differences in the scale proposed that might exist between the political parties It is relevant, too, if the new Minister or Ministers are to be sure of firm support when their financial proposals are placed before their colleagues in the Cabinet No differences of opinion as to particular details of the recommendations of the Trend Committee or the Robbins Committee should be allowed to hinder the swift formulation of definite proposals for an adequate ministerial and administrative structure to serve the needs envisaged alike in education and in civil science, or to impede the informed discussions of the many difficult and complex issues involved Prompt action and imaginative and forward thinking are imperative, alike in dealing with long-term problems as in dealing with the short-term emergency arising out of the 'bulge' over which the Robbins Report perhaps passes too cursorily

HEALTH IN THE TROPICS

Tropical Health

A Report on a Study of Needs and Resources Pp xxxi+540+9 plates (Washington, D C Division of Medical Sciences, National Academy of Sciences—National Research Council, 1962) 6 50 dollars

THIS volume is published by the U S National Academy of Sciences, National Research Council, as a report sponsored by the U S Army Medical Research and Development Command, the National Institutes of Health, U S Public Health Service and the Rockefeller Foundation

The main objectives of the survey, which was first conceived by the American Society of Tropical Medicine and Hygiene in 1954, were to evaluate the important problems of medicine and hygiene relevant to the future development of the tropics, and to define the resources and research needs as a basis for recommendations regarding the future activities of the United States in these fields

The geographical areas examined were (1) Caribbean, Central and South America, (2) Africa, (3) South West Asia, (4) South Central and South East Asia, (5) Oceania One hundred and sixty-nine countries and territories were included in the survey

So far as possible data covering the period 1954–58 were collected and presented on a regional basis and not in terms of individual countries or political groups The survey was restricted to the most significant public health problems and diseases in the foregoing geographical areas

The subjects investigated were infectious diseases, nutritional diseases, tropical physiology and climatology, environmental hygiene and the zoonoses and other diseases of domestic animals Chronic and metabolic diseases (other than those related to nutrition) were excluded The work finally concerned 34 human conditions ("the great diseases of the tropics") These were considered in four categories diseases of high endemicity, diseases of relatively low endemicity but potentially great because of possible epidemic character, diseases of relatively low endemicity but high mortality or serious complications, new diseases concerning which present knowledge is limited, but which appear to be of considerable health importance

An examination was also made of significant diseases of domestic animals in tropical and sub-tropical areas and of human and animal tropical biometeorology (included in the section on "Research Needs")

Part 1 of the book is concerned with the significant human diseases The difficulties involved in analysing the data were immense, and the authors freely recognize the shortcomings of some of their conclusions which were based on inadequate and inaccurate information provided from underdeveloped areas in which professional personnel was in desperately short supply Unfortunately, except on certain specific points, information was not obtained from mainland China The lack of international uniformity in the notification of diseases was an important source of unavoidable error For example, only five diseases (yellow fever, cholera, plague, typhus and smallpox) were notifiable in all 169 countries and territories of the survey

The four categories of disease are discussed in separate chapters, each condition in terms of distribution, incidence and prevalence in the major geographical areas, morbidity and mortality and public health importance Control is briefly discussed

The first 10 diseases of importance to public health in the five geographical survey areas were tuberculosis, malaria, influenza, nutritional deficiencies (excluding kwashiorkor), cholera, bacillary dysentery pneumonia, measles, whooping cough and amœbic dysentery Tuberculosis, influenza, malaria and pneumonia were among the first ten in the entire survey area Only three of these (malaria, cholera and amœbic dysentery) belong to the classical 'tropical' diseases Only four "have not been demonstrated to be controllable by known means", namely schistosomiasis, leptospirosis, certain arthropod-borne viruses and measles

Part 2 covers significant diseases of domestic animals in tropical and sub-tropical areas An attempt was made to evaluate the importance of diseases of animals in human health problems in terms of zoonoses and protein nutrition Sources of information were generally poor, probably because, in general, veterinary services and facilities for training veterinarians were lamentably deficient On the 23 important diseases of domestic animals studied only one (sweating sickness of cattle) was without known methods of control and prevention The percentage of total budgets expended in veterinary services in the countries investigated was considerably less than that appropriated for health services

Part 3 contains information concerning the numbers of medical and associated personnel, expenditure on health services, numbers and types of hospitals and clinics, and the preventive medical programmes in the survey countries There was serious lack of staff of all categories and of hospital beds and clinics Health expenditure averaged 5–6 per cent of the total budget in most areas which provided reliable relevant figures

Preventive programmes concerned especially immunization against smallpox, typhoid, yellow fever, cholera diphtheria and tuberculosis These varied widely in magnitude and geographical extent More than 500 million vaccinations for smallpox, 95 million for tuberculosis (BCG), 52 million for cholera, 46 million for yellow fever and 27 million for typhoid were performed in the total survey area in the period 1954–58

Data regarding medical missions and medical organizations set up by American corporations working in the tropics indicated that these bodies contributed significantly to medical care and preventive operations in the tropics

Information on water supplies and sewerage systems in the areas of the survey was largely deficient In the Caribbean and Central and South America, which is the most materially developed area surveyed, 39 per cent of the population lacked piped water, the deficiencies being most evident in communities of fewer than 10,000 inhabitants Sewers were non-existent in most rural communities

The impact of disease and the low living standards of tropical populations on the internal economy of the United States was examined in terms of material and strategic interests including foreign trade, market potentials, etc Natural increase in population resulting from successful reduction of morbidity and mortality might be significant unless economy improves in proportion, expected increase in individual capacity for work associated with improvement in health may be an important factor in this balance

Movements of individuals and populations arising from travel, migration, religious pilgrimages, socio-economic development, etc , have important bearings on the distribution and activity of disease processes There are many such examples of the dissemination of disease in war and peace

International sanitary regulations have markedly limited the distribution of the quarantinable diseases, but many 'tropical' diseases which constitute risks to the community or the individual, or both, may still be imported or exported This challenge must be met, especially in view of the speed and frequency of modern travel (Its importance in Europe was recently the subject for discussion at a meeting of representatives of the European schools of tropical medicine held in London and Liverpool)

The practical problems of the United States are discussed in this respect At present, Americans travelling to the tropics are mainly at risk in the Caribbean and Central and South America Increasing interests in the other areas will mean increasing exposure to health risks

Parts 5 and 6 of the report contain surveys of research programmes in tropical problems in progress in the United States and elsewhere, sponsored by the United States and other Governments and organizations

The teaching of tropical medicine and hygiene and opportunities available for training in the United States medical schools and in foreign institutions are summarized and discussed On the whole, incentives and career opportunities in the field of tropical medicine are lacking in the United States

There has been little recent expansion of interest in tropical medicine in the United States The number of workers involved is "such a small fraction of the total professional and scientific personnel engaged in medical and biological sciences that any future expansion in personnel should not constitute any strain on total manpower needs over the next decade"

The remainder of the report deals with an analysis of research needs in human and veterinary medicine in the tropics, based on written opinions obtained from advisers in the various fields Thirty-one human diseases and ten veterinary problems are discussed

Most of the comments made by the authors are self-evident and readily acceptable A series of recommendations is made by the Advisory Committee These include (1) The contribution of the United States to international effort against tropical disease could best be channelled through a national programme for research in tropical health, developed by an advisory organization within the National Research Council (2) Grants should be available for approved foreign institutions to support programmes of research and training in tropical health (3) More effort is recommended to stimulate interest in medical schools at undergraduate and post-graduate levels which should include overseas programmes and tropical field studies (4) The World Health Organization should be invited to intensify its statistical coverage of tropical health, possibly with increased support by the United States (5) The economic aspects of tropical disease should receive closer study, and tropical veterinary education and research should be expanded, particularly in Africa and South East Asia

There are numerous text figures and nine plates demonstrating in maps, with the usual limitations, the tropical world distribution of the major diseases These maps are beautifully drawn and presented, which makes it all the more the pity that the American Geographical Society of New York, which permitted their publication, should have consistently misplaced Surinam and British Guiana

A remarkable amount of well-collated and clearly presented information is contained in this report, which constitutes a valuable reference volume for workers engaged in the research and field problems of tropical health The Appendix detailing the needs for research contains many comments and suggestions which are at once acceptable and familiar to those concerned with tropical problems There is little that is new Omissions were probably determined by the limits within which the survey had to be confined The material published appears to have been decided by the volume of the individual contributions from consultants, and this has led to some imbalance in certain fields, particularly in biometeorology, which appears over-emphasized The almost complete omission of hæmatological and genetic aspects of tropical life and disease is unfortunate

The absence of an index is a disadvantage

However, this is undoubtedly a remarkable and comprehensive report Its recommendations are sound and should be investigated by other Governments concerned in helping developing countries

B G MAEGRAITH

THE DEVELOPMENT OF HISTOCHEMISTRY

Enzyme Histochemistry and Its Application in the Study of Neoplasms

By M S Burstone Pp xii + 621 (Figures) (New York and London Academic Press, 1962) 160*s* 6*d*

AT the present moment, histochemistry is suffering from a wave of popularity Although it is delightful that the subject has been taken up so enthusiastically, it is becoming a little difficult to know how it should be defined This is not to decry the various facets which are becoming disclosed by different workers but to make two pleas first, that if it bears a chemical connotation, at least it should be a science, secondly, that since it relates to tissue its methods should preserve histological structure

Thus there is a view, to which I contribute, that histochemistry is concerned with visualizing cellular chemistry, that it is an extension of biochemistry which can be performed on histologically acceptable sections, and that it has already a certain amount of scientific information which is related to the special conditions concerned with the chemistry of intact sections There is another view that it is predominantly an extension of histological staining, in which the stains have a rational, chemical basis and hence may be indicative, but nothing more, of the chemical activity of the tissue Either way, a professional histochemist tends to study a new work on his subject with some anxiety to ensure that, whatever the views of the author, the work will advance this new subject and not lay it open to ridicule

Dr Burstone is a distinguished histochemist, who has contributed considerably to the histochemical armamentarium As might have been expected, his chapter on proteolytic enzymes, in particular his discussion of aminopeptidase, on which he has done outstanding work, is of real value, of only less importance is his section on cytochrome oxidase, which his own studies have made readily susceptible to histochemical investigation He seems to see histochemical progress as limited solely by the lack of suitable substrates and, consequently, much of the book is devoted to methods by which the newer substrates may be synthesized Although I sympathize with this view, I wonder if this is, indeed, histochemistry For example How many histochemists will be advanced by knowing the X-ray diagram of various naphthol *AS* compounds (pp 78, 79, 80), or the fact that the first aryl glycoside was synthesized in 1879, or will be helped by the chart (IX, p 128) of a multitude of "azine type dyes" which are figured but not even named ? The organic chemistry seems too abstruse for histochemists and on occasion it is even misleading If he succeeds in exciting histochemists to synthesize their own reagents, without training them first in analytical methods for proving the purity of their products, he is indeed liable to spread alarm and confusion

Unfortunately, the book may be criticized on many counts The language is slovenly and often confusing, there is too much qualitative reporting of the "one says this although another disagrees" type, without analysing the contentions and the evidence underlying them, too often a surprising fact is reported without a reference to the source being given But most serious of all are, first, the lack of critical standards for assessing the histochemistry and, secondly, either an apparently total ignorance of tissue biochemistry, or a refusal to relate histochemical findings to those of the biochemists It may be that the biochemical evidence is erroneous, but it would be comforting to have an explanation of how the error arose and how histochemistry has avoided it For example, in the discussion of acid phosphatase, the whole point of de Duve's work on lysosomes has been either missed or ignored, if the latter, then it would have been illuminating to have known on what grounds it was discarded. One is, however, forced to suspect the former. Holt's analysis and modification of the metal-salt method have been misunderstood, and Burstone's obsession about the need to "eliminate spurious staining" would be set to rest if he recognized the value of controls in histochemistry. But even more startling is his insistence on fixation for even this enzyme. Fixation with calcium formol is recommended on p. 207, although formaldehyde is listed among the inactivators of acid phosphatase on pp. 213-215, 218. Even more surprising is the recommendation (p. 207) of a method involving fixation in acetone and embedding in paraffin wax which has been estimated, in another recent text-book of histochemistry, to produce as much as 90 per cent loss of this enzyme. Indeed, there is justification, from this sort of histochemistry, for the jibes that the subject is nothing more than "histo-cookery" or "histo-alchemy".

Burstone's major contribution to histochemistry has been in relation to the azo-dye coupling reactions. He has synthesized and tested many compounds which are likely to be useful. It was to be hoped that his histochemical understanding would have matched his organic chemistry. One of the main worries about these coupling reactions is whether appreciable diffusion of the components or even of the complete dye can occur, so giving rise to spurious localizations. In part, this depends on the 'substantivity' of the dye. On p. 138, Burstone gives a correct definition of this concept as applied in the dye-stuff industry, but proceeds to so modify it for histochemistry as to render it valueless. He then proceeds to argue (on p. 140) apparently by polemic alone, that the naphthol which is released by enzymatic reaction within the tissue binds to the tissue component from which it is released.

Although he is aware of the dangers of diffusion artefact, which could be very severe if the coupling reaction is not extremely fast, he admits he has no scientifically acceptable evidence and offers only pious hopes (p. 95). "With an azo-dye technique the potential qualities of naphthol may be nullified if the coupling with the diazonium salt does not take place promptly. Although there is no actual knowledge about the submicroscopic nature of a 'capture reaction' involving a stain precursor (e.g., liberated naphthol), we can assume that this reaction should take place as rapidly as possible."

There then follows a remarkably ineffectual discussion of diffusion artefact, which is not given any objective analysis. An example of his critical histochemistry occurs on p. 150. "The sites of enzyme activity in a tissue section when visualized by means of good histochemical techniques should be sharp and clear cut. All available evidence at our present state of knowledge indicates that sharp histochemical localizations are significant even when there is overall cytoplasmic activity (Figs. 5, 6). *Histochemists trained as histologists or pathologists should always expect to see clear cut microscopic localizations provided that proper techniques have been employed*." Regrettably this is typical. One is left to wonder what histochemists who have not been trained as histologists or pathologists may expect to see. One may also wonder how "sharp and clear cut" localization can be induced for weak enzymes of the type that are normally found in the microsomal or supernatant fractions of the biochemists.

Paradoxically, however, it deserves to be reported that it is indeed where he uses totally irrational methods, as for acid phosphatase, that he does get some "background staining"; it is amusing that it should be in this particular case that he considers diffuse staining is permissible.

The contents of the book cover all aspects of enzyme histochemistry. The opening chapter is concerned with fixation and embedding techniques but cannot be recommended. Naphthalene derivatives are then considered briefly as histochemical reagents. The third chapter is useful in that it deals with diazonium salts and azo-dye coupling, which subjects are taken up more fully in relation to tissue sections in the fourth chapter. There then follow chapters devoted to phosphatases, esterases, sulphatases, glycosidases, transglycosylases (which forms a valuable review of these enzymes), proteolytic enzymes, oxidases and dehydrogenases. The additional chapter devoted to the application of enzyme histochemistry to electron microscopy will be welcomed by many, the purpose of the chapter on electrophoretic procedures is less obvious, while that relating carcinostatic agents to histochemical substrates makes little impact. There are a number of printing errors and the photographs often are inconclusive. Apart from the organic synthetic chemistry and the section on aminopeptidase it is not apparent in which way this book appreciably advances the subject technically or theoretically, beyond what has been achieved by Pearse's justly famous work. Moreover, it is very difficult to comment on a work on histochemistry which figures stained muscle bands as mitochondria (Fig. 1, p. 488).

J. CHAYEN

THE MARCH OF SCIENCE

Science in Progress

Thirteenth Series. (SIGMA XI–RESA National Lectureships, 1961.) Edited by Wallace R. Brode. Pp. xiii+305. (New Haven and London: Yale University Press, 1963.) 56*s*.; 7.50 dollars.

To review at all adequately a book which consists of ten lectures on unrelated themes and of the excellence of those contained in this volume is rarely possible within reasonable space. At best all that can be done is to indicate the scope of the volume and the high standing of the lectures. Seven of those contained in this volume are specialized and comprise those of Prof. E. R. Biel, on microclimate, bioclimate and comparative dynamic climatology, Prof. N. F. Ramsey, on nuclear interactions in molecules, Prof. S. Chapman, on Sun storms and the Earth: the Aurora Polaris and the space round the Earth, Prof. T. Dobzhansky, on man and natural selection, Prof. L. M. Beidler, on biophysical approaches to taste, Prof. J. L. Greenstein, on stellar evolution and the origin of the chemical elements, and Prof. S. C. Brown, on plasma physics. Together they provide a fascinating picture of some of the frontiers of science which should be readily intelligible to the non-specialist, and, although delivered in 1960–61 they are none the less to be commended to the general reader.

Three of the lectures have, however, a common theme. From different angles they consider the problems with which the present advance of science confronts society. In the first of them Dr. A. T. Waterman, forecasting the shape of science in the sixties, emphasizes the vital importance of fundamental research, including research in the social sciences, over the whole spectrum of knowledge, and urges that while our organization must be such as to ensure such a distribution of effort it is unlikely to be secured in the absence of effective international co-operation.

Next, Prof. H. Brown examines some of the problems which arise in the relations of science and Government, and he too urges the imperative necessity for international co-operation, stressing first, however, that an effective system of disarmament is an urgent priority, transcending in importance all other aspects of relations between Government and science. Secondly, he directs attention to the contribution of science towards meeting the needs of the under-developed countries and then to the importance of attention to the conservation of natural resources. He concludes that the agencies already established in the United States for providing scientific advice to the Government and concerting national effort and policy in science and technology should be adequate if sufficient attention is directed to these aspects of the situation.

In the third lecture, Dr W R Brode discusses the growth of science and a national science programme, considering what is implied in such a programme and the effect of the growth of science on supporting and performing institutions He suggests that there is a limiting factor to the growth of science and that this is likely to set a limit to the proportion of scientists and engineers, in the United States, he believes this limit is already approached In contrast to Prof Brown, he suggests that national and Government organization of science and science policies in the United States calls for independent review, but he agrees as to the importance of international co-operation, particularly in respect of technical assistance to the underdeveloped countries

These three lectures are thus of general interest, not so much from the information point of view, like the other seven, as from that of a stimulating contribution to a discussion which ranges far outside the United States and is particularly lively in Britain at the moment

R BRIGHTMAN

WHAT GOES UP . . .

Throw Out Two Hands

By Anthony Smith Pp 272+32 plates. (London George Allen and Unwin, Ltd , 1963) 30*s* net

A BALLOON has seemed an uncomfortable thing to me ever since, when I was very young, one came low over a field on my father's farm, a rope was thrown to the men hoeing turnips, but they "weren't touching them things—'tweren't nat'ral", and the balloon deposited its bruised passengers on a gorsy common some miles away Most of East Africa would be much less comfortable for landing than an English turnip field or common, one man told me that the most worrying minutes of his life were in a small aeroplane, the engine of which failed over a sisal estate in Tanganyika, gliding over miles of large erect needle-pointed leaves before they reached the grass beyond

So I admire anyone who has courage to learn ballooning for a few flights over tracts of country where mountains and escarpments rise for thousands of feet, where an equatorial Sun produces violent thermal currents and where forests, lakes and thorn trees make balloon landings very dangerous That was done by Anthony Smith science correspondent to the *Daily Telegraph,* and it is well described in this book

The accumulation of backers, of equipment and funds, the training flights in England and Holland and the arrival in Africa—all that takes up one-third of the book When the prime object was to photograph game in and around the Serengeti Park, I cannot understand why a start was made from Zanzibar, for a short flight to the mainland, in order to face hundreds of miles of bad Tanganyika roads to the game reserves Moreover, it is surprising that, when there was so much expensive equipment, there were no spare ropes so that it should not have been necessary to buy in the Zanzibar bazaar those old cotton ropes which nearly wrecked the balloon at the start

However, Zanzibar seems to have enjoyed the start, and it gave the expedition some adventure before more adventures on the muddy roads of Tanganyika People who visit Africa for adventure usually find it; we who worked there used to look for as much comfort as possible on our limited means, in all my camping I never had the excitement of having my tent flooded by a single storm, with bread, cameras and books floating about

But we were not balloonists and we had not the writer's gift for describing the sensations of ascent, of silent travel and perilous descent, so well portrayed by Mr Smith. Everyone is shocked by their first hearing of the tree hyrax's poignant call, but few could describe it so well. Few could have had the aid of such skilled photographers with such fine equipment, there are some beautiful photographs in colour, for the animals, the clouds and the orange and silver balloon were all photogenic

As I am a botanist, I would have liked to see more of the patterns of vegetation below the balloon and less of the passengers Moreover, I would like to have read more non-ballooning matter, for there are very interesting references to the bogus films of African game, to the cruelty of predators, to the problems of game conservation and counting of animals More emphasis might have been made concerning the great help given to Mr Smith and his party by many people It was surprising that, when Mr Smith had a chance to help a party stuck in the Ngorogoro Crater mud, he delayed that party by leading them into more soft ground, this exploit may have seemed funny to Mr Smith and his companions, but it cannot have seemed funny to the worried African driver, to his belated passengers or to the people who were waiting for them It does not seem funny to me, for I have spent a night in a car stuck in Serengeti mud I am glad that Mr Smith afterwards felt "slightly humble"

The book gives definite results of a trial of ballooning in tropical Africa "Any puff of wind was to be dreaded", there are many puffs of wind in Africa "I do not think balloon photography, to sum up, is very easy, but it is most interesting " So is the book, and, even if the project did little to advance the study of game in Africa (for most of the best photographs were taken on the ground), it may well lead to a revival of ballooning in Britain

A S THOMAS

A SOLUTION FOR MAGNETIC CIRCUIT PROBLEMS

The Nomographic Computation of Complicated and Highly Saturated Magnetic Circuits

By Prof Otto Benedikt Translated by G P Dienes Pp xviii+275 (London and New York Pergamon Press, 1962) 105*s* net

SINCE the earliest days of electrical machine design, one of the frequently recurring problems has been that involving iron under saturated magnetic conditions. Methods have been devised to solve such problems, but invariably the techniques use gross approximations and/or assume linear magnetic conditions which do not exist Generally, in machine design empirical relationships are used, demanding a great deal of experience on the part of the designer in order to obtain reasonably accurate results, while, in more theoretical problems, the custom is to assume linear magnetic conditions in order to render the problem more tractable In the latter case it is usual to add that the conditions are not linear, but that it is necessary to start somewhere, and that a knowledge of the idealized problem will assist in the understanding of the non-linear case Altogether, the subject, up to recent times, has proved to be an unfruitful one for those who would like accurate solutions Of recent years, the importance of finding solutions to these problems has been making itself more and more apparent, and at the present time much effort is being produced to place the subject on a higher level of understanding

The Nomographic Computation of Complicated and Highly Saturated Magnetic Circuits, by Otto Benedikt, would appear to be the result of close co-operation between industry and university It has already been published in Hungarian and German, besides the original Russian, and it is a pity that it was not translated and published in Britain sooner

The author states that the nomographic method, which he sets out in great detail, has been developed over a long period of time He also claims that, whenever it has been possible to check by experiment, the agreement between his theoretical and the experimental results has been good The purposes of the book are to show that the nomographic method is suitable for solving a large number of problems arising in the design and construction of electrical machines and also that the method may be used with some profit to solve other allied problems, some of these he lists in the final chapter

The first chapter deals with the shortcomings of the methods generally used in solving magnetic circuit problems, followed by chapters showing how the nomographic method may be applied to ferromagnetic bodies of varying cross-section and series connected bodies

Chapter 4 considers the much-debated problem of the magnetic relations in the region of the tooth and the magnetically parallel slot This leads naturally to the problems in the next chapters involving the series connexion of a tooth and a magnetically parallel slot, the magnetic relations in the region of tooth, slot and air-gap, the problem of saturated pole-shoes and the estimation of the transverse slot fluxes due to saturated teeth

In Chapter 9, it is claimed that the 'exact' eddy current and hysteresis losses in rotating electrical machines may be calculated by the nomographic method The word 'exact' is perhaps the choice of the translator rather than the author, for it is difficult to see either how the losses could be exactly calculated, or how they could be exactly measured in order to check the calculations However, methods are developed for calculating the various losses in the different parts of the magnetic circuit and also in the copper conductors

Throughout, the mathematics is set out in great detail, with many worked examples to aid understanding, and to illustrate the use of the three sheets of nomograms which are included at the back of the book

The units used are those of the c g s e m u system, which might provide some initial difficulty to those used to the m k s or mixed industrial unit systems It is pleasing to note that Carter's coefficient is mentioned, so presumably Britain is credited in the U S S R with some original work The book was printed in Hungary—this is reflected in the standard of printing which is not quite so good as one generally expects in a modern text-book No bibliography is provided This is unfortunate, since it would be interesting to see what other publications had appeared in connexion with this subject

To those engaged in any branch of non-linear magnetic circuit work, including machine designers, this book should prove to be a useful introduction to the nomographic method Many will find their problems already solved for them and set out in great detail One looks forward to more publications in this particular field

E M Freeman

QUANTUM MECHANICS

Quantum Mechanics for Mathematicians and Physicists

By Prof Ernest Ikenberry Pp xii+269 (London and New York Oxford University Press, 1962) 64s net

Mathematics for Quantum Mechanics

An Introductory Survey of Operators, Eigenvalues, and Linear Vector Spaces By Dr John David Jackson Pp x+97 (New York W A Benjamin, Inc , 1962) 3 50 dollars, paper , 4 75 dollars, cloth

According to his preface, Prof Ikenberry's intention is to provide a text on quantum mechanics for graduate mathematicians with little or no background in physics and for physicists with a minimum of mathematics Although the former may feel forsaken by the detailed physical content of the first three chapters on the dual nature of light, the dual nature of matter and the pictorial description of the atom they will find themselves well catered for in the remainder of the book The physicist, on the other hand, will visit the rough in the chapters on wave equations and wave packets, observables and linear operators and quantum mechanical operators but, with diligence, at the end will find his mathematical knowledge no longer minimal

After the introduction of the Schrodinger wave equation in Chapter 8, this book deals with most of the topics which are usually included in books of this kind, the harmonic oscillator, the hydrogen atom and spin, and ends with a very good introduction to relativistic quantum mechanics I myself found the chapters on sectionally constant potentials and eigenfunctions and eigenvalues quite excellent in presentation, scope and completeness Criticism can, however, be directed at the chapter on time independent perturbation theory which is ludicrously short, only six pages, and completely ignores some simple applications of this technique which are of considerable physical interest Indeed, in general, the attention of the reader is not sufficiently directed to the physical interest of the topics considered Comparable books on quantum mechanics contain sections on the quantum mechanics of simple many-particle systems, on applications of variational principles and on simple collision theory These are omitted here

Despite these defects, this book will provide teachers of quantum mechanics with a valuable manual There are 352 problems listed to form an integral part of the text, and abundant references are provided to guide the student in supplementary reading There seem to be few errors or misprints, and the presentation and notation are clear and precise One can hope, however, that the use of the symbol ' Lap" for the Laplacian will not become popular

Dr Jackson's monograph is a mathematical supplement to the course in quantum mechanics for first-year graduates which he gives at the University of Illinois He can justifiably claim to have presented concisely the mathematical methods of quantum mechanics in a form which emphasizes the unity of the different techniques Although Prof Ikenberry's and Prof Jackson's books are being jointly reviewed they are scarcely companion pieces, and it is clear that the courses in quantum mechanics given at Auburn and Illinois are considerably different in emphasis This is not a criticism of either course but is to make the point that maximum value from Prof Jackson's mathematical supplement will only be derived when read along with a companion volume which covers physical topics requiring this kind of mathematical development Such treatises usually provide their own mathematical supplement, either in the text or in appendix form, as in the case of Prof Ikenberry's book Accordingly it is difficult to believe that this book will command a wide sale among students, most of whom will prefer a single comprehensive treatment of the physics and mathematics of quantum mechanics

Considered, however, on its own as an applied mathematical text-book with chapters on eigenvalue problems in classical physics, orthogonal functions and expansions, Stum-Liouville theory and linear operators and linear vector spaces, it is very good Prof Jackson has a way of illustrating and developing mathematical concepts which I found refreshingly novel The reader will take particular pleasure in the use of the Schmidt process of orthogonalization to construct the important polynomial functions of mathematical physics Problems are interspersed throughout the text, and appendixes on the properties of the special functions are provided Teachers and students will find the tables of roots of the Bessel functions especially useful, and at last somoone has thoughtfully provided a key to the choices of spherical harmonic phase factor used in various standard texts on quantum mechanics This key could be more comprehensive, but it seems clear that in this connexion Prof Ikenberry prefers to be with the minority

A L Stewart

The Evolution of Living Organisms

Edited by Prof G W Leeper (A Symposium to mark the Centenary of Darwin's "Origin of Species" and of the Royal Society of Victoria, held in Melbourne, December, 1959) Pp v+459 (Melbourne The University Press London Cambridge University Press, 1962) 126*s* net

THIS interesting volume includes thirty-six contributions from workers distinguished in a wide variety of fields including evolution, genetics, and population dynamics The volume is divided into two parts of approximately equal length In the first part, twenty papers are collected under the heading, "Evolution and Selection" Although the scope and content of these papers differ very widely they are impressive contributions in their respective fields The first paper, "Accident or Design, the Paradox of Evolution", by E Mayr, is a brief but important, summary of the history of ideas on the origin of adaptation As Mayr points out, evolutionists are now closer in their thinking and in their entire interpretation of evolutionary events to the Darwin of 1859 than they have been at any time in the past hundred years Some of the other papers deal with very restricted aspects of the subject, for example, chromosome breakage in *Astroloma* and effect of chromosome inversions on size and viability of grasshoppers, while others, such as those on the origin of life, the evolution of dominance, and the survival of the mediocre, are clearly of more general interest

The 16 papers included in the second part of the volume are concerned with the distribution and evolution of the Australasian fauna and flora, and provide a wealth of detailed information indispensable to anyone with an interest in the fauna and flora of this area

In considering the origin and distribution of the animals and plants of the southern continents, one's attitude to the theory of continental drift becomes crucial in a sense that it scarcely ever does when dealing with groups restricted to the northern hemisphere, where the movements of the continents relative to one another have been much less Acceptance or non-acceptance of the theory of continental drift not only affects at various levels the manner in which factual evidence of past and present distributions is interpreted, but it also seriously affects conceptions about the phylogeny of many groups For these reasons it seems a pity that more attention has not been directed to this basic problem, although the few authors who have touched on it seem to reject the possibility of continental drift H E HINTON

Symbiotic Associations

Thirteenth Symposium of the Society for General Microbiology held at the Royal Institution, London, April 1963 Edited by P S Nutman and Barbara Mosse Pp x+356 (London Cambridge University Press, 1963) 50*s* , 9 50 dollars

THE Society for General Microbiology has a well-deserved reputation for its symposia They are planned to cover a wide field and to point toward future developments, and not simply to summarize existing knowledge on a narrow topic This, on symbiosis in micro-organisms, is another worthy successor in the series It shows both the strength and weakness of this approach The topics range from bacteriophages to lichens Every microbiologist will surely find fascinating new facts on material which is unfamiliar to him This wide appeal is the result of the wide range The weaknesses are also apparent Few of the articles are detailed and up-to-the-minute reviews of the kind that are so much in demand nowadays This is expected as a necessary consequence of the general policy of these symposia However, more serious defects are seen in a few of the contributions In some the subject-matter has not been related carefully to the problem of symbiosis This is particularly marked in the section on bacteriophages In others the theory of symbiosis is skimmed over The standard of writing is nevertheless commendable, and nearly all the articles are easy to follow

The chapters fall into three sections First, there is an introduction by R Dubos and A Kessler This, on general factors of symbiosis, is stimulating but not very clearly written The second section is entitled "Plant Associations" It includes sections on lichens (surely among the most remarkable forms of life), on legume nodules, and on mycorrhiza The third section is on animal associations, many of which are fascinating, for example the symbionts of insects, discussed by Marion A Brooks, and the chapter on algae and invertebrates by M R Droop J M Baker contributes a fine chapter on the ambrosia beetles and their fungi Two articles deal with associations in the rumen The book is well produced, and errors are commendably few P H A SNEATH

Quantum Statistical Mechanics

Green's Function Methods in Equilibrium and Non-equilibrium Problems By Dr Leo P Kadanoff and Dr Gordon Baym (Frontiers of Physics a Lecture Note and Reprint Series) Pp xi+203 (New York W A Benjamin, Inc , 1962) 4 95 dollars, paper, 6 95 dollars, cloth

THE principal problem of quantum statistical mechanics is the many-body problem It consists essentially of finding methods for evaluating equilibrium and non-equilibrium characteristics of large assemblies of particles Recently new methods for investigating this problem have been developed which are based on the mathematical techniques of quantum field theory One of the most effective of these techniques is the quantum mechanical Green's function method developed by Martin and Schwinger This book consists of a set of lectures on this Green's function method by two of their former students

In all, there are thirteen chapters The first five are devoted to the original work of Martin and Schwinger on the equilibrium Green's function and to methods for deriving approximations to the Green's function The following six chapters outline a theory of non-equilibrium phenomena, including a generalization of Boltzmann's equation with applications to sound propagation and the Landau theory of Fermi liquids These six chapters owe much to the original research of the authors The last two chapters discuss the use of the shielded potential to describe a Coulomb gas and the application of the two-body scattering matrix approximation to systems with short-range interactions

Being essentially a collection of lecture notes the style is informal and at times brief, with an emphasis put on a mathematical analysis of the problems discussed There is no index and, except for a list of supplementary reading matter at the end, there are few references The lectures are suitable only for the serious student of statistical mechanics who is familiar with quantum mechanics and with second quantization Such a student should find this a useful addition to his library The book would be of little use to the student who wishes only to apply statistical mechanical results to specific problems F J SMITH

Abstract Harmonic Analysis

By Prof Edwin Hewitt and Prof Kenneth A Ross Vol 1 Structure of Topological Groups—Integration Theory—Group Representations (Die Grundlehren der Mathematischen Wissenschaften, Band 115) Pp viii+519 (Berlin Springer-Verlag, 1963) 76 D M

IN the theory of Fourier series one deals with periodic functions, which may be considered as functions defined on a circle, with the circular functions *sine* and *cosine* taking a special role The subject of this book, abstract harmonic analysis, can be regarded as a vast generalization of the theory of Fourier series in which the circle is replaced by any topological group, which is either compact or locally compact and Abelian (The case of a non-Abelian group, which is only locally compact, has a less complete theory and is outside the scope of the present work) In the generalized theory, the role of the circular functions is played by the matrix elements of the irreducible matrix representations of the group It is remarkable how many of the theorems about Fourier series survive such a broad generalization

The development of the theory requires a great deal of special knowledge about topological groups, integration theory and invariant measures on groups Even though the authors do not hesitate to refer to standard texts whenever possible, their treatment of this preparatory material occupies the first half of the volume, and constitutes a valuable addition to the text-books of these subjects Only in the last two chapters, on convolutions and group representations and on characters and duality of locally compact Abelian groups, does the harmonic analysis proper begin Full details of the subject are reserved for the second volume There are appendixes on Abelian groups, topological linear spaces and normed algebras, and a good bibliography

Text-books at this advanced level not uncommonly make very heavy reading, and so it is a pleasure to report that the authors have obviously made great efforts to ease the way for those readers who are not already experts Thus the proofs are written out in very good detail An abbreviated first route through the book is recommended Nearly all the twenty-six sections contain a large collection of examples and miscellaneous theorems to round out the general theory There are also notes commenting on the history of the leading ideas of the section, and giving references to alternative treatments and further developments Thanks are due to the authors for producing a book which will serve well both students and experts in its subject T A S JACKSON

Textbook of Organic Chemistry

By Prof Alexander Gero Pp ix+679 (New York and London John Wiley and Sons, Inc , 1963) 72*s*

PROF. GERO has had long experience of industrial research and university teaching, and the latter is the basis of this book Although it starts with an introductory survey of organic chemistry and of electronic theory it is a university book rather than one for schools.

A preliminary fifty-page chapter deals with relatively simple compounds and their reactions A second one deals with fundamental theories, explaining the nature of organic reactions on the valence-bond theory rather than on molecular-orbital theory which Prof Gero finds less suitable for many of his students owing to their inadequate background of physics and mathematics The latter theory is included in a penultimate chapter A final chapter is on physical methods used in organic chemistry It discusses concisely the use of the polarimeter and *p*H-meters, of ultra-violet and infra-red spectroscopy, molecular resonance and various resonance spectra

Separation of aliphatic from aromatic compounds is abandoned after the preliminary chapter, and related types, for example, alcohols and phenols, are considered together Physical properties receive full consideration, but the aim is not a load of facts but a balance of them with theory so that the student shall "understand how and why reactions take place" Practical instructions are not part of the book The main part of the book comprises twenty-three chapters on groups of compounds selected for their importance in giving a worth-while picture of the subject, two on stereochemistry and one each on the hydrocarbon-based industries and the plastics industry and polymerization There are two chapters on polyfunctional compounds, three on heterocycles and one each on isoprenoids and steroids Many chapters conclude with a useful summary and almost all of them with a selection of questions

The book is produced to the high standards that are to be expected from American text-books H DUNNICLIFF

Birds of Hampshire and the Isle of Wight

By Edwin Cohen Pp xii+278+16 plates (Edinburgh and London Oliver and Boyd, Ltd , 1963) 30*s* net

ONE of the signs that birds are the most popular group of animals among British naturalists is the existence, for every county in England and most of those in Wales, of a book or at least a booklet devoted entirely to their birds But so great has been the increase in our knowledge of the migration and breeding distribution of British birds resulting from the remarkable upsurge of amateur observation in the past thirty years, that any county avi-fauna not published within that period (or indeed within the past ten years) is likely to be seriously out of date This was certainly the case with Hampshire and the Isle of Wight, where the standard work dated from 1905, and this new book by Edwin Cohen, who has for so long been associated with the county's ornithology, will therefore be most welcome to bird watchers both in and out of Hampshire

Much of the value of such a work depends on the thoroughness with which the author has both combed the vast and scattered literature and vetted the records of rarities, in these respects Mr Cohen has been exemplary Though the main part of the book consists in the usual annotated systematic list of the individual bird species, there are also some useful introductory chapters D W Wray writes on geology, Mrs A K Hunt on botany, and six authors deal with birds in relation to habitat and land use in the county divided into seven regions J S Ash on the Fordingbridge area, D F Billett on the south-east corner, E L Jones on north-west Hampshire, J Stafford on the Isle of Wight, J H Taverner on the Winchester area and part of the Solent shore, and the author himself on his home region, the New Forest R S R FITTER

A Study of Reptiles and Amphibians including their Care as Pets

By Alfred Leutscher Pp 80 (London Blandford Press, Ltd , 1963) 10*s* 6*d*

ALFRED LEUTSCHER has written yet another popular account of reptiles and amphibians intended for the lay reader Three-quarters of the text is a thumbnail sketch of the origin, evolution, classification, biology and habits of the reptiles and amphibians, with a short chapter on popular misconceptions The remaining quarter concerns the care of these creatures in captivity and simple experiments and observations that nature groups and others might carry out For the person to whom herpetology is a new interest a fair background to the subject is given, but some wildly inaccurate facts and figures are also presented and an outdated classification of the amphibia used, which suggest that the author is unfamiliar with the literature and with many of the animals

A few examples will suffice In describing caecilians, the author states that "*behind* each eye is a small tentacle on the face" The leathery turtle is defined as having "a soft leathery skin with only *a few* bones in it", there are in fact many hundreds The pineal eye of the tuatara is reported as possessing an *iris* (My italics) But the most astonishing definition is that given to the Reptilia "they are distinguished from all other vertebrate animals in having lungs and limbs, and their bodies covered in a protection of scales". Into this category the uninitiated might gleefully place the armadillo, pangolin and the lung fishes While the photographs are good, the line drawings are of limited use and some are even grossly inaccurate If a competent illustrator was not available, reproduction of early figures of snake skulls would have been infinitely more reliable than the author's diagrams, which feature a puzzling arrangement of bones with an apparently fractured pterygoid hinged to the lower jaw

A G C GRANDISON

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Sir HOWARD FLOREY, P.R.S.

AWARD OF MEDALS, 1963

Copley Medal: Sir Paul Fildes, F.R.S.

SIR PAUL FILDES has the enviable distinction of being the pioneer chiefly responsible for opening up a vast new field of microbiological research. His early studies, more than half a century ago, on the growth factors of *Haemophilus influenzae* and related organisms and his elucidation of conditions requisite for the sporulation and germination of anaerobic bacteria of the tetanus group, both *in vitro* and in the infected animal, were notable achievements in their own right. Their chief importance, however, lay in the fact that they convinced Fildes that the key to the rational control of microbial infections lay in a full knowledge of the nutritional requirements of the causative organisms and elucidation of the biosynthetic precursors involved in the utilization of essential nutrients. He was probably the first to recognize the necessity of close collaboration between bacteriologists and biochemists for fruitful investigations along this line of approach. To this end he established in 1934, under the auspices of the Medical Research Council, a team which, under his leadership, pioneered the way into the field of bacterial chemistry.

Of the many notable discoveries resulting from the work of his unit perhaps the most outstanding was the demonstration of the inhibitory action of the sulphonamide drugs on the utilization of *p*-amino-benzoic acid in bacterial synthesis. This discovery provided the stimulus for the intensive search for analogues of essential growth factors for use as chemotherapeutic agents, a search which is still being carried out on a world-wide scale.

The influence which Fildes's work has had on biological research extends far beyond the realm of medical microbiology. Stemming from his discoveries came the realization that bacteria provide the best material for the investigation of many of the fundamental, and interlinked, enzymatic processes on which the life of higher organisms, including man himself, depends. Equally the development of bacterial genetics would have been impossible without the background of knowledge derived from the exploitation of his early concepts.

Fildes still continues in active research, and during recent years has published important papers on interactions between bacterial viruses and bacterial host cells.

A Royal Medal. Prof H. H. Read, F.R.S.

Prof Read has achieved an international reputation for the contributions he has made to the understanding of metamorphism and the interpretation of granites. Trained in the hard practical school of the Geological Survey before he entered academic life, he has always appreciated that theories of space and time in geology must stand up to the observed facts of field work. It is for this reason that his work on low-load metamorphism, metamorphic differentiation and polymetamorphism has placed him in the acknowledged position of a leader with a large and successful school of workers following the directions in which he has pointed.

His work on migmatites led him to look at the problems posed by the existence of granites and to develop the concept of the Granite Series. In his famous book *The Granite Controversy*, he fitted granites according to their composition, form and environment into the framework of mountain, fold or mobile belts. From an international point of view, it is for this work that he is probably best known, but we should not forget the many pieces of carefully detailed field interpretations of Scottish metamorphic and igneous rocks, nor, on a different plane, his ability to write excellent text-books.

A Royal Medal: Dr Robert Hill, F.R.S.

Dr Robert Hill is distinguished for many original contributions to biochemistry but particularly for his outstanding work on the processes involved in photosynthesis. His first research on the affinity of different hæmoglobins for oxygen led to the development of a sensitive quantitative test for measuring oxygen produced in reaction systems, and by applying this test to isolated chloroplasts he discovered the now famous Hill reaction. He demonstrated that isolated chloroplasts have oxido-reductive systems activated by light, that oxygen production is independent of carbon dioxide fixation but requires an electron acceptor, which need not be specific because naturally occurring compounds could be replaced by such substances as ferric oxalate. His identification of the light-induced reaction with the photolysis of water initiated current investigations in cell-free systems of the photochemical phase of photosynthesis, and his lucid formulations of the problems contain most of the concepts on which work fruitfully proceeds. Hill recognized an enzyme involved in the oxygen-yielding system, and this was later purified and crystallized. He also identified cytochromes peculiar to chloroplasts, and it is generally accepted that these are concerned in an electron transport system connected with photosynthesis. Although Hill is best known for his work on photosynthesis, he has also contributed much to knowledge about the role of cytochromes in respiration, and he was one of the first to investigate isolated mitochondria from plants. His remarkable insight into biochemical processes has kept him consistently as a pioneer, able to design experiments that seem simple because they are made without elaborate equipment but that produce decisive and novel results.

Davy Medal. Dr E J Bowen, F.R.S.

Dr Bowen has made many contributions to physical chemistry, but undoubtedly his main work has been the investigation of phenomena involving the interaction of matter and radiation. He was among the first to examine the mechanism of photochemical processes in terms of quantum theory at a time, forty years ago, when the significance of Einstein's law of photochemical equivalence was not clear. He investigated the photo-decomposition of a number of simple gases such as ozone, the hydrogen halides, and the oxides of chlorine. Some of these reactions show simple behaviour, and Bowen's results, together with those of Warburg provided some of the earliest verifications of Einstein's law. Other reactions, however, showed deviations from the law, and Bowen studied further the decomposition of aldehydes and ketones and the photo-oxidation of hydrocarbons and alcohols. Low quantum yields in liquid-phase reactions were attributed to primary recombination, and high yields to chain reactions.

His attention later turned from photochemistry to the phenomena of luminescence, phosphorescence and fluorescence, and particularly to the quenching of fluorescence. Much of his work was done with anthracene and other polynuclear hydrocarbons such as rubrene, naphthacene, pentacene and coronene. For anthracene he demonstrated elegantly the interrelation between fluorescence, photodimerization and photo-oxidation. The relative efficiencies of different molecules in quenching fluorescence gave information about the variety of interactions involved, ranging from perturbation by van der Waals forces to highly specific transfers of electronic energy. A knowledge of the absolute magnitudes of quenching efficiencies, together with their variation with temperature and viscosity, yields information about collision processes in liquids which is difficult to obtain in any other way.

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* Delivered at the Royal Society on November 30

Some of Bowen's earliest work was on chemical reactions and fluorescence in solids, and recently he has returned to an investigation of fluorescence in solids and gases. In particular, his work on solid solutions of naphthacene in anthracene shows how a photon absorbed by one component can be transferred over considerable distances in the solid before it excites fluorescence of the other component; this kind of energy transfer is of importance for theories of the solid state. He has always been interested in the application of fluorescence and other optical measurements to other sciences, and has published papers on the use of fluorescence for analysis, the action of light on dyes, photosynthesis, and a method for making visible streamlines in fluid flow.

A marked characteristic of Bowen's experimental work has been the skilful use of simple apparatus.

Hughes Medal: Prof. F. C. Williams, C.B.E., F.R.S.

Prof. Williams's war-time contributions to the development of radar were inevitably somewhat obscured by the circumstances of the time, but it was generally understood that they were of considerable importance. They were recognized by the award of an O.B.E. in 1945, and he received the premier premium award of the Institution of Electrical Engineers, the 'Institution Premium', for a group of papers on pulse techniques and associated circuitry which he presented to the I.E.E. Radar Convention held in 1945.

On returning to the University of Manchester in 1946, his primary interest became the development of digital computing machines, in collaboration with T. Kilburn, and he soon perfected a cathode-ray-tube storage system which formed the central part of the Manchester computor which began to operate in 1948. This was the prototype of the first Ferranti *Manchester Mark I* machine which was installed in the University in 1951, and this machine was unquestionably the first commercially made saleable machine in the world. In addition to the cathode-ray-tube store, several other novel features were incorporated which are common to most present-day digital computors. Williams was the inspiration behind the early-stage development of digital computors in Great Britain and his work exercised a considerable influence on similar developments in other parts of the world.

Later his interests turned from electronics to electrical machines. His objective is to devise a brushless variable-speed a.c. motor and some progress has been made towards this. It appears that practically any pair of 'even pole number' speeds can be provided by his method using a simple switching system, and that at least five speeds can be obtained at the cost of rather more complex switching arrangements. There seems little doubt that these machines will find rapid acceptance in industry, since the two-speed version shows a saving of one or two frame sizes over the double-wound equivalent.

This work is now being extended to cover multi-frequency alternators by pole-changing methods, and various schemes are under consideration for application to electric and Diesel electric traction. Present experiments are concerned with the application of semi-conductors to rotating machines. In all this machine work, Williams has been ably assisted by many colleagues, but the inspiration has again come from him.

The quality of his work and of his authorship may be judged from the fact that he has been awarded, either individually or in association with his staff colleagues and research students, no less than nine premiums of the Institution of Electrical Engineers, including the Kelvin and the Sylvanus Thompson Premiums each on two occasions.

Leverhulme Medal: Dr. A. J. P. Martin, C.B.E., F.R.S.

Dr. A. J. P. Martin has been the central figure in the development of chromatography, the outstanding advance in the techniques of chemistry for the past quarter of a century.

The most important part of this work was carried out during his service as a chemist at the Wool Industries Research Association in Leeds (1938–46) and later on the staff of the Medical Research Council in London (1948–56). Through this period, and particularly during the past fifteen years, chromatography has emerged in a variety of forms as a method of analysis, separation and purification of the greatest value to chemistry. Dr. Martin's outstanding contributions to this explosive growth have been widely recognized by numerous awards including that of the 1952 Nobel Prize for chemistry (jointly with Dr. R. L. M. Synge), the Berzelius Gold Medal of the Swedish Medical Society (1951) and the Franklin Institute Medal (1959).

The methods of chromatography have become so much a part of chemical practice, and in so short a time, that they scarcely call for description. Like distillation, crystallization and titration, they form part of chemical operations in laboratories throughout the world. In all the main sections, paper chromatography, column or partition chromatography, and the later gas chromatography, Martin played a leading part, and his work led on to later technical modifications and extensions such as ion-exchange and reversed-phase chromatography. His inventiveness, imagination and resourcefulness have been linked with a full understanding of basic principles, and have enabled earlier disconnected observations to be converted into sound general methods of extraordinary and sweeping range. Paper and partition chromatography enabled new fields of organic, biochemical and medical research to be successfully attacked, and later applications to inorganic analysis have been of great benefit. Gas chromatographic procedures, with their auxiliary methods of detection and measurement, have attained a remarkable elegance and have presented the chemical and petroleum industries with a most valuable analytical tool.

The applications of chromatography to-day are fantastically diverse. They range from the investigation of cosmology to that of disease, and from the evaluation of mineral deposits to that of Old Masters.

ACCOMMODATION OF THE ROYAL SOCIETY

This year I have been faced with more than the usual difficulties in deciding what I should say in this anniversary address. I wrote one version in a euphoric frame of mind engendered by perfect weather enjoyed in the shade of banana trees not very many miles from the monument to Captain Cook's landing in Tahiti to observe the transit of Venus. In that respect the address might well have been unique. But a return to the less salubrious climate of Great Britain has forced me to admit that the full course of the negotiations for improved accommodation for the Society has not yet been run. Nevertheless I have some reason to believe that they will be successful, so that with the agreement of the officers I will try to tell Fellows what is happening and where we stand.

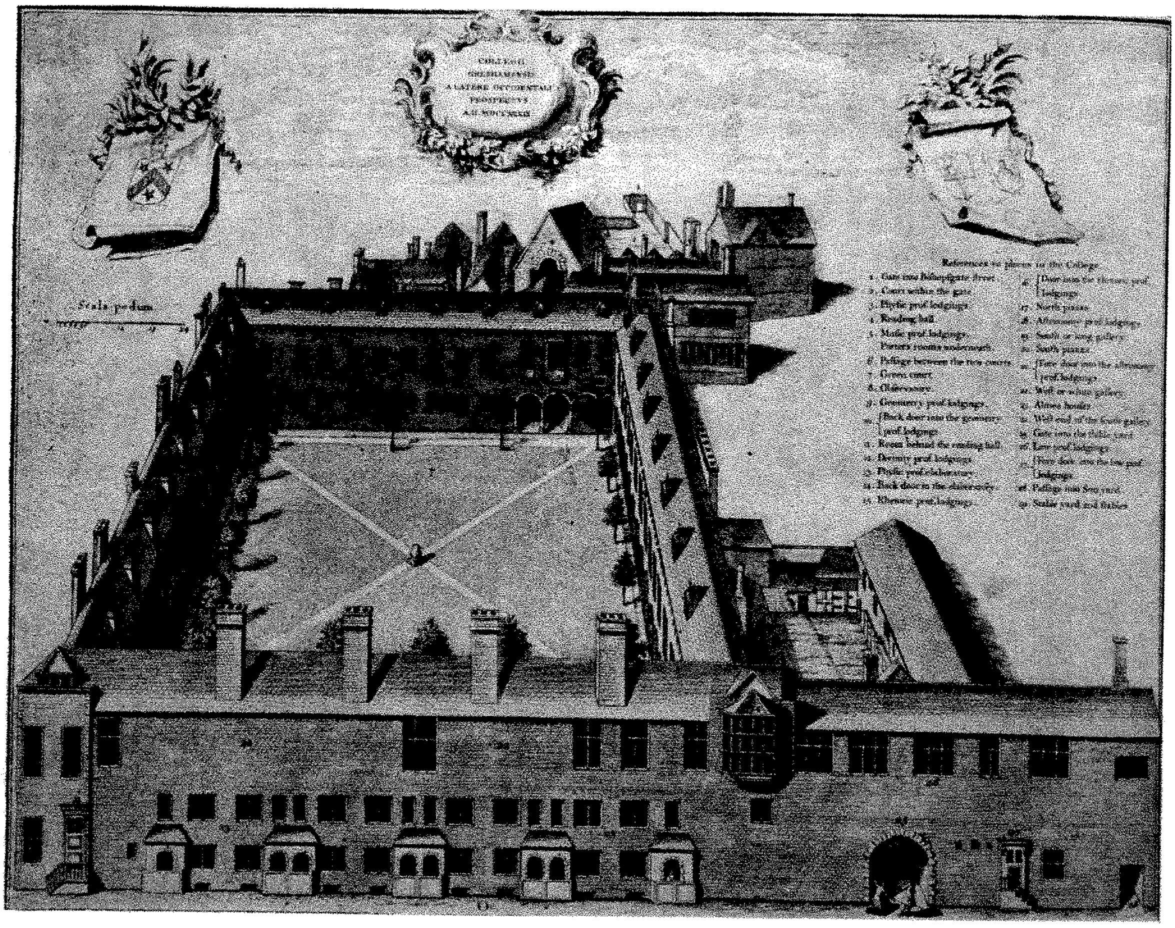
Scala pedum
References to places in the College
1. Gate into Bishopsgate Street
2. Court within the gate
3. Physic prof. lodgings
4. Reading hall
5. Music prof. lodgings. Porters rooms underneath.
6. Passage between the two courts
7. Green court
8. Observatory
9. Geometry prof. lodgings
10. Back door into the geometry prof. lodgings
11. Room behind the reading hall
12. Divinity prof. lodgings
13. Physic prof. elaboratory
14. Back door to the elaboratory
15. Rhetoric prof. lodgings
16. Door into the rhetoric prof. lodgings
17. North piazza
18. Astronomy prof. lodgings
19. South or long gallery
20. South piazza
21. Fore door into the astronomy prof. lodgings
22. Well or white gallery
23. Alms houses
24. West end of the South gallery
25. Gate into the stable yard
26. Law prof. lodgings
27. Fore door into the law prof. lodgings
29. Stable yard and stables

Fig. 1. Gresham College

Some of the older Fellows will remember that Sir Henry Dale devoted his presidential address of 1943 to a consideration of the accommodation of the Society, which was not only inadequate for its activities at that time but had been so for many years. He gave a sketch of the previous arrangements for the housing of the Society and drew some conclusions not only about our own post-War needs but also about those of numerous other scientific societies. To give a background to what I have to say about present developments, I propose to repeat some of his remarks in the hope that the older Fellows will not have a clear recollection of what Sir Henry said and in the confident belief that few of the younger Fellows read former anniversary addresses.

The question of a building in which it could function effectively has exercised the Society on and off ever since its beginning. Soon after its foundation the original rooms which had been put at its disposal by Gresham College (Fig. 1) had to be evacuated because the College was needed for the Royal Exchange—the buildings of which had been destroyed in the fire of London in 1666.

Mr. Howard, who was later to be the sixth Duke of Norfolk, then offered the Society rooms in Arundel House (Fig. 2) the site of which is now marked by Arundel Street and Norfolk Street near the Strand. This offer was gratefully accepted by Council, and the first meetings were held in Arundel House in January 1667. These rooms were occupied for six and a half years, during which time Council began to discuss the provision of more permanent accommodation, and, in the modern manner, appointed a Committee which, as is sometimes the case in our own day, accomplished little. It was perhaps not to be unduly blamed for this, for great hopes were raised by the gift of Chelsea Hospital to the Society by Charles II. But the buildings were unfortunately dilapidated and in need of repair, and in spite of strenuous efforts money could not be raised for their restoration. Generous donors were, however, not entirely lacking, for Mr. Howard gave the Society 4,000 sq. ft. of the grounds of Arundel House on which to build. Unfortunately difficulties arose with regard to this site and, what was ultimately more serious for the Society, with regard also to the Chelsea Hospital site. So, in spite of apparently great efforts, nothing came of the negotiations, and to the lasting and serious deprivation of the Society the Chelsea site was handed back to Charles II in 1682 for the payment of £1,300. It was, to be sure, quite reasonable for the President and Council of the day to consider this to be in the best interests of the Society, and no doubt we should have taken the same course to-day if confronted with the same situation.

During this period Hooke made proposals for building what was described as a "handsome fabric for the Society's use, with a library, repository, laboratory and other conveniences for making experiments". But it appears that he never put his plans on paper.

Another effort which came to nothing was to include rooms in a rebuilt Gresham College. Wren was asked to consider what accommodation the Society needed and to produce a suitable design. Unfortunately the proposals, which had the full support of Gresham College, were among a number connected with the College which needed an Act of Parliament for their implementation, and the Act when brought before the House was rejected at the first reading. This made it necessary for Council to take vigorous measures to find premises, their efforts being stimulated when the Mercers' Company let it be known in 1705 that they were not prepared to let the Society have any room at all in Gresham College. Many explorations were made, including a petition to Queen Anne for a grant of land at Westminster; but they came to nothing

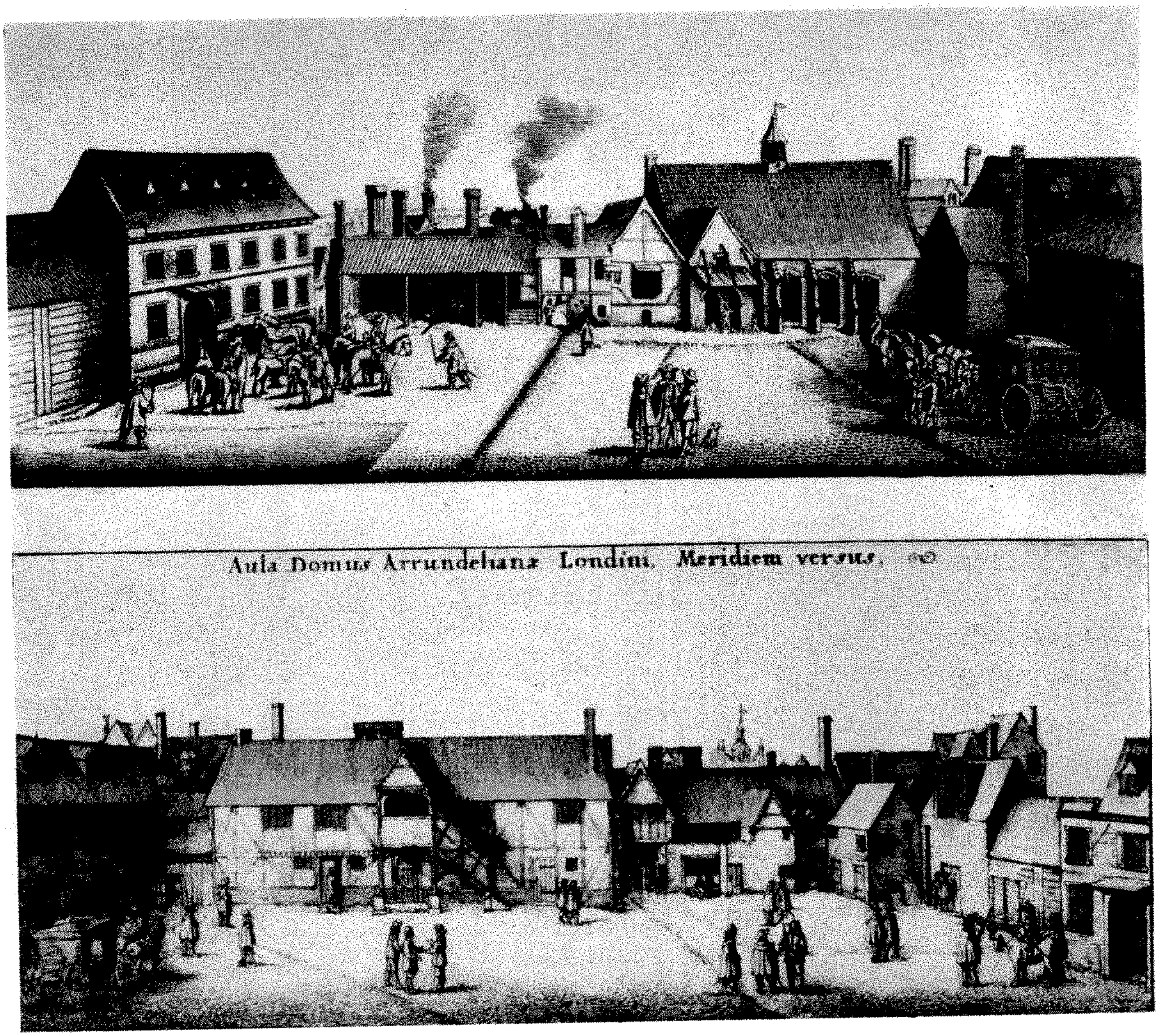
Aula Domus Arrundelianæ Londini, Meridiem versus.

Fig. 2. Arundel House

until in 1710 two houses were bought in Crane Court where the Society remained until 1780 (Fig. 3).

The bald statement that the houses were acquired scarcely conveys the effect on some of the Fellows of this decision by the President, Sir Isaac Newton, and Council. There was a stormy meeting about the proposals and a good deal of acrimonious comment was made; but this did not deflect the President and Council in whose competence the decision rested. The President, it seems, behaved with mule-like obstinacy. But within a short time all this was forgotten, and although the accommodation of Crane Court was never adequate the Society flourished there to such an extent that it acquired a reputation as a body of national importance, with the result that in 1778, thanks to the personal interest of George III, the Government agreed to provide rooms rent free and indeed free from any financial obligations at all, an arrangement which has happily continued ever since.

Discussions took place to accommodate the Society in the new Somerset House (Fig. 4), which was to be designed by the architect Sir William Chambers to house a number of bodies, including the Royal Academy. The space offered to the Society seemed to the Council to be inadequate, but after some rearrangement of the rooms had been proposed it was concluded that the conditions were so favourable that the Government's offer should be accepted. The houses in Crane Court were consequently sold; and it is not without interest that they brought in only £1,000, although seventy years before they had cost £1,450. This is a sharp and interesting contrast with the modern trend in property values.

The rooms in Somerset House were never sufficient for the Society's needs, although the meeting room was certainly elegant. By 1852 the congestion had become so acute that the Library Committee recommended that Council should raise with the Government the whole question of the future accommodation of the Society.

About this time the idea took root that it was desirable to bring the major scientific societies under one roof, by which arrangement they could share meeting rooms of various sizes and have co-ordinated library facilities without impairing the independence of the societies. Considerable pressure towards this goal was exerted by a newly formed dining club, the Philosophical Club, which had started to consider the preparation of a memorandum on the subject as early as 1847. It appears that it was through this Club that a proposal was made that the Society should endeavour to obtain Burlington House from the Government in place of the rooms in Somerset House, and in 1856 this was proposed in an official letter. The Government were not then in a position to give a definite undertaking, but agreed that at least temporary premises should be provided in Burlington House for the Royal Society and some others. This offer was accepted (Fig. 5). Burlington House has undergone a number of changes. Originally it must have been a very desirable and commodious town residence. It was altered by the Lord Burlington of the day according to the plans prepared in 1717, after which it probably remained essentially unaltered so far as its exterior was concerned until the middle of the nineteenth century when it appeared as shown in Fig. 6.

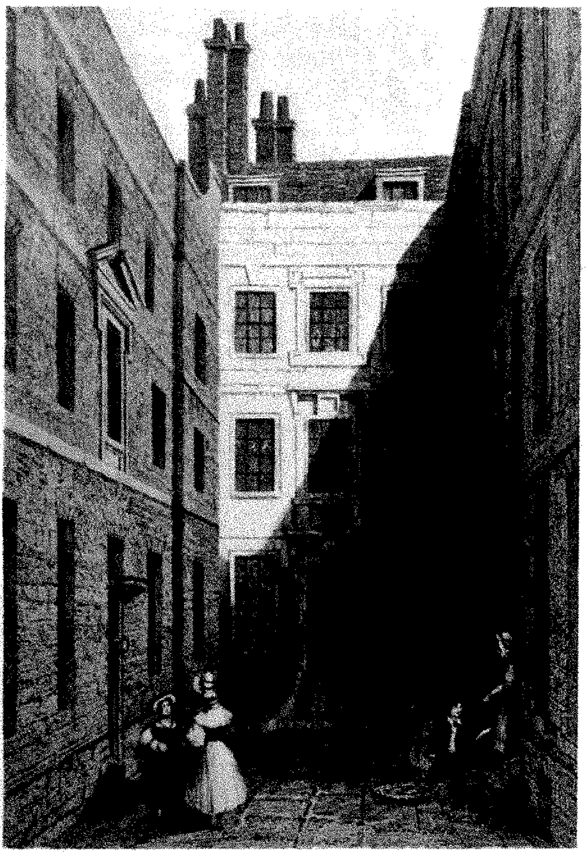

Fig. 3. The Houses of Crane Court

In the interval between the Great Exhibition of 1851 and the acceptance of the Government's offer of Burlington House another suggestion had been made. With the profits of the Exhibition, land had been purchased in Kensington Gore, and it was proposed to group the most important scientific societies there. However, the scheme was rejected by the interested societies, who wanted a more central position, for at that time Kensington Gore was almost in the outer suburbs. The idea of what has recently been called a 'Science Centre' was thus clearly formulated by our predecessors more than a century ago.

The occupancy of Burlington House by the Royal Society, which began in 1857, did not long remain undisturbed, for in 1866 the Government decided to allocate it to the Royal Academy. The manner in which this event was announced seems to indicate that the Royal Society was not esteemed as highly in ruling Government circles as the Fellows might have wished or may indeed have imagined, or that in some way it had been outmanœuvred. The first information that the Society had of the impending move came from the columns of *The Times* of 1867 in the form of a statement that the Government had decided to give the Royal Academy a permanent lease of Burlington House with the right to extend northwards. Sir Edward Sabine, the President, was away at the time—let me hasten to add very properly so, as it was during the summer vacation—but then, as now, the Society had an alert treasurer, Prof. W. A. Miller, who promptly wrote to the Prime Minister, Lord Derby, to enquire if the announcement was true. It was.

The Government wished to use the building the Royal Academy occupied in Trafalgar Square for what is now the National Gallery, and so the Academy had to move. To what better place than Burlington House? The Royal Society then went through five years of considerable

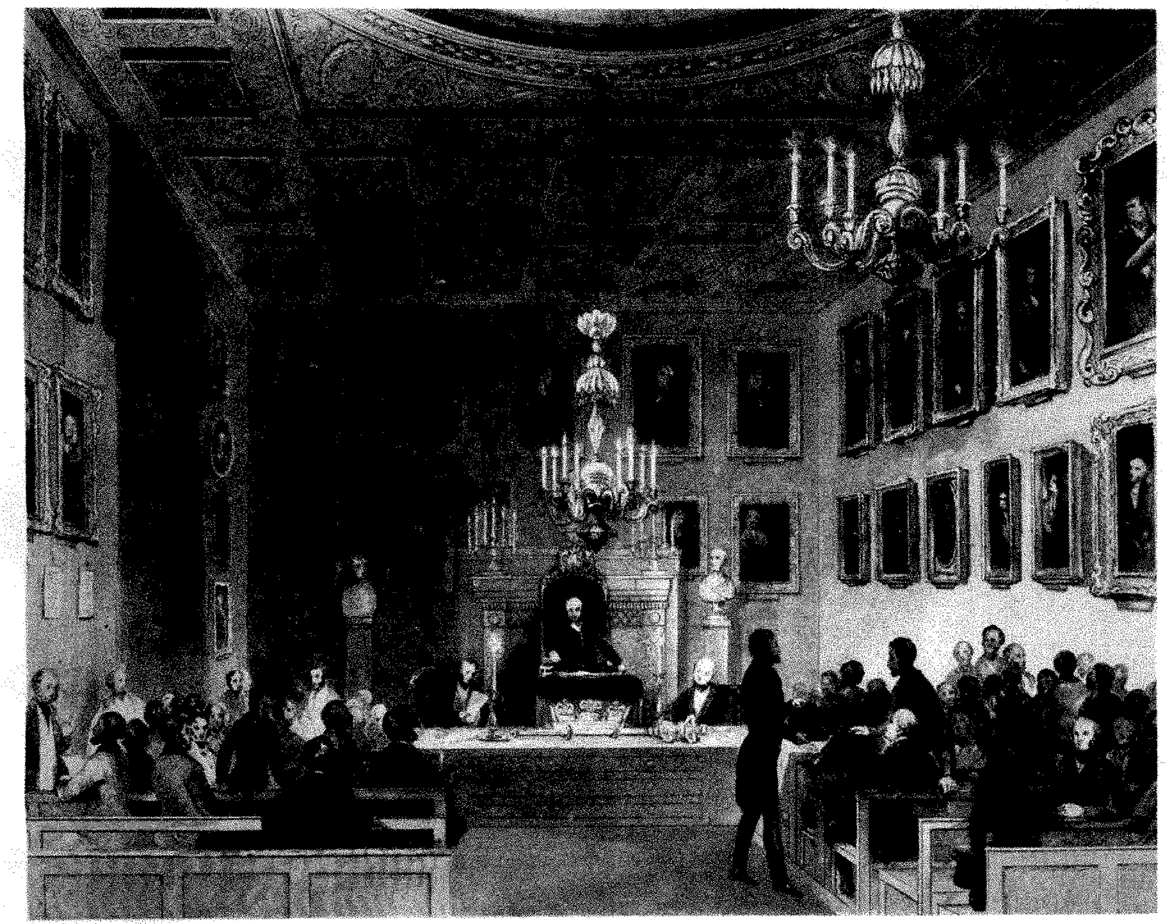

Fig. 4. The Meeting Room in Somerset House

difficulty, and, I have no doubt, discomfort and annoyance while the Burlington House that we know was constructed.

The Royal Society was then allotted its present rooms, the occupation of which was completed by November 30, 1873, when the anniversary meeting was held here.

The utilization of the magnificent site surrounding Burlington House for scientific societies was further complicated by the construction of what is now known as Burlington Gardens on the northern portion of the area. Burlington Gardens was built to accommodate the University of London, and as it was principally to be used for examinations it was constructed with a number of extremely large rooms.

As Sir Henry Dale pointed out, a magnificent opportunity to provide adequately for the growing scientific societies was lost, for the buildings allotted to them around the quadrangle on the Piccadilly side could not be enlarged, and even when first constructed they were not more than sufficient for present-day needs. Nor could the original conception of some integration of the facilities of the societies be carried out.

The Society had not occupied its present rooms for long before pressure on space began to grow once more. This was already evident by 1900 when at a meeting of the Council of the Society held on March 22 "the Senior Secretary reported that, having learnt in an indirect manner that the Government was considering how to make use of the building in Burlington Gardens, about to be vacated by the University of London, he had written privately to Lord Esher explaining that the present rooms occupied by the Society were rapidly becoming inadequate for its proper accommodation and desiring him to consider whether the Society might not with advantage be transferred to the above vacant building". But this was not to be, and the Civil Service Commission was installed in Burlington Gardens (Fig. 7).

The pressure on space has continued to build up. Some indication of the growth of the activities of the Society is given by the fact that there are now over 100 committees—committees, let it be emphasized, which actually do something—and that to serve them and the other functions of the Society the permanent staff has grown from 2 in 1873 to 70 in 1963. Fellows who have inspected the premises in detail in recent years must have been astonished at the cramped and inadequate conditions under which the constantly growing executive staff have been doing their work with such efficiency and success.

In the anniversary address of 1950 Sir Robert Robinson gave a résumé of the negotiations that had been going on since 1944 for the improvement of the accommodation of scientific societies. These negotiations culminated in proposals to establish a 'Science Centre' which was to house a number of societies including the Royal, as well as Government scientific organizations such as the Department of Scientific and Industrial Research. This centre was to act as a focus for both national and international scientific activities. The site chosen for the centre was on the South Bank, and the Government offered to finance the project. The President said: "All the fundamental aspects have been agreed in principle and, if we are now to be disappointed, it can only be as the result of some grievous calamity". The fact is that the offer of the Government was withdrawn in 1958. What the judgement of posterity will be it is impossible to say, but I sometimes have a fleeting thought when looking at the splendid panorama of the Thames from the Festival Hall that our successors may want to know why this scheme did not come to fruition, and no doubt this will furnish an interesting article for *Notes and Records* in, say, fifty years' time.

The problems of accommodation continued to press, and so about three years ago I enquired from the Execu-

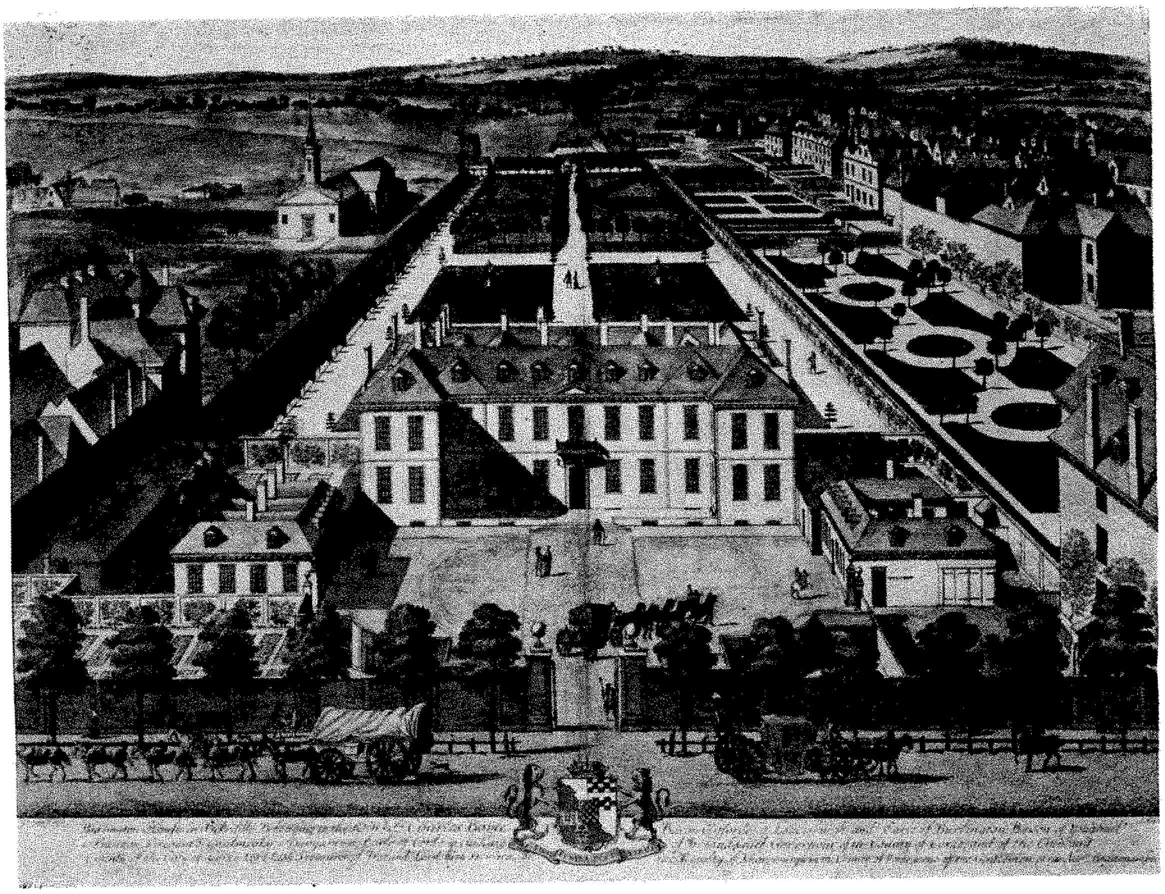

Fig. 5. Old Burlington House

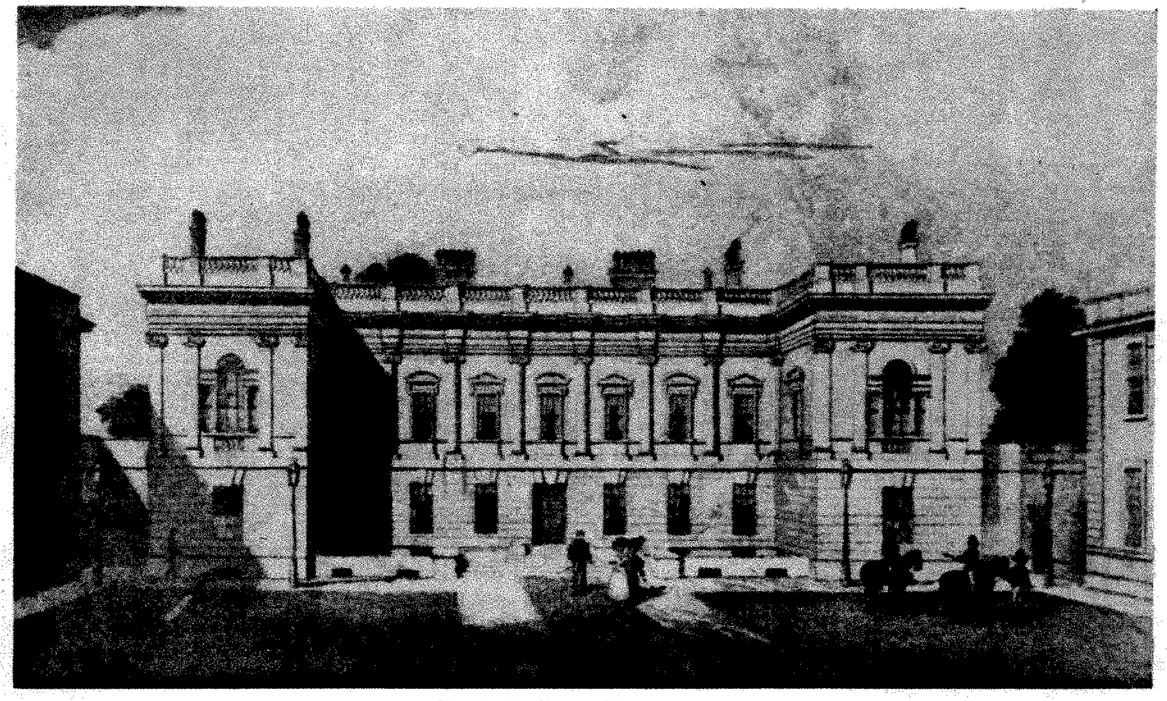

Fig. 6. Burlington House in 1868

tive Secretary what the position was. He told me that a plan had been produced by the Ministry of Works in 1960 for sharing out the space in Burlington Gardens, which was about to be evacuated by the Civil Service Commission, among the scientific societies and others. The plan was considered to be inadequate by Council, and the Ministry of Works was asked for other suggestions. In December 1960 the Executive Secretary placed on my desk an article which had appeared in *The Times* directing attention to the fact that as the Government did not propose to build a new Foreign Office on the site of Carlton House Terrace the future of the buildings there was uncertain. A spokesman for the Crown Estate Commissioners, who was apparently somewhat vague, mentioned that it might be suitable as headquarters of cultural bodies. As we all consider that even to-day science has not totally lost its place as a part of the national culture, the Royal Society seems to qualify and Carlton House Terrace was in due course inspected by the Officers (Fig. 8).

In the meantime revised plans for cutting up Burlington Gardens were produced by the Ministry of Works. The presidents of the societies around the courtyard of Burlington House were informed, and a composite inspection party invaded Burlington Gardens. Suffice it to say that the officers of the Society considered the proposed arrangements were unsatisfactory and that accommodation in Carlton House Terrace would be much preferred. Accordingly, an application was made to the Treasury for Government assistance to enable us to occupy houses Nos. 6–9. This application was refused on January 25, 1962.

There the matter might have rested if it had not been for a lunch-time conversation at Oxford, the details of which are perhaps not suitable for an address such as this. But Fellows may like to be reminded that apparently trivial events can affect their corporate life just as much as their experimental results.

For the first application to the Government the officers had prepared documents setting out why the Royal Society needed more accommodation. A copy of this was forwarded to the Nuffield Trustees, who with very little delay came forward with the magnificent offer of £250,000 towards the £500,000 which it was estimated that it would cost to transform the interior of Nos. 6–9 Carlton House Terrace for the needs of the Society.

With this backing we again approached the Treasury to see whether it would agree to pay the rent, as it has done for our premises for more than 180 years, if we raised a substantial amount for the capital cost of alterations. It agreed to consider the matter again. In the meantime further generous promises were given us—£50,000 from the Wolfson Trust, £60,000 fron the Wellcome Trust, £1,000 from Mr. L. F. A. d'Erlanger and £500 from Hambro's Bank Ltd.

In view of this backing from private sources, Her Majesty's Government, as was announced in Parliament on November 28, has agreed to pay, subject to Parliamentary approval, a sum of the order of £45,000 per annum to the Crown Estate Commissioners, who act for the Crown in respect of Carlton House Terrace. This will pay the rent and for the upkeep of the exterior of the building.

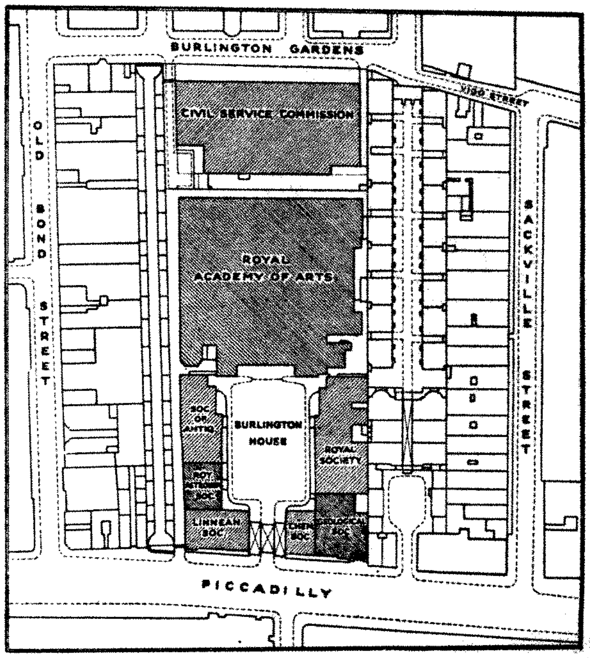

Fig. 7. Plan of Burlington House in 1943

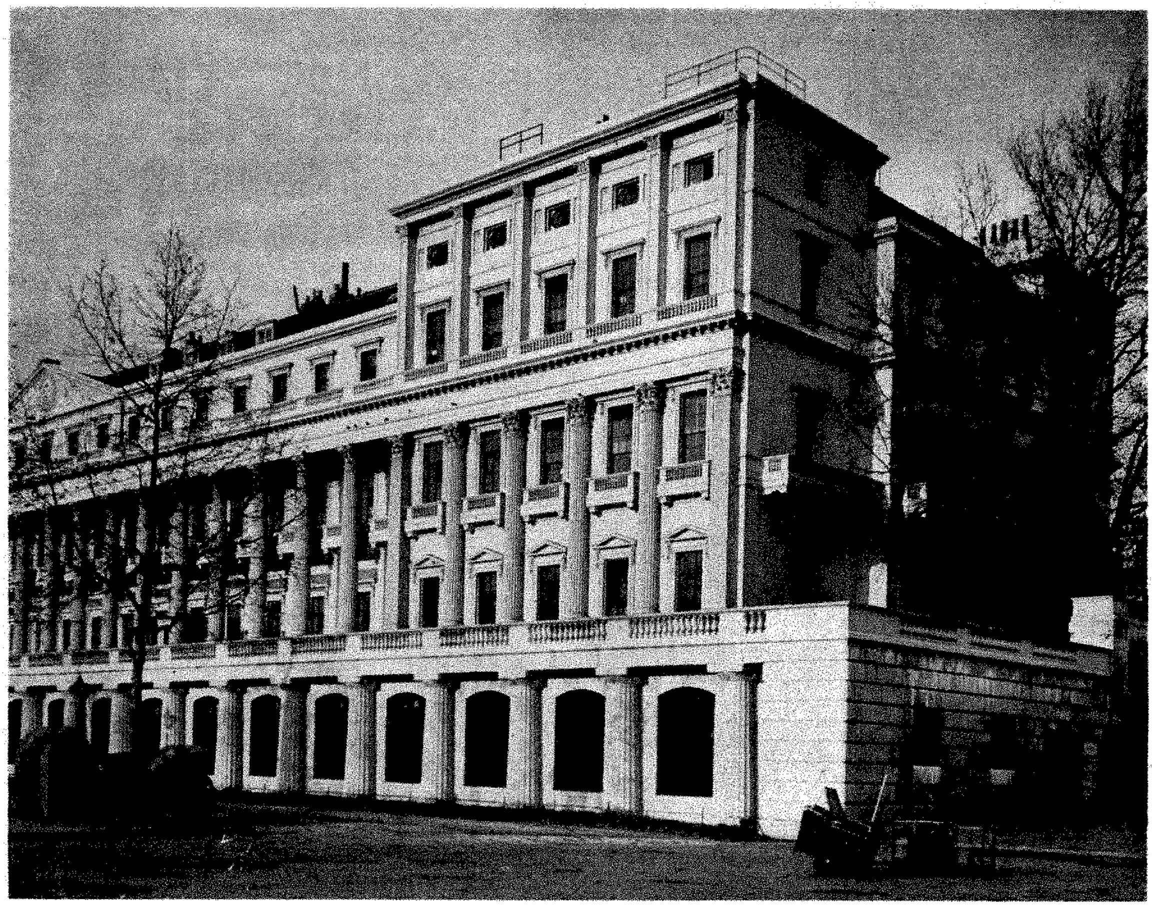

Fig. 8. Carlton House Terrace, 1963

Clearly Council has had to satisfy itself that the new accommodation will be better than the rooms we are at present occupying. In making this assessment we have had the very great advantage of securing the advice of Sir William Holford, who, as a fraternal gesture from the Royal Institution of British Architects, prepared some preliminary plans. Indeed, if it had not been for his backing of the idea that the Society could be satisfactorily housed in Carlton House Terrace, it may well be that the Treasury would have remained unconvinced of the desirability of our schemes.

Tentative plans show that in Carlton House Terrace we can have an ample number of committee rooms and one meeting room to hold about 280 people, another to hold about 100 and a third for lesser numbers. It may be possible to use the two larger meeting rooms as one if the occasion should demand this. There will, of course, be a library. There will be adequate office accommodation for the officers and the permanent staff. An innovation will be the provision of a bar and a dining room so that the social activities of the Society can be enlarged. Some six bedrooms, which can be occupied by Fellows and which may be of particular value for visitors from overseas, are planned, and also a flat for the use of the Executive Secretary.

Wren recommended, when considering his designs for a building for the Society, the provision of "a place . . . so seated in the said ground that the coaches of the members (some of which are of very great quality) may have easy access . . .". In the Carlton House site there will be some garages and parking space for additional cars—for modern Fellows without reference to their quality; and, of course, we shall occupy one of the finest sites in London.

These in bare outline are the main proposals. Fellows will, no doubt, realize that much of the negotiation associated with trying to acquire the new premises has had to be quite confidential, but three successive Councils have been kept fully informed of what was afoot. No dissentient voice has been raised. It is earnestly to be hoped that the Fellows will accept the new arrangements if they can be completed as being a substantial improvement in the Society's resources, although we shall all have regrets at leaving our present splendid library and council room.

Negotiations are still proceeding with the Crown Estate Commissioners on some architectural arrangements. Until these are completed no agreement can be initialled. We are not unhopeful of a successful outcome.

We are all immensely grateful for the extraordinarily generous response to our appeals by the Foundations and individuals I have mentioned, but it should not be forgotten that we still have at least £100,000 to collect for the adaptation of the building, and continuous efforts are being made to find this. If any Fellows can aid in bridging the gap this would be a signal service to the Society.

The generosity of the Foundations would have been of no avail but for the very substantial and continuing undertaking by Her Majesty's Government to pay the rent and outside upkeep of the new accommodation. This is not only very welcome and generous assistance in itself but it is a splendid recognition of the important part the Royal Society still has to play in the nation's scientific life.

In undertaking the present negotiations, the position of numerous scientific societies, both those now around the courtyard of Burlington House and those founded more recently, has been kept in mind. Some societies are in dire need of space to be able to carry out their functions, for in many instances the premises now occupied are quite insufficient and often occupation is of a temporary nature. It is clearly the duty of the Royal Society to assist other societies in this matter in any possible practicable manner.

At the present time there are some schemes on foot to provide London with what might be called a conference centre which among other things would contain auditoria in which individual scientific societies could hold their meetings if they so desired and which would be invaluable for international gatherings These schemes vary from the grandiose to the inadequate, but it is possible that if all scientific societies could agree to support the idea of a building in the nature of a conference centre something might be accomplished This matter is under consideration by the officers

In an ancient society it is perhaps easier to look backwards than forwards, but in the rapidly changing world in which we live it becomes urgent to adapt ourselves to the prevailing scientific environment Perhaps new and elegant accommodation will give us a base from which the Society can move forward into the exciting times that await us

As Fellows can see from the annual report of Council, the activities of the Society have been maintained and indeed they have had to be increased in view of the turmoil surrounding the reorganization of science and technology This has thrown a great burden on the secretaries in particular It is with regret that we part with an able, amusing and energetic biological secretary, Sir Lindor Brown I am grateful to him, for he has been of the greatest assistance to me personally and has, on many occasions, propped me up when I clearly did not know what I was doing He has served the Society not only by his wise advice in its internal affairs, but also by acting as its representative on many external bodies The surviving officers will all miss him and not least for the anecdotes with which he has cheered us up from time to time

The work of the secretaries has been somewhat lightened by the so-called active vice-presidents Sir Gordon Sutherland has held this office for two years and is now retiring He has been of very great help to us and particularly in directing attention, through his Committee on Emigration, to the seriousness of the loss of our scientists and technologists I would like now to thank him for all he has done for the Society and for science in a wider context

It remains only for me to welcome the new biological secretary, who is just beginning to realize the strenuous nature of his new office, but he is, I am happy to say, a very vigorous man [*Nature*, 200, 1049, 1963]

No year can close without recording our continued indebtedness to the Executive Secretary for his unremitting labours on behalf of the Society and its manifold interests He is backed by an able and well-informed staff, and to them also we give our sincere thanks

With the blurring of the conception of the British Commonwealth one of the functions of the Society, which may become more important with the passage of time, is to see that our close associations with actively growing and sympathetic countries are fostered in every possible way In this connexion, the Executive Secretary paid a visit to New Zealand in August and I joined him in Australia I need scarcely say that we were both treated with unbounded hospitality and that our glimpses of antipodean scientific activities were greatly stimulating We hope that the close contacts we have with the Australian Academy may be strengthened by visits of a similar character at not too infrequent intervals

OBITUARIES

Prof F E. Zeuner

Prof F. E Zeuner, head of the Department of Environmental Archæology in the University of London Institute of Archæology, died on November 5 at the age of fifty-eight

Zeuner came to London from Germany in 1933 He joined the staff of the British Museum (Natural History) as a research associate in palæontology in 1934 and with the aid of a Leverhulme studentship of the London Museum, also worked on the problems of the Quaternary deposits of the Thames Valley The results of this work were later incorporated in *The Pleistocene Period*, first published by the Ray Society in 1945 In 1937 he joined the newly founded Institute of Archæology, then established in St John's Lodge, Regent's Park, as honorary lecturer in geochronology, and thus established on a systematic basis the study of human environment in the past as it had not previously been attempted After the Second World War (in which he worked with the locust control unit at South Kensington) Zeuner's was one of two chairs created by the University when it accepted full responsibility for the Institute, the term 'environmental archæology' being substituted for geochronology

Zeuner's breadth of interest was already apparent before he left Germany as a young man, and he maintained and developed this throughout his career He visited many parts of the world in pursuit of his subject, usually returning with material which then became the subject of research undertaken by himself or by his students He was associated with many foreign learned societies and institutions, and as a result of his many contacts—he was, incidentally, a good linguist—had built up a collection of separates which was invaluable to his students, who were allowed free access to it Although at times the centre of controversy, his best-known book, *Dating the Past*, demonstrates his grasp of the environmental sciences Its fourth edition appeared in 1958 and it has been translated into a number of foreign languages Among his other activities, for several years he directed the operation of the carbon-14 dating apparatus which the Royal Institution set up and maintained in Albemarle Street It was this machine, which was functioning at a time when others in Britain were having difficulties, which produced the then surprising date for the early appearance of an agricultural community at Jericho in the early seventh millennium B C Just before his death he was in process of inaugurating, with the aid of a grant from the Department of Scientific and Industrial Research, a big programme of research into sea-level and soil evidence for climatic fluctuations in the Quaternary period His last book, *The History of Domesticated Animals*, was published earlier this year (*Nature*, 198, 45, 1963)

Zeuner's work and example must have a lasting effect on the study of the past in its scientific aspects Many archæologists owe their introduction to the scientific connexions of their subject to him He was an excellent teacher, with an enthusiasm for all branches of natural history which he communicated to all who listened to him, whether in his class-room or in the field Students from many parts of the world were attracted to the Institute of Archæology by his reputation, and returned to their own lands equipped to apply his ideas to their own problems

W F Grimes

Dr Frank L Howard

Dr Frank L Howard, chief of the Pure Substances Section in the Analytical Chemistry Division of the National Bureau of Standards, U S Department of Commerce, died suddenly on October 15, at the age of fifty He was a staff member of the Bureau for more than twenty-six years

Dr Howard was born in Pueblo, Colorado, in 1913 He was educated at the University of Maryland, completing a B S in chemistry in 1934 and a Ph D in organic chemistry in 1938 He joined the Bureau in 1937 and became leader of the group engaged in synthesis of hydrocarbons During the Second World War he pursued research aimed at improving aviation petrol Following the War he turned his attention to the study of jet fuel and the phenomena of combustion He was appointed acting chief of the Engine Fuels Section in 1949, and during that same year was the recipient of the Commerce Department's Silver Medal for Meritorious Service In 1951 he was appointed chief of the Engine Fuels Section, and remained in that post until 1960, when he was appointed chief of the Pure Substances Section

Dr Howard's special fields of interest covered synthesis and purification of organic compounds, petroleum and fuel technology, combustion of fuels, distillation, and automotive antifreezes He was author and co-author of many technical publications

Dr Howard is survived by his wife, two daughters and three sons

NEWS and VIEWS

Shell's Thornton Research Centre:

Dr T. M. Sugden, F R.S.

Dr T M Sugden has been appointed research director, Thornton Research Centre, Chester Dr C B Davies will continue to be director, Thornton Research Centre The Thornton Laboratories are the largest of the five research laboratories operated by "Shell" Research, Ltd, in the United Kingdom The work of the Thornton Laboratories is concerned with basic and applied research related to oil products Dr Sugden has been a reader in physical chemistry in the University of Cambridge In this capacity he has been responsible for a major contribution to high-temperature chemistry by the development of flame photometric techniques for studying controlled traces of metallic elements and their compounds in flame gases These techniques proved to be extraordinarily powerful, allowing the development of methods of measuring the stability of many simple compounds of metallic elements at high temperatures, of investigating new types of chemiluminescent reactions, and of determining rate constants for the reactions by which free radicals recombine after their production by chain-type processes

Some five years ago, Dr Sugden and his collaborators developed a mass spectrometer capable of sampling ions directly from flame gases Many interesting species, some positively and some negatively charged, were shown to be associated with the flames of hydrogen and hydrocarbons, with and without metallic additives Apart from being of great interest to students of chemical kinetics, these results have a practical importance in rocket and magnetohydrodynamics technology All Dr Sugden's work has been characterized by determining the essential chemical features of complicated reacting systems and submitting them to quantitative examination by new methods

The Prix Jecker

Dr. Léo Marion, F R S.

Dr Leo Marion, vice-president (scientific) of the National Research Council, Canada, has been awarded the Prix Jecker by the Academy of Sciences, Paris, for his work on the structure of alkaloids Dr Marion was born in Ottawa He gained an honours B Sc in 1926 and an M Sc in 1927 from Queen's University and a Ph D degree from McGill University in 1929 The same year he began his career with the National Research Council of Canada, continuing in the field of organic chemistry, principally alkaloids, on which he carried out his post-doctorate work at the University of Vienna in 1934–35 Since 1947, Dr Marion has served as editor-in-chief of the *Canadian Journals of Research* and, from 1952 until 1963, as editor of the *Canadian Journal of Chemistry* He also serves on the editorial board of other scientific journals He became head of the organic chemistry section of the Division of Chemistry in 1943, director of the Division of Pure Chemistry in 1952, senior director in 1960, and vice-president (scientific) of the National Research Council in 1963 Dr Marion has many honours to his name, including fellowships of the Royal Society of London, the Royal Society of Canada, the Chemical Institute of Canada (of which he is a past-president), l'Association Canadienne-Française pour l'Avancement des Sciences, and the Chemical Society of Great Britain, and membership of the American Chemical Society Other honours for his contributions to the field of alkaloid chemistry include the Médaille Léo Pariseau of l'Association Canadienne-Française pour l'Avancement des Sciences, the Palladium Medal of the Chemical Institute of Canada, and the Gold Medal of the Professional Institute of the Public Service of Canada

Founded in 1851, the Prix Jecker has been awarded, since 1857, to the author of the most useful work on organic chemistry, or to the chemist whose collective works have contributed most to the progress of organic chemistry The recipient receives a monetary award of 6,500 French francs (1,300 dollars)

Geography in the School of Oriental Studies (University of London)

Prof C A. Fisher

Prof C A Fisher, head of the Department of Geography in the University of Sheffield since October 1959, has been appointed to the newly created chair of geography in the School of Oriental Studies in the University of London A graduate of the University of Cambridge, he first developed special interests in the geography of south-east Asia and far-eastern regions during the Second World War, when he was on active service in Malaya and, later, from 1942 until 1945, a prisoner of war in Japanese-controlled Thailand These regional interests he has since developed, he has published numerous papers in various journals on aspects of the regional and political geography of eastern Asia and was a joint editor of the text, "Geographical Essays on British Tropical Lands" More recently, in a volume of some eight hundred pages, he has contributed a major work on the social and political geography of south-east Asia, scheduled for publication in January 1964 In 1962 he led the British delegation of geographers attending the regional conference on south-east Asia, organized by the International Geographical Union at Kuala Lumpur In 1961 he visited Japan in an extended tour, at the invitation of the Japanese Foreign Office, and in 1962 he was appointed as the first director of the Centre of Japanese Studies now attached to the University of Sheffield

The Defence (Transfer of Functions) Bill

The Defence (Transfer of Functions) Bill, which received its second reading in the House of Commons on November 21, is intended to implement the proposals of the White Paper published in July to establish a unified Ministry of Defence Although in moving the second reading Mr P Thorneycroft, the Minister of Defence, emphasized the importance he attached in the new organization to having scientists built in the administration and with the military at every level, so that the scientists were not merely considering purely scientific problems but putting forward their ideas on many aspects of strategy and challenging the established views, there was little comment on this aspect in the debate. Mr. N. Birch expressed serious misgivings about the continuance of the office of Chief of the Defence Staff, preferring a secretariat organized rather on the lines of that established by Lord Hankey and Lord Ismay. Mr. Aubrey Jones also appeared to hold rather similar views, referring to Lord Hankey's staff of the Secretariat of the Committee of Imperial Defence. Apart from this doubt as to whether the Minister of Defence and also the Cabinet Office might not require strengthening on some such lines, it was left for Sir Harry Legge-Bourke to stress the importance in the new structure of the Chief Scientific Adviser. However, he was more concerned that effective control should be political, though he thought that the changes were in the right direction and, like Mr. R. T. Paget, welcomed the prospect of bringing scientific and technical skill into all Government departments, believing that this would facilitate speedier action. In replying on the debate, Mr. J. Hay, the Civil Lord of the Admiralty, strongly upheld the importance of scientific advice being available at all stages of the policy-making process in defence.

Potential Scientific Manpower

In a written answer in the House of Commons on December 11 the Financial Secretary to the Treasury, Mr. A. Green, gave the number of full-time students in universities at the beginning of the autumn terms in 1961–63 as 111,385, 116,610 and 124,002, respectively. Of these, 12,209, 13,273 and 15,323, respectively, were in social studies, 28,082, 30,791 and 33,212 in pure science, 16,934, 16,652 and 18,183 in applied science, 12,424, 12,875 and 12,892 in medicine, and 2,028, 2,101 and 2,013 in agriculture and forestry. Full-time students in colleges of advanced technology on courses leading to recognized qualifications in the same terms numbered 9,098, 10,346 and 11,871, respectively; of these, 5,451, 6,138 and 7,205, respectively, were in technology, 3,040, 3,602, 4,103, respectively, in science, and 607, 606 and 563 in arts and social studies. Of 35,109 first-degree and first-diploma students entering universities for the first time in October 1963, 4,915 were in social studies, 9,416 in pure science, 5,357 in applied science, 2,726 in medicine and 560 in agriculture and forestry. For October 1962, the corresponding figures were 10,470, 4,121, 9,060, 4,872, 2,717 and 594, and for October 1961, 10,180, 3,932, 8,433, 5,118, 2,530 and 560. Of the 4,481 first-year students in the colleges of advanced technology in October 1963, 2,523 were in technology, 1,542 in science and 416 in arts and social studies; for October 1962, the corresponding figures are 4,081, 2,326, 1,422 and 333, and for October 1961, 3,458, 1,983, 1,165 and 308.

The Plant Varieties and Seeds Bill

The Plant Varieties and Seeds Bill, which received its second reading in the House of Lords on November 26, deals first with the rights of plant breeders and, secondly, regulates transactions in seeds and seed potatoes. As Lord St. Oswald, Joint Parliamentary Secretary to the Ministry of Agriculture, Fisheries and Food, explained in moving the second reading, the Bill as a whole originated in the reports made by the Committee on Transactions in Seeds in 1957 and 1960. The first part provides essentially a scheme for encouraging plant breeding and also derives from the International Convention for the Protection of New Varieties of Plants, to which the Government was signatory two years ago, subject to ratification after discussion in Parliament. The provisions of the Bill follow closely the arrangements proposed in the Committee on Transactions in Seeds report (Cmnd. 1092) of 1960 and grants the plant breeder a form of proprietary rights in his new variety. These are such that when the breeder has succeeded in registering the variety and has given it a distinguishing name, he alone, during the period of protection, has the right to grow it for producing seed or other reproductive material for sale, and the right to sell such material, except under licence from the breeder. The Bill also provides wide co-active powers by means of the issue of compulsory licences to prevent unreasonable restriction. Consent of the breeder will not be required for use of his variety for further breeding or research. For these purposes the Bill establishes the Plant Variety Rights Office as a semi-autonomous body standing in much the same relation to the Agricultural Departments of the United Kingdom as the Patent Office does to the Board of Trade.

The second part of the Bill is largely concerned with measuring, entrusting to regulations certain of the details written into the Seeds Act, 1920, and making the statutory control more flexible. The essential principle that the seller of seeds should give an adequate and reliable description of his goods to the buyer is retained and strengthened, particularly by the civil warranty provisions of Clause 17. A further Clause enables the Agricultural Ministers to make regulations controlling the import of certain kinds of seed, when necessary, to safeguard Britain's indigenous strains against deterioration by admixture and cross-pollination, or to prevent the import for sale of seed unsuitable for growing in Britain. The Agricultural Ministers are also empowered jointly to prepare an index of names of plant varieties for use in connexion with the sale of seeds and to require new varieties of plants to undergo performance trials before they are put on the market. In replying on the debate, Lord St. Oswald referred to the valuable work of the National Institute of Agricultural Botany and said that the Bill, which was generally welcomed in the debate, was an attempt to support the Institute's work.

"New Courses for New Students"

An address, "New Courses for New Students", delivered by Prof. H. Rée, of the University of York, at the annual conference at Margate to a meeting of Grammar School Teachers has been issued as a booklet by the National Union of Teachers (*New Courses for New Students*. Pp. 12. London: National Union of Teachers, 1963). Prof. Rée was concerned with the effect on the universities, particularly the new universities, of the immense increase in the demand for university education, and he believed the reason for changes in university courses, as well as in sixth-form courses, lay largely in the attitude of the sixth-formers themselves. The new sixth-former was much less dependent either on his parents, or on teachers, or on any of the older generation, than in the past, and Prof. Rée believed that the sixth-former resisted the compartmentation of his studies and the barrier between arts and the science sides. The new universities were attempting to deal with this situation and to provide world citizens rather than local citizens. At York the aim was to limit the number of studies offered and students were encouraged to take one main subject and another subsidiary to it—about two-thirds of the time being spent on the main subject and one-third on the subsidiary. Where this subsidiary course was education, it was not designed for those necessarily becoming teachers but rather to help introduce the undergraduate to the world in which young people were growing up—the youth clubs, the youth employment service, the juvenile courts, etc. This was a general feature of the new universities; another was the determination to ensure direct contact with teachers and eliminate the "nine to five" students. While it was hoped to secure that all students had at some time in their university life the experience of living together in groups in colleges or halls of residence, it was not intended to eliminate living in 'digs' or flats. Another problem which the new universities were facing, and in which experiment was needed, was that of the social uncertainty of the student this might be particularly difficult while the new universities were still small

Computers in Industry

A VALUABLE service to all who are interested in the use of computers in industry and commerce has been performed by the Library of Watford College of Technology Following a symposium, which was held at the College during September 1963, under the auspices of the Watford and District National Productivity Year Committee, two booklets have been prepared which should be useful to many people in industry and commerce The first, *A Select Bibliography on Computer Applications in Commerce and Industry*, has been prepared by C R Randall to show the uses of computers over a wide range of industries References are to books and articles in journals and are up to date The bibliography is intended for readers who are not computer specialists but who wish to obtain a basic knowledge of computers and applications in their own specialist fields The second, *A Directory of Computer Service Bureaux in the United Kingdom*, has been prepared by J T Graham, and gives information about the geographical coverage of many bureaux, their charges, the services they provide, whether or not there are penalty clauses for work not completed on time, and the advisory and technical research services available The main gap in coverage is the computer time available from governmental and educational establishments throughout Britain The booklets may be obtained from the Hertfordshire County Council Technical Library and Information Service, Hatfield, Herts

The National Central Library

THE forty-seventh annual report of the National Central Library, covering the year ended March 31, 1963, refers to progress with the foundations of the new buildings and to a considerable increase in applications for inter-library loans to 98,690 (91,311 in 1961–62)—the highest number recorded for ten years (Pp 24 + 3 plates London The National Central Library, 1963) While the easier type of request, chiefly for current British books, is now dealt with locally in the regional system, the demand for the more difficult type of material such as older works, those in foreign languages or published in the United States and elsewhere, continues to increase However, the Inter-lending Department succeeded in satisfying some 77 per cent of the applications, compared with 78 per cent in 1961–62 Of the satisfied applications, 15,743 were from university libraries 10,341 from special outlier libraries, 2,504 from Government departments and 4,066 from overseas Besides the 15,743 loans just noted made by or through the National Central Library to university libraries, the university libraries made 17,855 loans at the direct request of the National Central Library and lent a further 16,381 as members of regions to libraries in that region or to libraries outside their region Loans to libraries overseas totalled 3,443, and 2,686 items were borrowed—the corresponding figures for 1961–62 were 3,699 and 2,550, 603 photocopies were supplied and 936 obtained (633 and 786 in 1961 and 1962) Acquisitions by purchase totalled 7,902 volumes (1,011 for adult class purchases), 6,891 being current British Government publications, 636 American books and 628 works in foreign languages Catalogued books are now estimated at 214,000 with some 46,000 uncatalogued The Treasury grant was increased from £75,000 in 1961–62 to £82,500, including £6,500 for renting a building at Woolwich Arsenal, which was formally occupied in November 1962 as an overflow bookstore, and which will facilitate the later acceptance of larger acquisitions of material in foreign languages and older British books The Executive Committee, however, considers that the Library's income still falls far short of what is needed for it to function efficiently as a national lending library and centre of library co-operation

Melting Points of the Metal Oxides

Compilation of the Melting Points of the Metal Oxides, by Samuel J Schneider, deals comprehensively with the literature on the melting points of the metal oxides (National Bureau of Standards Monograph 68, October 10, 1963 Pp 31 U S Government Printing Office, Washington, D C 25 cents) It includes a critical evaluation of the published data as well as the designation of certain preferred values Chemists and physicists, particularly those involved in high-temperature applications, will find this Monograph especially valuable It represents the first step in a programme recently initiated by the National Bureau of Standards to re-evaluate these melting points The programme tentatively includes the acceptance of previously published values after experimental verification, as well as accurate determinations of ill-defined or completely unknown melting points Monograph 68 presents the melting points of 70 metal oxides as given in the literature published up to January 1963 It gives both the original melting point and the equivalent value based on the International Practical Temperature Scale of 1948 Information on pertinent experimental details, such as the method of temperature measurement, purity, furnace type and environmental conditions, is included The ultimate aim of the National Bureau of Standards programme is to secure national and, it is hoped, international recognition of the melting points of several specific oxides for use as calibration standards

Radioactive Fall-out

RECENTLY a report has been published by R S Cambray and Miss E M R Fisher, of the United Kingdom Atomic Energy Authority, entitled *Radioactive Fallout Short-lived Fission Products in Air and Rain, August 1962–April 1963* (*AERE-R*4384 Pp ii + 32 London H M S O. 1963 5*s* 6*d* net) This report is the most recent of a series, extending over several years, and covers the period following the tests carried out by the United States in the Pacific, which started in April 1962, and the U S S R tests of 1962 The amounts of total beta, barium-140, iodine-131 and zirconium-95 activity were estimated in atmospheric dust and rain-water derived from five stations in Great Britain, and in troposphere samples, collected by aircraft over the eastern Atlantic Brief notes on the sampling media and methods are included The American tests gave no significant amounts of barium-140, but this isotope was detected fourteen days after the resumption of testing by the U S S R It is interesting that there was a considerable increase in the amount of barium-140 washed out in snow, in late December and early January, without a correspondingly marked increase for zirconium-95, in addition, the debris arrived from the testing site in a very few days No cause is suggested for this phenomenon All the data with which this report is concerned are tabulated in considerable detail, and graphs are also included where applicable

Drugs and the Health Services

Two recent publications have directed attention to the central part played by drugs in the development of an effective health service The first, issued by the Association of the British Pharmaceutical Industry, London, is purely factual and shows that, contrary to popular belief, the percentage of money expended on pharmaceutical services related to total National Health Service expenditure has shown no significant change over the past ten years (*Facts on Drugs Information on Drugs and the National Health Service* Pp 16) 70 per cent of to-day's prescriptions could not have been written in 1935; the drugs did not exist Antibiotics, which were only made available in the 1950's, now account for 25 per cent of the drug bill The second, issued by the Office of Health Economics, is mainly concerned with the place of the general practitioner in the National Health Service and shows how his position has altered with the development of drugs (*The Personal Health Services a Perspective of the General Medical and the Pharmaceutical Services* Pp 31. 2*s*) As Lord Taylor has said, "To-day the practitioner in the gloomier slum practice can treat pneumonia more effectively than the most eminent specialist was able to do before the War" The most important development in the future should be more concentration on personal preventive medicine Early detection of such diseases as cancer and diabetes is now possible The publication states that general practitioners need improved facilities to help them carry out this early diagnosis

Antibiotics in Food and Feedstuffs

At the instance of the Joint Expert Committee on Food Additives of the World Health Organization and the Food and Agriculture Organization, the World Health Organization convened a committee to examine the public health problems likely to arise in connexion with the increasing use of antibiotics in animal feeds as growth promoters, for food preservation and in plant disease control The Expert Committee appointed has recently reported on its findings and conclusions in a booklet entitled *The Public Health Aspects of the Use of Antibiotics in Food and Feedstuffs* (Technical Report Series No 260 Pp 30 Geneva World Health Organization, London H M S O , 1963. 1 Sw fr , 1*s* 9*d* , 0 30 dollar) The report points out that most of the antibiotics used in food preservation or added to animal feedstuffs are of low toxicity, and, provided regulated quantities are used, the quantities likely to be present as residues in food are very small The danger exists, however, that penicillin, for example, may cause allergic reactions in humans already sensitized by medical treatment Chloramphenicol is in a special category, as fatal damage to the bone marrow has occurred in patients treated with therapeutic doses The Committee believes that further research is desirable in this field The rapid spoilage of meat in hot climates might, for example, be averted if tetracyclines were used, either by giving the animal pre-slaughter injections, or by treating the carcass The report stresses that such uses should be regarded as an exceptional and temporary measure pending the eventual provision of adequate refrigeration Two useful annexes to the report summarize the practices in different countries in relation to the use of antibiotics in animal feeds and for food preservation

Farm Forestry

Farm forestry plays an important part in land use in Sweden, and during 1953–60 an economic study of this subject was conducted in three localities—south, centre and north Sweden (*Kungl Skogshogskolans Skrifter* (*Bulletin of the Royal School of Forestry, Stockholm*), Nr 39 *Bondeskogsbruket · Ekonomisk Undersokning Grundad pa Bokforing 1953–1960* (*Farm Forestry in Sweden an Economic Study 1953–1960*) Av Th Streyffert och Chr von Malortie Pp 207 Stockholm Skogsbiblioteket, Skogshogskolan, 1963) Farm woodlots occupy almost half the forest area of Sweden and some of them are in the best forest area, namely, south Sweden The practice of farm forestry is an excellent example of integrating different land uses, and the forest properties are regarded as a means of increasing the income of the farmers In fact, the law prohibits the selling of forest land where it is needed for the support of the farm This investigation has produced some interesting statistics, but care should be exercised in interpreting them The woodlots examined were those where the owners were keen enough to undertake the large amount of record work necessary, and this meant that, on the whole, they were the best managed Therefore, the statistics may be considered as above average It was revealed that there was a tendency to overcutting of the prescribed yield in the smaller forests and it is suggested that the lower yield per unit area from the larger forests is due to the owners not being in such need for the money or the labour force being fully occupied with normal work But efficiency seems to be better on the larger properties where a higher rate of return is recorded As is to be expected, there are divergences from averages and many of these are due to the woodlots being under the personal management of the owners This investigation should concern not only the forest economist but also anyone interested in the integration of agriculture and forestry

Archæology in Ontario

Bulletin No 184 of the National Museum of Canada consists of a detailed report of the excavation of the Donaldson site by J V Wright and J E Anderson (*The Donaldson Site* (Anthropological Series No 58) Pp viii + 113 (26 plates) Ottawa Queen's Printer, 1963 1 50 dollars) The site is located in the Bruce Peninsula of Ontario, and traces of the settlement here include middens, a cemetery and house foundations The cemetery yielded both single and multiple inhumations in pit graves, some accompanied by personal ornaments Two house structures, demarcated by post-holes, were recovered, these were rectangular, the largest approximately 25 ft by 15 ft , and supported in all by more than 100 posts No trace of rebuilding was noticed, and some of the interior posts probably represent a sleeping platform Pottery from the middens belongs to the Saugeen Focus of the early Middle Woodland period, *c* 500 B C The stonework includes projectile points, scrapers and choppers, and copper objects were rare The material seems to show elements allied to other Woodland complexes, and is representative of an economy comparable with the northern Algonkians of historic times

Earthwork on Overton Down, Wiltshire

A recent report of a research committee of the British Association for the Advancement of Science describes the construction of a ditch and bank of chalk in Wiltshire, built partly with primitive tools, partly with modern equipment (*The Experimental Earthwork on Overton Down, Wiltshire, 1960* An account of the Construction of an Earthwork to Investigate by Experiment the way in which Archæological Structures are Denuded and Buried Pp viii + 100 + 8 plates Edited by P A Jewell London The British Association for the Advancement of Science, 1963 25*s* , 4 50 dollars) The object of the exercise, carefully outlined in meticulous detail in this book, is to allow archæologists both of the immediate and of the distant future to record through observation and excavation the development of the monument, the degree of erosion and denudation of the bank, and the degree and manner of silting of the ditch This evidence should be of great importance in the interpretation of sections obtained during the excavation of prehistoric and early historic monuments A number of objects, including textiles, food, flints and potsherds, have been buried in the bank, and future excavation will show how the various materials have reacted to the conditions of burial The first of a number of sections has already been cut, although the report on this will not appear until it has been combined with another excavation in 1964

Joseph P Kennedy, Jr Foundation Awards

The Joseph P Kennedy, Jr Foundation was established in 1946 by former Ambassador Joseph P Kennedy in memory of his son, Lieut Joseph P Kennedy, Jr The Foundation engages in a programme of support of scientific research, care and treatment in the area of mental retardation Major research projects have been established at Johns Hopkins University, Massachusetts General Hospital, Harvard University, Stanford University, the University of Wisconsin, George Peabody College, and the Kennedy Memorial Hospital in Brighton, Massachusetts Educational, diagnostic and treatment centres are located in New York City, Washington, Chicago, and Santa Monica, California In 1962, a programme of International Awards was instituted by the Foundation to recognize outstanding contributions in the field of mental retardation and to support activities in this field Awards are made in three general 'areas' (1) *Scientific Research Award*, to an investigator who has made outstanding contributions to the knowledge of mental retardation in the biological and behavioural sciences, (2) *Service Award*, to the individual or group developing outstanding programmes in the identification, care, rehabilitation, or education of the mentally retarded, (3) *Leadership Award*, to a civic leader, public official, author, or any other person whose activities on behalf of the mentally retarded have awakened the public conscience or led to increased individual and community effort Awards will be in amounts of 5,000–25,000 dollars. In addition, up to 50,000 dollars will be awarded for the furtherance of current or proposed programmes More than one Award may be offered in a single 'area' Institutions, organizations, and individuals working in the field of mental retardation are invited to send nominations to the Foundation These nominations should include biographical information, *curriculum vitae*, bibliography, and all pertinent supporting data Nominations received by August 1 will be considered for the current year's Awards, which will be announced in December Further information can be obtained from International Awards, the Joseph P Kennedy, Jr Foundation, 1413 K Street, N W , Suite 306, Washington 5, D C , to which nominations should be sent

1963 Awards

PROF L S PENROSE, Galton professor of eugenics, the Galton Laboratory, University College, London, has been awarded the Scientific Research Award The Service Award is to be shared by Dr R P L Lafon (Montpellier) and Dr G F Powers (Yale) Senator Lister Hill (Alabama), Representative J E Fogarty (Rhode Island) and Governor B T Combs (Kentucky) are to share the Leadership Award

Bursary for British Women Engineers

THE Caroline Haslett Memorial Trust is offering a bursary to assist the representation of British women engineers at the first International Conference of Women Engineers to be held in New York in June 1964 The sum of £250 will be made available for this purpose, and will be awarded to a British woman engineer, or will be shared by two, depending on the decision of the interviewing panel Application forms can be obtained from the Secretary, Caroline Haslett Memorial Trust, 25 Foubert's Place, London, W 1, and should be completed and returned by January 31, 1964 Applicants should be qualified in any field of engineering or should be pursuing a recognized training in engineering They should be prepared to make a real contribution to the Conference by reading a paper or leading a discussion, and to give some lectures in Britain after the Conference In 1960 the Trust sent two women engineers to study the training and employment of women engineers in the U S S R

The Emmie Clough Scholarship

THE Royal Horticultural Society is offering a scholarship provided out of a bequest from the late Mrs Emmie Clough to enable a girl to take a course of training in horticulture which would otherwise be beyond her means The scholarship, valued at £352 a year, will commence in September 1964 It will be open to women between the ages of eighteen and thirty and will be tenable for two years at Studley College, Warwickshire Candidates will be required to have attained a standard of education which will permit them to take full advantage of the course of study, and should normally have completed, or be prepared to undertake, such periods of practical training in horticulture as may be considered necessary The form of application, which can be obtained from the Secretary, should be completed in the candidate's own handwriting and should be returned so as to reach the Secretary, Royal Horticultural Society, Vincent Square, London, S W.1, not later than February 10, 1964

Atmospheric Radiation

THE International Radiation Commission of the International Association of Meteorology and Atmospheric Physics is holding a conference at Leningrad during August 10–15, 1964 There will be both invited and contributed papers on a wide variety of topics of current interest in the field of "Atmospheric Radiation". It is hoped to include in the programme sessions on infra-red spectroscopy of the atmosphere, theory of radiative transfer in planetary atmospheres, energy balance of the Earth's atmosphere, including (*a*) experimental investigations of the radiation field in the free atmosphere, and (*b*) radiation problems as related to atmospheric dynamics and the general circulation, radiation climatology, surface and network instrumentation Further information can be obtained from Prof J London Department of Astrophysics and the Atmospheric Sciences, University of Colorado, Boulder, Colorado; or Prof M I Budyko, the Main Geophysical Observatory, M Spasskoya 7, Leningrad *K*-18 Prof. Budyko is chairman of the local arrangements committee

Announcements

PROF E CHARGAFF, professor of biochemistry at the College of Physicians and Surgeons, Columbia University, has been awarded the Charles Leopold Mayer Prize of 80,000 N F by the French Academy of Sciences, for his work on nucleic acids

A MEETING of the Food Group of the Society of Chemical Industry will be held in the University of Liverpool on January 16 The programme will include "Whaling" by Mr C E Ash Further information can be obtained from the Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S W 1

A MEETING of the Food Engineering Panel of the Society of Chemical Industry will be held in London on January 22 The programme will include "Dehydration other than A F D ", by Mr G Evans Further information can be obtained from the Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S W 1

A ONE-DAY symposium on "Chemical and Nutritional Aspects of Heated Fats", arranged jointly by the Oils and Fats Group and the Food Group of the Society of Chemical Industry, will be held at the Imperial College of Science and Technology, London, on March 25 Further information can be obtained from the Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S W 1

THE one hundred and thirty-fourth course of Christmas lectures for young people will be delivered by Prof Ronald King on "Energy" The lectures will include "Energy in Atoms and Moles" (December 31), "Electrical Energy and Magnetism" (January 2), "Energy in Waves" (January 4), "Energy Deep Inside the Atom" (January 7), "Persuading Energy to do the 'Right' Thing" (January 9) Further information can be obtained from the Royal Institution, 21 Albemarle Street, London, W 1

MEASURING AIRBORNE DUST AND MIST

AN international colloquium on this subject was arranged jointly by the Staubforschungsinstitut des Hauptverbandes der gewerblichen Berufsgenossenschaften, Bonn, and the Institut National de Recherche Chimique Appliquée, Paris, it was held on October 22–23 at Freiburg (Breisgau) and was attended by about 60 scientists and engineers. The programme was divided into three sections, the first of which was concerned with number and mass concentration and the distribution of particle size. Introductory speeches were made by Prof. A. Winkel (Bonn) and A. P. Avy (Paris).

Dr. F. Hartogensis (The Hague) discussed the influence of microscopic techniques on the number of particles counted and outlined a case for the use of a well-defined system to eliminate the considerable variations in the numbers of fine particles which were visible. There was little evidence on the liability of 0·5μ particles to cause silicosis, but there was no doubt that large numbers of much finer particles were found in silicotic lungs.

The error in mean dust concentration as a function of the number of measurements, based on a log-normal distribution, was evaluated by J. Juda (Warsaw) and could be applied to gaseous pollutants. He also spoke of a method of size analysis based on pressure measurements up to 3 mm water while particles were sedimenting in liquid. Size analysis by measuring the light scattered by individual particles, while airborne, was possible with apparatus described by J. Bricard and J. Pradel (Paris). A very uniform zone of light was traversed by a jet of aerosol particles which scattered light at 90° into a microscope provided with a photomultiplier cell. An advantage of the apparatus was that the aerosol did not have to be diluted so that natural clouds of liquid particles could be measured, unexpectedly, fine liquid and solid particles had been found in city fogs which formed at 90 per cent relative humidity, the saturation deficiency being due to sulphur trioxide.

The use of membrane filters for mine dust was explained by H. D. Bauer (Bochum), and H. Gessner (Zurich) had a new sedimentation apparatus for size analysis by weighing fractions. A. Berner and O. Preining (Vienna) presented a new calibration of the 'Cascade Impactor' which they used for sampling atmospheric radioactivity, on account of its ability to handle a large volume of air. W. Stober (Gottingen) explained a theory of the deposition of particles in a conical aerosol centrifuge.

The Stokes diameter of a particle, evaluated from its sedimentation rate, is smaller than the projected area diameter, which is assessed microscopically. New measurements of the dust penetrating a sedimentation chamber between horizontal plates had been made, using a thermal precipitator, by W. Walkenhorst (Bochum), who demonstrated that the ratio varied from 1/1·3 to 1/2·2 for glass quartz, talc and coal, and exhibited a considerable scatter in any one sample of dust.

W. Matla (Heerlen) presented a number of correlations between the quartz content and ash of airborne dusts which showed that the easily determined ash value was a good indication of the amount of quartz in the dusts. Before laying down permissible limits of dust concentration, due consideration would have to be given to the standardization of measuring techniques which at present varied widely, although the principle of selective sampling for the elimination of the coarser airborne dust was generally accepted.

The second section, dealing with selective sampling, was opened by Dr. C. N. Davies (London), who showed how a curve of lung retention against particle size could be derived by considering all the available distributions of airborne dust, and of dust recovered *post mortem* from the lungs, which had been obtained by electron microscopy. The British 'Hexhlet' selective dust sampler worked on a simple falling velocity separation formula which did not represent selection in the human bronchi and bronchioles but could be redesigned to do so. A cyclone separator, described by H. Breuer (Essen), collected much finer dust which resembled lung dust more closely.

M. Landwehr (Bochum) had used a pre-impinger to separate the coarse fraction of dust, while finer particles, below 5μ, passed through and were collected in a scrubber or on a membrane filter.

Two papers dealt with practical aspects of the British 'Hexhlet'. R. J. Hamilton (Isleworth) explained how it had been found necessary to increase the separation of the selector plates to 1·2 mm to avoid pick-up of deposited particles and to reduce the width of the entry to prevent external wind effects. K. G. Schmidt (Bonn) was critical of the performance of the instrument and had been troubled by corrosion of the aluminium plates which carried over into the fine fraction of dust.

The final section covered recording dust apparatus and opened with a description by A. P. Avy and M. Benarie (Paris) of a novel device in which an aerosol was sucked through an orifice so that the particles struck a microphone and could be recorded. F. Hartogensis had found that a compensated photoelectric smoke recorder was of some use for measuring industrial dust and had also used a paper-stain recorder. M. Raillière (Paris) compared the principles of measuring sideways or forwards scattered light and E. Walter (Bonn) claimed that photoelectric recording of dust concentration was possible for concentrations ranging from less than 1 mg/m^3 to 30 mg/m^3.

Messrs. Leitz (Wetzlar) announced a new Tyndallscope incorporating a photomultiplier which obtained its high voltage from a potted transistor flip-flop condenser circuit. This remarkably sensitive dust recorder retains the sensitivity and portability of the well-known visual instrument.

The recording of aerosol particles by the contact E.M.F. which is developed on impact has been developed by R. Prochazka (Vienna and Munich) and A. Schutz (Bonn). The former described a finished instrument in which wall collisions as the aerosol passed through a tube were amplified, concentrations down to 0·5 mg/m^3 at flows up to 60 m^3/h and particles down to 0·2μ could be dealt with. The apparatus of Schutz operated on collisions with a metal wire and was even more sensitive, when the dust concentration fell below 0·1 mg/m^3 fluctuations developed owing to the ion content of the atmosphere which depended on temperature and humidity. It could advantageously be preceded by a selection device when used for recording industrial dusts.

The use of Zeleny tubes to establish the size distribution of fine atmospheric particles marked with thorium *B* was described by G. Madelaine, J. Bricard, J. Pradel and F. Billard (Fontenay-aux-Roses and Paris). Using this aerosol the efficiency of a filter paper was found to change from 99·997 per cent to 99·993 per cent when the particle size increased from 10^{-3} to 10^{-1}μ. W. Coenen (Bonn) deposited small ions on dust particles and recorded the dust by the ion current.

The meeting concluded with a paper by W. Neuwirth (Freiburg), who had investigated correlations of atmospheric pollution with prevailing weather conditions.

The papers which were presented at Freiburg will be published in *Staub* early in 1964 and another meeting will be held in Paris next autumn.

C. N. Davies

GENERATION, DETECTION AND PROPERTIES OF COHERENT RADIATION OF WAVE-LENGTHS LESS THAN 1 MM

THE autumn meeting of the British Radio Spectroscopy Group was held at Bournemouth, Hampshire, during September 24–25 The meeting was concerned with the generation, detection and properties of coherent radiation of wave-lengths less than 1 mm Nineteen papers were presented during three sessions while the fourth session was taken up with a visit to the Quantum Physics Division of the Signals Research and Development Establishment, Christchurch, Hampshire

Mr R J Lees, director of the Signals Research and Development Establishment, who gave the address of welcome to the 130 delegates, comprising approximately 45 from the universities, 55 from industry and 30 from Government service, saw the essential function of the meeting to enable the delegates to exchange ideas on mutual interests so forming cross-linkages between the various sections of this wide field

The nineteen papers presented can be divided into four general groupings, eleven on generation, two on detection, three on spectroscopy and three concerned with gaseous lasers As can be seen, the generation of sub-millimetre radiation attracted the most attention

M. G Convert (Compagnie Générale de Télégraphie sans Fil) discussed the main design features beam geometry, beam velocity and construction of the elements that have to be considered in the development of a carcinatron The power output, which depends approximately on the reciprocal of the frequency squared, is limited by the imperfections in them together with any ohmic losses Results were presented of output power against wave-length for a range of values. In particular the 0 5-mm carcinatron gave a few milliwatts and had a 10 per cent tuning range.

Dr K D Froome (National Physical Laboratory, Teddington) outlined the design of his plasma–metal junction harmonic generator It rectifies and therefore generates harmonics by field emission from a liquid platinum cathode due to the proximity of a very dense plasma This plasma is formed in an argon–hydrogen atmosphere at high pressure and has an ion density that has to be kept to less than 10^{20} in order to avoid back conduction When the junction was driven with 10 W at 8 5-mm, 0 3-mm radiation, the twenty-ninth harmonic, was generated

The construction and operation of such a generator as a tunable source in a high-resolution sub-millimetre spectrometer was described by Mr. J Knapp (Queen Mary College) It was found not practical to use it in an automatic scan system Dr A J Bastin (Queen Mary College) reported measurements made by a number of workers, including himself, of atmospheric absorption in the sub-millimetre region These measurements showed that atmospheric absorption was due to (1) water vapour broadened by nitrogen collisions; (2) water droplets of varying radii, (3) oxygen, ozone and other trace gases, (4) collision-induced absorption between oxygen and nitrogen atoms It was commented that more experiments were needed in which some attempt had been made to match path-length to absorption coefficients

Mr G S. Waters (Signals Research and Development Establishment) described the construction and operation of an optical line-of-sight communication link using a helium neon laser on 6328 Å as source Measurements were made comparing the laser as a source with a mercury-arc lamp, both being shone 'backwards' through a mirror telescope over 1,300 yd The results showed that the spatial coherence of the laser made the laser superior to the arc lamp for ranges longer than 300 yd The laser's narrower line-width seemed to be only of secondary importance Dr D C Laine (Keele) examined the feasibility of an ammonia maser oscillating at 0 125 mm on the $J = 4$, $K = 3$ to $J = 3$, $K = 3$ rotational transition A relation was derived giving the minimum number of molecules required per sec for oscillation threshold The substitution of values into this relation gave that the number required was a factor two thousand times larger than the number needed in the microwave case This factor can only be obtained by the use of multiple beams

Dr J G Baker (Bristol) discussed the spectroscopy of short-lived free radicles and gases in the lower millimetre and sub-millimetre regions A free-space absorption cell suitable for the observation of electric and magnetic rotational transitions, many of which fall in these regions, was described together with a superheterodyne system of detection. Mr A W Horsley (Standard Telecommunication Laboratories, Ltd) discussed two possibilities for generating sub-millimetre radiation using bulk non-linear properties of semi-conductors The first rested on charge density gradients and electric field gradients in the semiconductor giving rise to harmonic currents The second involved the mixing of axial modes in laser beams by an extension of Pantell's centimetre wave mixing techniques Mr. C H Carmichael (Signals Research and Development Establishment) proposed the use of a Fabry–Perot etalon within the parallel mirror resonant cavity of a laser to suppress the emission except within the narrow pass bands of the etalon Then with a laser material, the fluorescence line width of which is 10 cm^{-1} or greater, the plate separation of the etalon can be chosen to yield frequencies, which, when mixed in a suitable non-linear system, will yield sub-millimetre radiation

Mr I B Bott (Radar Research Establishment) described the construction of a tunable source of millimetre radiation 0 93–2 mm Electrons, when injected into an axially symmetric magnetic field, undergo cyclotron resonance and the radiation emitted in a strong forward lobe is partially coherent In the case described the field was pulsed to 100 kG and remained reasonably steady for 7 msec The output at 1 1 mm was 1 mW Mr A H Beck (Cambridge) suggested that a spiralling electron beam when synchronized to an electric field having the correct time dependence should be a method of generating millimetre radiation Mr P M Robson (Sheffield) suggested that millimetre radiation could be generated by Doppler shifting radiation from a moving mirror of low-voltage electrons If the electrons were to be bunched at the matching frequency then both the electron mirror's reflectance would be increased and there would be some harmonic output Dr J V Jelley (Atomic Energy Research Establishment) discussed the use of Čerenkov radiation as a source of sub-millimetre waves A Fourier analysis of the radiation produced as electrons are fired through a dielectric shows that there is a component at a sub-millimetre frequency This component may be enhanced by bunching the electrons at the required frequency This method has produced 20 μW at 1 6 mm

Mr M A C S Brown (Radar Research Establishment) described two possible tunable detector mechanisms; one depending on changes in number of carriers and the other depending on changes of carrier mobility Both these mechanisms are suitable for use in narrow band far infrared detectors A description of the indium antimonide detector was given together with its narrow-band characteristics

Dr. M. R. Brown (Signals Research and Development Establishment) gave a brief report on the observation of infra-red quantum counter action in praseodymium doped fluoride lattices at room and liquid nitrogen temperatures. It was hoped to use the reported schemes as a basis for the development of an infra-red detector. Mr. D. M. Clunie (Services Electronics Research Laboratory) reported results of some experiments on helium–neon gas mixtures in which more power was obtained under pulsed conditions than under continuous excitation. This was attributed to the population in the laser terminal level being higher under the continuous excitation. Furthermore, by having two sections separately pulsed at 2.5 kc/s a continuous output at 0.25-W mean level was obtained. Doubling of the infra-red outputs in ammonium dihydrogen phosphate to give a yellow spot was announced. Mr. J. Smith (Mullards) reported gain measurements made in helium–neon gas mixtures using the 1.15μ laser line. It was found that the optimum gain in the negative glow region of a cold-cathode glow discharge within a hollow cathode was achieved at much higher pressure (20-mm mercury) and higher helium to neon ratios (1,280 : 1) than those used in radiofrequency or direct-current positive column excited devices.

Dr. E. L. Thomas (Signals Research and Development Establishment) explained the stimulated Raman effect and its use in the generation of radiation. When benzene is placed inside the resonant cavity of a ruby laser, stimulated Raman radiation is produced 991 cm^{-1} away from the ruby frequency. A second Raman radiation can also be generated approximately 1,982 cm^{-1} away. The sub-millimetre radiation, in this case 0.01 mm, would then be generated by mixing the Raman radiation with the ruby radiation.

Dr. D. H. Martin (Queen Mary College) reviewed the kinds of spectra that can be observed in solids with sub-millimetre radiation. These ranged from lattice absorptions in ionic lattices (30μ for lithium fluoride) to anti-ferromagnetic resonance (1.2 mm for manganous fluoride).

M. R. BROWN

THE GEOLOGISTS' ASSOCIATION

SEVERAL important geological finds made during 1963 were among the exhibits shown at the annual reunion of the Geologists' Association held in the Large Hall of Chelsea College of Science and Technology on November 2.

The most outstanding of these discoveries was the remains of a large pliosaur from the Oxford Clay exhibited by the Department of Palæontology, British Museum (Natural History). This comprised a right-hand paddle 7 ft. 6 in. in length. Many pliosaurs have been found in the Oxford Clay especially near Peterborough[1]. However, complete specimens of large pliosaurs lying *in situ* are rarely come by. It is thought their size prevented them from quickly sinking in the mud and hence they were eaten by their fellow scavengers. The pliosaur was found in the London Brick Company's clay pits at Stewartby, Bedfordshire, during June 1963, but the front half of the reptile had been taken away by the digger before the bones were noticed. Some of them were later recovered from the spoil heap by a party from the British Museum (Natural History), and the London Brick Co. has presented the remains to the Museum. It is hoped that the whole reptile will be recovered. The British Museum (Natural History) also showed dinosaurian footprints from the Purbeck, Devonian trilobites from the Spanish Sahara and cobalt pellets from the stomachs of sheep.

In 1961 the longest continuous trackways yet found were discovered in a quarry at Herston on the outskirts of Swanage, and in June 1963 after the overburden had been removed 75 ft. of trackway was exposed. Until now it has been generally assumed that these footprints were made by the herbivorous bi-pedal dinosaur, *Iguanodon*. But, as shown by the exhibit, it is apparent that the bird-like prints forming the main trackway are more akin to a bi-pedal carnivorous dinosaur, such as *Megalosaurus*.

Until recently, trilobite remains were almost unknown from the Spanish Sahara, but during oil exploration work there E. Rod collected almost 30 specimens. The best were given to the British Museum. They came from strata of the Middle Devonian and all belong to the genus *Phacops*. With the exception of a single specimen they belong to one species, probably new but related to *P. papulatus* from Morocco. The remaining specimen is a related form but is distinguished by a finer ornamentation of the test and a large number of eye facets.

S. E. Ellis (Department of Mineralogy) exhibited phosphate minerals deposited on cobalt pellets from the stomachs of sheep. The sheep is subject to a disease known as 'pining' due to a deficiency of cobalt in herbage. This is combated by either the expensive method of spraying or inserting into the young lamb's stomach a pellet of baked clay impregnated with cobalt oxide.

Ammonites and literature recently acquired in the U.S.S.R. were among the items shown by the Geological Survey and Museum. Ammonites are very important in the identification of zones or sub-zones, which are often named after them. They included the following species from the Upper Aptian of the Lower Greensand (Lower Cretaceous): *Parahoplites melchioris, Colombiceras caucasicum* and *Australiceras ramososeptatum*.

J. N. Carreck (Queen Mary College, London) showed specimens, maps, etc., illustrating copperas, as vanished Kentish industry. From about A.D. 1320 until 1914, copperas (an obsolete name for ferrous sulphate or green vitriol) was manufactured from iron pyrites occurring in the London Clay (Lower Eocene) on parts of the coasts of Essex[2], Kent, Dorset and, perhaps, Hampshire. It was used for the preparation of sulphuric acid, black ink and dye (both as a pigment and a mordant), dressing leather, as a sheep die, for the making of paints—prussian blue (ferric ferrocyanide) and spanish brown (ferric hydroxide), and after refinement was in much demand among both apothecaries and physicians. Mrs. Carreck exhibited photographs of past and present members of the Association.

Dr. G. P. L. Walker (Imperial College of Science and Technology, London) showed several xenoliths of wood collected from Tertiary basalt larvas. He explained how timber, when enclosed in basalt, chars and the basalt is consequently injected into the network of contraction cracks which result. Sometimes the charred wood survives, at other times it is fossilized, for example, replaced by zeolites, or it disappears to leave cavities in the basalt and these may afterwards be infilled with zeolites, etc.

With the west Weald earthquake of October 25 and the Glen Spean earthquake of August 31 still in the news, Dr. A. T. J. Dollar (Birkbeck College, London) exhibited a map illustrating the intensity of the quakes, together with data based on reports from observers of the British Earthquake Enquiry. Other earthquakes demonstrated in the same manner were those occurring in the County of Inverness in 1947 (ref. 3) and the Midlands in 1957 (ref. 4). Together with F. G. Berry, Dr. Dollar also showed a new hand-operated continuous coring tool with a sample core.

A. G. Nicholson showed specimens illustrating the geology of the Lizard Peninsula. These included the 'serpentine' of the local lapidaries which is the altered and attractively coloured part of bastite serpentine found chiefly at quarries near Kennack and Lizard Town, although some of the lapidaries' stock consists of beach boulders Rare invertebrates obtained from a very large 'pocket' of fossiliferous sand of the Lower Bartonian (Eocene) at Highcliffe, Hampshire, were exhibited by F. C Stinton They included a sea urchin *Spatangus omalii* and bivalves *Tellina scalaroides* and *Abra glabra* The 'pocket' was contained in a very sandy blue clay

Kingston College of Technology showed rocks collected in Aberdeenshire at the British Association Meeting and fish from the Lower Old Red Sandstone (Lower Devonian) at Kerrera, near Oban According to P R Gurr (Kingston College) and Dr L B Tarlo (University of Oxford), the fish fauna consisted of *Cephalaspis lornensis*, as well as a new species of this genus possessing long cornua projecting from the postero-lateral corners of the head shield Two new cephalaspid genera, also represented, indicated that forms with cornua were not merely primitive animals giving rise to true *Cephalaspis*, but that they had an important evolutionary development of their own

Some Tertiary mammals from France were shown by W H Bennett, including teeth of *Mastodon angustidens* from the Helvetian (Middle Miocene) and teeth and jawbone of *Lophiodon rhinocerodes*, a relative of the tapir, from the Bartonian (Eocene) Other items included casts of recently described American species of crustaceans, etc , by Mrs J I Collins, *Arcoscalpellum comptum*, a cirripede new to the Gault (Lower Cretaceous), by J S H Collins, a pyrites nodule from the chalk (Cretaceous) containing a terebratulid by A Gunner, fossil reptile remains from the Lias (Lower Jurassic) at Ravenscar by A J R Snelling, igneous and associated rocks of the Lake District by the Cumberland Geological Society, a cheap method of recording serial sections at minute intervals by F. J W Holwill (Imperial College of Science and Technology, London) and new German Devonian fossil finds, etc , by Mr and Mrs E P Bottley of the well-known geological firm of Messrs Gregory, Bottley and Co

J L GILBERT

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[1] Leeds, E T , *The Leeds Collection of Fossil Reptiles from the Oxford Clay of Peterborough*, 53 (1956)

[2] Shenstone, J T , "Copperas Industry" *The Victoria History of the County of Essex*, 2, 411 (1907)

[3] Dollar, A T J , *Nature*, 159, 648 (1947)

[4] Dollar, A T J , *Nature*, 179, 507 (1957)

INSTITUTE OF PERSONNEL MANAGEMENT

GOLDEN JUBILEE

THE last major event in the Golden Jubilee Year of the Institute of Personnel Management was its annual conference held at Harrogate during October 10–12 Again the attendance was about 1,000, made up of personnel and staff managers and directors from many companies throughout the world

The theme of the conference was management, productivity and industrial relations, and this was examined by Lord Shawcross in his opening address He suggested, first, that before advising industry how to organize itself, politicians should set themselves the task of organizing Government itself, for the machinery is cracking to the point of breakdown The weakest part of the organization is in the formulation of policy In the old days, perhaps up to the First World War, governmental activity was far less complex than it is to-day, and ministers with ample leisure and small departmental responsibilities were able to cope with problems reasonably satisfactorily To-day, with vastly increased occasions for governmental intervention, with problems of immense complexity and with heavy departmental pressures, this is far from the case To-day, policy is settled by debate in a Cabinet consisting of twenty-two members, each armed with a departmental brief and either tending to look at the matter mainly from a departmental point of view or expressing an opinion without much real knowledge of the subject-matter Any business run in this way would collapse Efficiency demands a much smaller Cabinet, say perhaps ten people, selected not primarily because they happen to be the heads of particular departments but because they are clearly the best ten people at the Prime Minister's disposal Among these ten people, the Prime Minister must lead clearly and firmly and accept the responsibility of being the ultimate arbiter of policy Obviously this arrangement would place on the Prime Minister a heavy burden· he would need assistance much beyond what can at present be given to him by his small private office He should have, as number two in his Cabinet, as a kind of 'chief of staff', a minister without direct departmental responsibilities, possibly the Lord President or the Lord Privy Seal, whose task it would be to assist the Prime Minister in formulating planning and assessing policies and surveying the main field of governmental activity This minister should himself have a small staff of top-level people, largely drawn from outside the Civil Service and including some possessing what politicians usually lack completely, a trained understanding of the technological age in which we live, who would help to formulate policies having first formed a political view as to what it was desirable and expedient to do

Turning to industry, Lord Shawcross said that, although there are gratifying signs of an upturn in the economy, Britain is not doing well enough Other countries have accelerated past her In the past eight years Britain's wages costs have risen about 3 per cent per annum faster than her main competitors, productivity has risen about 3 per cent slower In those years Britain's export prices went up by 15 per cent, her exports in quantity went up by 32 per cent In the same period, German prices went up only 5 per cent—but her exports rose in quantity by nearly 200 per cent

There is need for a supercharger to boost the industrial engine, and this must come from industry The Government, however, has a part to play in its planning function Government planning with knowledge of the overall economic picture ought to be of value to industry and there are things which Government can do, as varied as selective capital investment allowances, encouraging particular industries and special housing programmes and resettlement allowances, to encourage mobility of labour Subject to Government marking out the roads for advance, the self-correcting features of competitive private enterprise should not be destroyed The supercharger, however, is better management and better industrial relations leading to better productivity In the past quarter of a century there have been great improvements in standards of management Nowadays the pattern in big industry is increasingly for the boards of industrial operating companies, as distinct from holding companies, to consist entirely of whole-time executive directors who have worked themselves up to the top But the standard of management still needs to be raised considerably and management training should be organized not by industry alone, and not by the universities and technical colleges alone, but by both groups in consultation with a full knowledge of what each can provide and each needs. The crux is to enable executives to develop those qualities which make them more than merely good administrators, give them more powers of intellectual analysis and integrity and enable them to look for and assess the factors which are relevant to and on which they can take firm decisions and exercise clear leadership.

As usual, a series of sectional meetings at the conference provided a useful means of reviewing progress in a number of sectors concerned with improving human and industrial relationships. In discussing the merits of long-term agreements as a means of establishing better human relationships, for example, J. S. McGavin (Albert E. Reid and Co., Ltd.) suggested that, with the possible exception of the agreement in the building trade, long-term agreements differ from the old merely in that horse-trading occurs at greater intervals. The chief motive in negotiating a long-term agreement seems to be to reduce the frequency of negotiations which can occupy a large amount of the time of both employers and Trade Unions and lead to deterioration in relationships. There is now, however, a new force in evidence which must begin to exert an influence: the setting up of the National Economic Development Council and the National Incomes Commission. Particularly significant is the retrospective examination of any agreement which is part of the terms of reference of the National Incomes Commission. Private bargains between employers and Trade Unions may be subjected to close scrutiny based at least on some of the facts that tend to be forgotten in the heat of bargaining.

Among the disadvantages of long-term agreements are that, in a trade recession, profits may drop while labour costs are automatically increased, there is an increase in tension when an agreement comes to an end and a new one is to be negotiated, with the possibility of a strike if the negotiators are unwilling to make concessions, the experience of employers in the United States is that automatic wage adjustments under a long-term agreement have little effect on productivity but that there is some intangible or moral value, especially in disputes over technological change and its effects on remuneration. Not all trade unionists are keen on long-term agreements and some see them as seriously lessening the Trade Union bargaining position.

R. F. Marshall (Associated Electrical Industries, Ltd.) examined the Government proposals for industrial training as a means of improving Britain's inadequate supply of skilled manpower. The objects of the training proposals are to enable decisions on the scale of training to be better related to economic needs and technological developments, to improve the overall quality of industrial training and to establish minimum standards, to enable the cost to be more fairly spread. Among criticisms of Government proposals made by Marshall were that the provision of industrial training places in the appropriate numbers and of appropriate quality is an essential feature of the training arrangements for the technicians and technologists. The National Council for Technological Awards sent a comment on this to both the Minister of Education and to the Minister of Labour: "Industrial training plays as important a part in the training of a technologist as it does in the training of a craftsman and a technician. While a large number of firms have given considerable assistance to colleges in providing industrial training for students following courses leading to a Diploma of Technology, the further expansion of courses will require a substantial increase in the number of training places in industry".

It is proposed that the majority of firms scheduled within a defined industry shall contribute a levy and thus be directly involved, even though some of the firms do not actively contribute to training. The Minister of Labour has suggested that it would be uneconomical to extract the levy from very small firms. This point of view is criticized on the grounds that it is these very small firms which drain away skilled labour from the larger units. The industrial training boards proposed by the Minister will have the responsibility for all aspects of training, not excluding management training. Doubts have been expressed about the capacity of a board which, having been set up to promote craft training, would be able to consider and promote training at other levels of employment where standards have yet to be set. Marshall also made a number of suggestions to ensure that levies are fairly made in industrial organizations and also for the efficient running of the Industrial Training Boards.

In a plenary address, Prof. O. Hahn-Frend, professor of comparative law (elect) in the University of Oxford, examined the question of whether increased legal intervention in industrial relations is desirable in Britain at the present time and, if so, in what directions. He distinguished between four different kinds of such intervention which may be called: auxiliary, prohibitive, regulative and remedial. The law may seek to promote collective bargaining and the observance of collective agreements, for example, by imposing a legally enforceable obligation to bargain (United States), by enforcing as compulsory terms of the contracts of employment the terms agreed between employers and Trade Unions (France, Germany and other Continental countries), or by creating substitutes for non-functioning bargaining machinery which at the same time operate as stimuli to collective bargaining and to the observance of agreements (Britain). In all this the law is no more than an auxiliary to collective bargaining. It does not regulate, but assists the autonomous forces of industry in doing so.

The law may (and must) mark the outer limits of the area within which hostile action in industrial relations is permissible, and prohibit certain types of conduct connected with strikes, lock-outs, etc., while permitting others. This is the only contribution the Common Law has made, and one of the main functions of legislation in Britain has been to free industrial action from the shackles of the Common Law. Even so, this prohibitory function of law can nowhere and never be dispensed with entirely.

Thirdly, the law may itself create the standards prevailing between employers and the employed, that is, their mutual rights and duties. Protective legislation concerning health, safety, welfare, legislation on hours of work, on methods of wage payments and also on minimum wages and holidays, offers examples. Outside Britain, this covers rights of tenure, that is, security of job, payment in the event of sickness or interruption of work, notice of termination of the contract and many other matters. To some extent, the recent Contracts of Employment Act is also an example. Fourthly, and lastly, the law must provide enforcement machinery, that is, administrative authorities, such as inspectors, and courts and enforcement officers to translate rights into remedies and remedies into tangible advantages. The practical value of auxiliary, prohibitive or regulatory legislation may depend on the stringency and accessibility of the remedies provided by its enforcement.

There is a strong case for improved and increased regulatory and remedial legislation, but the case for further auxiliary legislation is weak and that for further prohibitive legislation non-existent. Trade Unions and employers' associations are well able to see to the enforcement of collective agreements, provided the law does not further deprive them of the extra-legal weapons at their disposal. With the growth of non-manual at the expense of manual work (which is the result of technical developments) the area of collective bargaining may dwindle and the need for regulatory legislation may grow. So may the need for state-provided enforcement machinery, less expensive, more accessible and more rapid than the existing courts.

With regard to regulatory legislation on security of jobs and legislation on the enforcement of rights between employers and employees, Britain falls between two stools

Britain does not have collective agreements as intensive in coverage as those existing in the United States perhaps owing to the extensiveness of their scope (industry-wide as distinguished from plant-wide agreements). British agreements are less substantial than their counterparts in the United States, and, in particular, do not comprise anything like the American codes of job security and seniority or the grievance arbitration machinery through which these and other rights are enforced. On the other hand, Britain has nothing comparable with the German legislation on job security or the French principles on abusive or unfair dismissals. The Contract of Employment Act, 1963, is no more than a feeble and insignificant first step in this direction: it gives a right to a maximum of four weeks' notice after five years of continuous employment, and no power to award compensation for unfair deprivation of job. German workers and employees can claim up to one year's salary or wages by way of compensation for undue dismissal.

Similarly, Britain has neither the American pattern of grievance arbitration nor the Continental pattern of labour courts, consisting either (as in France) entirely of laymen, or (as in Germany) of a lawyer chairman, one employer and one employee with equal rights. These operate very rapidly and very cheaply, and settle many cases, and are far better adjusted to handling employer–employee cases than the existing machinery of justice in Britain.

To-day, British legislation on labour relations which, at one time, was in the van of progress, has fallen in arrears. The scrutiny of the fairness of dismissals, coupled with a power to award a right to be reinstated or to receive compensation and the creation of labour courts, are two measures necessary to promote social justice as well as the smooth operation of industrial relations.

The conference was also significant for a plea by Mr. G. R. Moxon (United Glass Ltd.), the president of the Institute, that the time had come for all bodies dealing with personnel management to re-examine their structure and organization and pool their resources in order to provide better services, particularly in projects overseas where British participation would be invaluable.

T. H. HAWKINS

SOLID-STATE CHEMISTRY

THE phenomenal growth of solid-state chemistry in the past fifteen years owes much to the joint research ventures of chemists, engineers and physicists, and the impetus for this stems largely from what has been aptly termed "the explosion in electronics", the invention of the transistor. In a paper by Dr. N. B. Hannay, chemical director, Bell Telephone Laboratories, for many years past concerned with semiconductor chemistry (*Science and Technology*, New York, October, 1963), the present position of this intriguing subject is ably summarized. The basis of this discipline is, he says, that "all solids are imperfect, fortunately, with atoms missing, misplaced, or of the wrong kind serving to bring the seemingly stolid solid to vibrant, useful, chemically reactive life". The high state of purity and crystalline perfection of some semiconductors, combined with their unique electrical properties, have facilitated the quantitative investigation of chemical processes in the solid state; they have made chemical reactions in such materials not only of great scientific interest but also to assume the importance of model systems as first principles in as yet a wide and largely unexplored field of chemical behaviour.

Many chemical interactions in semiconductors are analogous to those known and described in solution chemistry. The solvent is weakly ionized, like water, but in this case solid instead of liquid. "Just as ionization of water produces hydroxide and hydrogen ions ($H_2O \rightleftarrows H^+ + OH^-$) so ionization in semiconductors produces conduction electrons and holes (Si lattice $\rightleftarrows e^+ + e^-$) in a closely analogous way. Conduction electrons, which are not bound to any lattice atoms but wander freely through the lattice, are created when valence electrons are excited out of their normal atomic orbits by thermal agitation or by optical excitation. Holes, which simulate positively charged particles in their electrical behaviour, simply represent missing valence electrons". Several examples of reactions in semiconductors resembling those in aqueous solution are given in this paper and the analogies between them are striking. They result from the essential similarity between the hole (e^+)–electron (e^-) equilibrium in semiconductors and the hydrogen–hydroxyl equilibrium in water. It is indicated that both equilibria are governed by the familiar law of mass action, that is, any change in concentration of reactants which are in equilibrium shifts the equilibrium in a direction that tends to neutralize the change.

As Dr. Hannay points out, there remain, apart from the 'glamorous' materials and devices already discovered in the course of these researches in solid-state chemistry, innumerable problems of major theoretical and practical importance that are still unsolved. Among these may be cited the better understanding of controls on reaction rates, diffusion mechanisms, nucleation processes, for example, as applicable to catalysis. H. B. MILNER

CENTENARY OF THE BIRTH OF W. H. YOUNG, F.R.S.

WILLIAM HENRY YOUNG was born on October 20, 1863. He entered Peterhouse, Cambridge, in 1881, was fourth Wrangler in 1884 and a Fellow of Peterhouse from 1886 until 1892. For thirteen years he coached for the Mathematical Tripos, a profitable but arduous occupation. He was an excellent coach, particularly with less than excellent pupils, but he gave, so far as mathematics was concerned, no sign during this period of the fertile originality that was to come.

In 1896 he married Grace Chisholm, a mathematician of no mean ability herself, a Wrangler of Girton and the first woman D.Phil. of Göttingen. Distinguished as her own work was, her greatest service to science was the influence she had on her husband, which, in the first instance, led him in 1897 to give up his Cambridge practice and devote most of his time to research. From then on, the family home was abroad, at first in Göttingen, later in Switzerland. Although Young spent some months in each year from 1901 until 1923 in some paid activity, mainly teaching mathematics in Britain, mathematical research (unpaid) dominated the rest of his life. In 30 years he published more than 200 papers. Young burst out in British pure mathematics like a supernova. He was elected a Fellow of the Royal Society in 1907.

His productivity is the more astounding in that it started so late and that his first work failed to bring him the credit it deserved. About this time, Lebesgue arrived at his definition of an integral. Young, working independently, arrived at a definition differing in form from Lebesgue's, but essentially equivalent. Lebesgue, however, was first, and it is called the Lebesgue integral. Fortunately, Young's name is attached not only to recondite theorems in the theory of Fourier series and the theory of surfaces, but to more than one result in undergraduate analysis, where the clarity of his mind created a new simplicity and a new elegance.

He raised a large and talented family. He died in 1942, alone in Lausanne, separated by the accidents of war from his wife and family. At a seminar on November 5, at Imperial College, Sir Graham Sutton (one of Young's pupils), Dr J C Burkill and Prof. J Proudman spoke on Young and his mathematical work, including his methods of teaching, and the centenary was commemorated in the evening by a small dinner at his old club, the Athenaeum. (See also *Nature*, 150, 227, 1942.)

H D URSELL

SHIFTING SAND BANKS OFF LOWESTOFT

LITTLE is known about the origin or stability of sand banks although some are serious hazards to navigation around the southern half of the British Isles. Accordingly, publication of a detailed account of 6 square miles of sand banks off Lowestoft* is a most welcome addition to knowledge, for both practical and academic reasons. It is fitting that this study was carried out by a member of staff of the department of the Admiralty which surveys the sea-floor and draws up navigational charts.

The method of approach adopted by Mr Cloet was to compare the 44 detailed surveys made between 1835 and 1962, using a standard datum. From these surveys he prepared figures showing the maximum and minimum depths detected at any place and then derived a further figure showing the maximum known changes in thickness which can be expected. Such changes may reach even as much as 80 ft. It is suggested that the channelled surface of maximum depth is made of bedrock or relatively immovable material such as pebbles.

Much of the analysis is concerned with a demonstration of the stability or motion of channels and sand banks and the smaller features associated with them. It is shown that the rate of movement of a feature need not be uniform and can vary with depth. Their direction of growth is particularly interesting since it is apparent that they approach Lowestoft both from north-north-west and south-south-west, and interfere off the harbour entrance. There are indications that their direction of growth corresponds to the direction of sand transport by tidal currents though the occasions of transport, and the relative importance of sea-waves cannot be revealed by such a study.

Diagrams are numerous, but it is a pity that so few are self-explanatory. It is rather unfair that only some of the localities mentioned in the text are shown on the figures, for not everyone has ready access to detailed Admiralty charts, or knows the region as well as Mr Cloet. A much more serious criticism concerns the difficulty of identifying the ornament and of matching it with that in the key on figures, such as 3–5. Such a style of reproduction is false economy.

A H STRIDE

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* Admiralty Hydrographic Department. Admiralty Marine Science Publications No. 6. *Hydrographic Analysis of the Sandbanks in the Approaches to Lowestoft Harbour*. By R. L. Cloet. Pp. iii+15+22 illustrations+12 graphs. (London: Admiralty, Hydrographic Department, 1963.)

LONG-RANGE WEATHER FORECASTS

THE present state of knowledge does not permit a satisfactory theoretical solution to the problem of the general circulation of the atmosphere, nor to the associated problem of forecasting for long periods ahead. Because of the practical importance of long-range forecasts, many national meteorological services attempt to deal with the problem empirically, mostly on a regional basis and, of course, avoiding over-precision. No country depends entirely on one method of prediction, but makes the most of a variety of aids towards a solution. In any country, however, there tends to be a concentration of effort along particular lines because of the work involved. A short description of the methods adopted by different meteorological services has recently been published[1] by the German Weather Service, following the report[2] published in 1962 by a working group of the Commission for Aerology (World Meteorological Organization).

Some methods used are statistical and objective, and can treat a wide range of observational material in attempts to find correlations between future weather and available measurements. Regression equations have been used, for example, in forecasting the Indian Monsoon, but simple and multiple correlation tables also have their uses. The French, German and Russian weather services make great use of statistical methods. The various chosen predictors may be values of anomalies of pressure, temperature or rainfall, or may be concerned with weather types, solar influences, or with the interactions of atmosphere and ocean. Climatological probabilities and persistence tendencies must also be taken into account.

Another of the favoured methods makes use of analogous situations from the past, and assumes that an existing weather situation will develop in the manner followed by a similar previous situation found in weather records. The finding of analogues is essentially the basis of the British method, which compares present and past patterns of temperature anomalies over a large part of the northern hemisphere, and also examines the sequence of weather charts during the month over the British Isles as well as the monthly mean pressure chart over the northern hemisphere. A forecast can then be made when a number of analogous situations lead to similar sequels. If their sequels are different, then the most likely sequel is chosen in the light of additional arguments which may be based on the limits of the polar ice-cap, sea temperatures, snow cover or some marked anomaly of the general circulation.

Other methods depend on extrapolation. For example, the United States Weather Bureau examines mean circulation patterns and extrapolates these by normal forecasting methods, thus obtaining an indication of the most likely main paths of depressions and anticyclones during a period. From this indication can be produced charts showing anomalies of temperature and rainfall. Other extrapolation methods make use of periodogram analysis and extrapolate any regular variations found in a chosen predictor, though some methods used in the United States make use of a theoretical formula for obtaining the speed of movement of a wave pattern.

Many of the methods are adaptable to the electronic computer, and the enormous possibilities of modern computing are causing some reconsideration of previous ideas on the use of statistical data. The computer also makes possible various researches on the use of dynamical theory to obtain a numerical prediction.

In all this work there is great need for international co-operation by the exchange of scientific papers, data and forecasts Verification tests are difficult to carry out even with the facilities of a meteorological service, but the various countries show a very similar (rather low) standard of success. The British Meteorological Office, on the basis of experience with experimental forecasts during the past decade, claims no more than forecasts which are significantly better than chance when considered over a period of time From December 1963 the forecasts will appear in a new Meteorological Office publication[3], though the methods used are still experimental The publication consists of a single sheet, one side of which contains climatological data for Britain for the forecast period, while the other side contains data for the month just past, followed by weather prospects for the coming month Used with understanding, these forecasts may be of value to various interests in industry, commerce and agriculture.

W S GARRIOCK

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[1] Deutscher Wetterdienst *Annalen der Meteorologie*, Neue Folge, Nr 2 *Der Internationale Stand des Problems der Langfrist-Vorhersage.* Alfred Hofmann. Pp 54 (Offenbach a M Selbstverlag des Deutschen Wetterdienstes, 1963)

[2] World Meteorological Organization, *The Present Status of Long-range Forecasting in the World* Technical Note No 48, Geneva, 1962

[3] Meteorological Office, *Monthly Weather Survey and Prospects* (Published monthly as a single sheet, with a mid-month supplement Price, including supplement, 1*s* 2*d* monthly, post free Annual subscription rate 13*s* 6*d* Obtainable from the Secretary, Meteorological Office (MWS), London Road, Bracknell, Berkshire)

ACCELERATION OF SINKING RATES OF RADIONUCLIDES IN THE OCEAN

By PROF CHARLES OSTERBERG, DR. ANDREW G. CAREY, JUN., and DR. HERBERT CURL, JUN

Department of Oceanography, Oregon State University, Corvallis

ZIRCONIUM-95–niobium-95 and cerium-141–144 were detected by γ-ray spectrometry in two samples of sea-cucumbers collected off the Oregon coast west of Newport in April 1963 Sea-cucumbers of the genus *Paelopatides* were found at 2,800 m, 65 miles offshore, *Stichopus californicus* were taken in 200 m of water 25 miles off Newport The γ-ray spectra of these two benthic sediment feeders were remarkably similar, except for the lower zinc-65 peak in the spectrum of *Paelopatides* sp (Fig 1) Since these animals are bottom feeders, the presence of short half-lived fission products in the deeper sample suggests a more rapid sinking rate for fall-out particles in the sea than would be expected from purely physical processes

All benthic animals were collected with a 22-ft shrimp-type otter trawl. Samples, preserved in formalin and freeze-dried, were ashed at 500° C overnight 12 c.c. of ash were counted in the well of a 5 in × 5 in sodium iodide (Tl) crystal, the signal from which was resolved with a 256-channel pulse height analyser

Recounts of both samples over a 114-day period indicated a 66-day half-life[1] for ^{95}Zr–^{95}Nb The calculated half-life of the $^{141-144}$Ce mixture gradually lengthened due to the decreasing percentage of 33-day ^{141}Ce compared to 282-day ^{144}Ce Potassium-40, the naturally radioactive isotope of potassium, has a very long half-life and showed no significant changes in activity over the period of observation

Particles of fall-out radionuclides exist in a wide range of sizes, but Sisefsky[2] found particles larger than 4 5μ from worldwide fall-out to be very rare These particles would not be likely to sink in sea-water at a rate faster than that determined from Stokes's law According to the calculations of Carritt and Harley[3] based on Stokes's law, a 10μ spherical granite particle would require almost a year to sink to 2,800 m and a 1μ particle would require 100 years, in the absence of turbulence

There are few measurements of the settling rate of fall-out particles in the ocean Near Eniwetok immediately after a nuclear test, Lowman[4] observed that radioactivity moved downward in the surface mixed layer (about 75 m thick) at 2 5 m/h Below the thermocline, it increased to 10 m/h At these high rates, a particle would sink to 2,800 m in approximately 12 days Such high sinking rates are probably due to the predominantly large particles present in local fall-out[5], most of which have a diameter greater than 25μ

Sugihara and Bowen[6] reported that cerium-144 in the Atlantic Ocean, away from local fall-out, is removed from surface waters on particles sinking at about 100 m per month, or slower Fall-out travelling at this rate would require 28 months to reach 2,800 m The half-life of zirconium-95 is only 65 days, and the peak due to this isotope in the spectrum of our deep sample is a prominent one Clearly the zirconium-95 in our collecting area, also far from any nuclear test site, must have descended at a rate much faster than 100 m/month

The similarity of the fall-out peaks (^{95}Zr–^{95}Nb and $^{141-144}$Ce) for the two samples (Fig 1) suggests a very short transit time downward for these radionuclides Unfortunately, however, absolute values for settling times require many assumptions For example, we may assume that both deep and shallow sea-cucumbers should have the same ^{40}K ^{95}Zr–^{95}Nb ratios, and attribute the small observed difference to radioactive decay The larger ratio for the sea-cucumbers from 2,800 m would result from a decay time (that is, transit time) of seven days

We might also assume that both groups of animals should have equal amounts of ^{95}Zr–^{95}Nb (per unit weight) if they were feeding together at the same depth However, the deeper sample was observed to have less ^{95}Zr–^{95}Nb

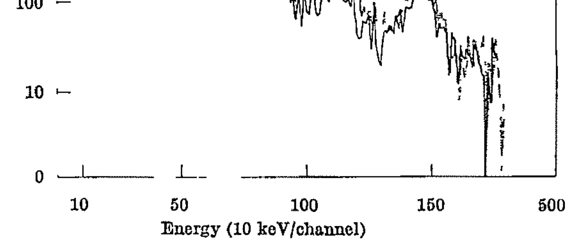

Fig 1 Gamma-ray spectra of the sea-cucumbers *Paelopatides* sp and *Stichopus californicus* To avoid the confusion of overlap, the spectrum of the sea-cucumber from 2,800 m has been displaced downward slightly Zirconium-95–niobium-95 and cerium-141–144 are from fall-out, zinc-65 is principally from the Hanford, Washington, reactors, and potassium-40 is the naturally radioactive isotope of potassium 100 min count, , 200 m, —— 2,800 m

than the shallow sample The observed difference in activity, if attributed to radioactive decay while the fall-out settled to the greater depth, yields a transit time of about 12 days Although neither assumption is known to be valid, the small difference in the spectra points to the need for a process which is capable of carrying fall-out down to the ocean depths before appreciable radioactive decay can occur

We suggest that biological processes are responsible for the relative abundance of fission products in deep bottom feeders Particulate fall-out radionuclides are known to be concentrated by herbivorous copepods, euphausiids, salps and pteropods[7-9] These zooplankton, which make up the bulk of second trophic level marine organisms, ingest fall-out particles attached to microscopic plants in the surface waters of the ocean Unassimilated radionuclides are released in fæcal pellets, which, because of their greater size, sink rapidly. The biological turn-over time for these radionuclides is quite short Chipman[10], in a laboratory experiment, observed that the copepod *Tigropus californicus* excreted 97 per cent of ingested cerium-144 in one day. He concluded that fission products in general were poorly absorbed from the digestive tracts of zooplankton Apparently most herbivorous plankton are rapid 'processors' of particulate fall-out radionuclides, continually converting small particles into larger ones

Preliminary measurements of the sinking rates of fæcal pellets have been made in our laboratory by Curl (in preparation) *Euphausia pacifica*, a very abundant euphausiid, was fed diatoms (*Skeletonema costatum*) The average sinking rate for the resulting fæcal pellets ($100\mu \times 1{,}000\mu$–$500\mu \times 3{,}000\mu$) was 43 m/day Under similar conditions (still water, temperature 10° C, salinity $33^0/_{00}$) these pellets would require about 65 days to reach 2,800 m. Of course, there are many factors in the ocean which could alter the sinking rate Nevertheless, radionuclides incorporated into fæcal pellets would reach the bottom much faster than 'unprocessed' fall-out particles

Predators which feed on herbivorous zooplankton carry fission products into higher trophic levels These predators are in effect concentrating fall-out radionuclides, since most of the ^{95}Zr–^{95}Nb and $^{141-144}$Ce is not assimilated[9]. It is conceivable that the excretory products of higher trophic level organisms are in such a form as to further accelerate settling rates of these radionuclides

The ratios of ^{95}Zr–^{95}Nb $^{141-144}$Ce, which were higher in the deep sample throughout the observed decay period, indicate that more $^{141-144}$Ce than ^{95}Zr–^{95}Nb was lost during the longer transit time to 2,800 m Preferential loss could result from a slightly greater solubility of $^{141-144}$Ce, either in sea-water or in the digestive tract

Zinc-65, from atomic reactors (Hanford Laboratories, Washington) on the Columbia River, did not behave in the same manner as the fission products It was abundant in sea-cucumbers from 200 m, but much less was found in animals from 2,800 m (Fig 1), despite a longer half-life (245 days) than ^{95}Zr–^{95}Nb Unlike zirconium, niobium and cerium, zinc is present in a number of enzymes Apparently the biological turnover time for zinc is quite long, and little radiozinc reaches the bottom Either it is not readily excreted, or, if excreted, most zinc-65 is lost from fæcal pellets before they reach 2,800 m

The possibility that our samples were contaminated with fall-out either at sea or in the laboratory was carefully checked. Of the 25 sea-cucumbers taken in the deep sample, only 16 were ashed to obtain the spectrum in Fig 1 Six others were dissected, and their digestive tracts (which would not be subject to surface contamination) were analysed separately The same radionuclides were present The spectrum of seven unidentified anthozoans (sea anemones) from the same trawl showed comparable levels of ^{40}K and ^{65}Zn, but the ^{95}Zr–^{95}Nb peak was only one-eighth as high This difference would not be expected if the sample were contaminated, but is typical of the discrimination against ^{95}Zr–^{95}Nb which has been observed in some oceanic animals[9] As a further check three and one-half months later a second collection was made from 2,600 m, 145 miles offshore The levels of radioactivity in *Paelopatides* sp were lower, but evidence of fall-out was clearly present Benthic worms, which are considered to be deposit feeders, were also collected in this trawl They too contained ^{95}Zr–^{95}Nb

These observations lead us to conclude that fall-out radionuclides can reach the ocean bottom quite rapidly under certain conditions We propose that the great numbers of herbivorous zooplankton in the upper layers of the ocean are largely responsible for this phenomenon

We acknowledge the assistance of Danil Hancock, Lauren Larsen and the captain and crew of the R V *Acona* We thank Elizabeth Deichmann and J McCauley for identification of our sea-cucumbers

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PALÆOMAGNETIC INDICATIONS OF AN INCREASE IN THE EARTH'S RADIUS

By DR. D. VAN HILTEN

Department of Mining, Technological University, Delft, The Netherlands

A NUMBER of authors[1-4] have proposed, mainly on geotectonic grounds, the hypothesis that the Earth's radius has increased during geological history The rate at which this increase should have taken place according to their views is depicted in Fig 3 The authors agree that the continents have not—or have scarcely—increased in area since the Palæozoic, and that the increase in global area must be sought in the formation or enlargement of the oceanic basins This implies that the distance measured along the Earth's surface between two points on the stable part of the same continent remains constant, but that the geocentric angle between these points decreases inversely proportional to the increase in the Earth's radius

Like most students of palæomagnetism, I assume that the ancient geomagnetic field had a dipole configuration From the direction of magnetization measured on rocks of one sampling site the ancient position of the geo-

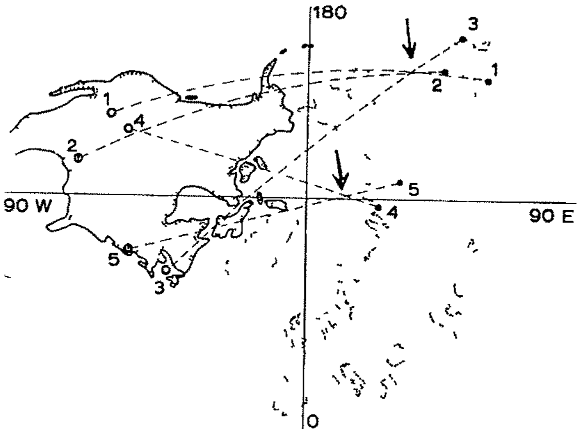

Fig 1 Carboniferous (1, 2, 3) and Triassic (4, 5) palæomagnetic data from North America in stereographic projection Sampling sites (open circles) are connected by great circles (dashed) with the corresponding pole positions (full dots), the distances between site and pole position have been calculated on the assumption of a constant Earth's radius ($R_a = R$) The scatter of the poles can be reduced by diminishing this Ra/R ratio to such a degree that the pole positions come to lie around the points of intersection (arrows) of great circles of equal geological age Numbers refer to Table 1

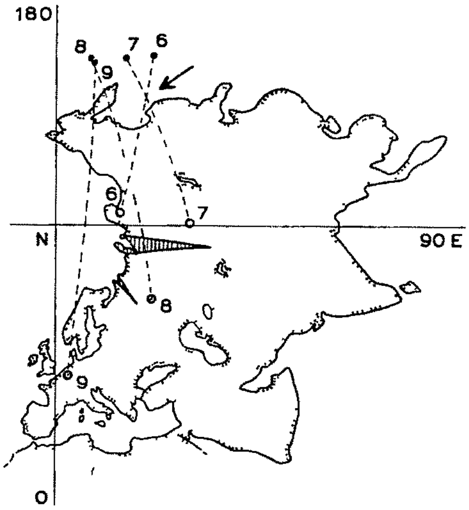

Fig 2 Permian palæomagnetic data from Siberia (6, 7), Russia (8), and western Europe (9) See also caption to Fig 1 and text

magnetic pole with respect to this sampling site may be reconstructed the declination of the direction of magnetization shows the direction (a great circle through the collecting site) in which the ancient pole was situated From the inclination (I) the ancient distance of the pole from the sampling locality can be calculated from

$$p = \frac{R_a}{R} \cot^{-1} (\tfrac{1}{2} \tan I) \qquad (1)$$

where p is the geocentric angle between sampling site and ancient pole position, adapted to the present-day radius of the Earth (R), R_a represents the ancient radius of the Earth at the time of deposition of the rocks investigated When palæomagnetic observations of equal age from two or more widely separated localities on the same continent are available R_a may be found Distinctions can be made in two different cases (1) the localities were situated on about the same ancient geomagnetic meridian, but on different geomagnetic palæolatitudes, (2) the localities were on about the same geomagnetic palæolatitude but on different geomagnetic palæomeridians

In the first case the ancient radius is known from

$$R_a = d/[\cot^{-1}(\tfrac{1}{2} \tan I_1) - \cot^{-1}(\tfrac{1}{2} \tan I_2)] \qquad (2)$$

where d is the distance measured along the Earth's surface between the two ancient geomagnetic latitude circles of the sampling sites, and I_1 and I_2 are the inclinations of the direction of magnetization measured at these localities In an earlier attempt to calculate the Permian Earth's radius, Cox and Doell[5] made use of this method; their results will be discussed here

In the second case the ancient geomagnetic pole position may be found by drawing the great circles through the sampling localities in the direction given by the declination (Figs 1 and 2) The ancient pole will be situated then on the intersection of these great circles, which are in fact geomagnetic palæomeridians From equation (1) R_a can be calculated, p being known as the distance between sampling site and point of intersection of the great circles

Scrutinizing the world's palæomagnetic record, five sets of palæomagnetic data can be found which seem favourable for an R_a evaluation (*a*) the Carboniferous of North America, (*b*) the Triassic of North America; (*c*) the Permian of Siberia, (*d*) the Cretaceous of North America, (*e*) the Permian of western Europe The first four of these sets should be classed under case 2, and the

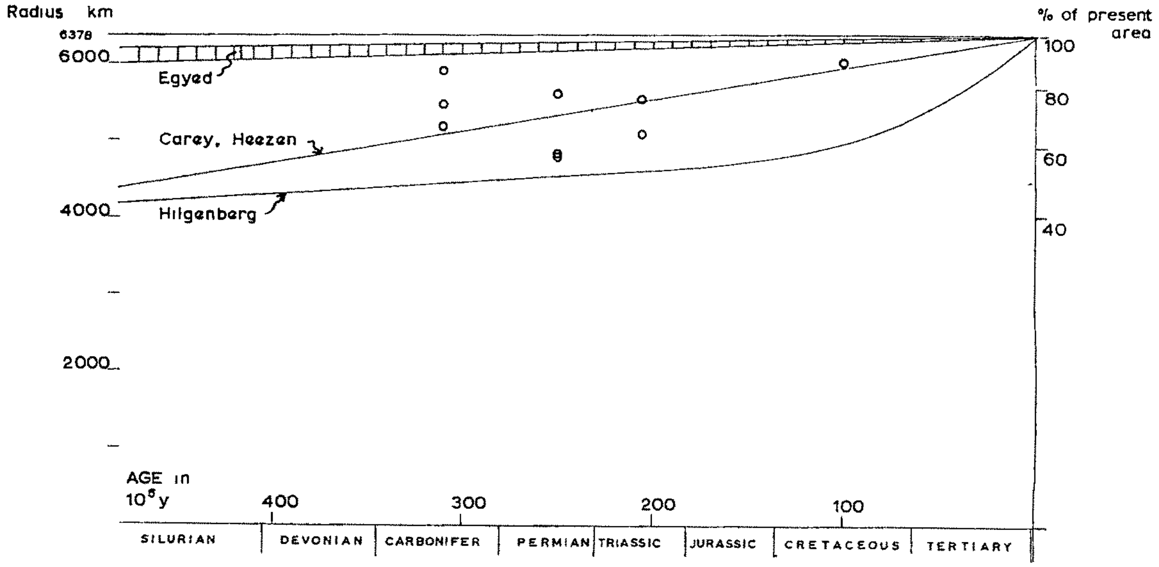

Fig 3 Change in the Earth's radius with time according to Egyed (ref 2) (0 5-1 0 mm/y), Carey (ref 1) and Heezen (ref 3) (45 per cent increase in area since the Permian), and Hilgenberg (ref 4) Time-scale according to Kulp (ref 23) Circles show the results of the analysis of palæomagnetic data discussed in this paper

Table 1

Age of formation	Mean sampling site position No in Fig				Mean pole position ($R_a = R$) Lat	Long	Distances between (1) and intersection (s) of great circles	(2)	R_a/R	R_a (km)
	No	in Fig	Lat	Long						
			(1)		(2)		(3)	(4)	(3)/(4)	
Carboniferous	1	1	35 N	112 W	35 N	123 E	76°	93°	0 818	5,217
(N America)	2	1	31 N	99 W	41 5 N	133 E	86°	93 5°	0 920	5,868
	3	1	48 N	62 W	33 5 N	136 E	83 5°	97°	0 861	5,491
Triassic	4	1	40 N	110 W	70 N	84 E	60°	69 5°	0 863	5,504
(N America)	5	1	41 N	74 W	64 N	100 E	59 5°	74 5°	0 799	5,096
Permian	6	2	72 N	102 E	40 N	150 E	30 5°	40 5°	0 753	4,803
(Siberia)	7	2	54 N	91 E	43 N	157 E	33°	43 5°	0 759	4,841
Cretaceous	10	–	36 N	115 W	77 N.	177 5 E	39°	50 5°	0 772	
(N America)							45 5°	50 5°	0 901	
	11	–	45 5 N	72 W	68 N	171 W	43°	52°	0 827	
							61°	52°	1 172	
	12	–	79 N	104 W	69 N	180	13°	21°	0 619	
							28°	21°	1 380	
	10–12							average	0 945	6,027

Calculations of the R_a/R ratios required to have the pole positions coinciding with the point of intersection of the great circles

fifth set of data under case 1, as the sampling sites of western Europe are situated about in line with the ancient pole position (same palæolongitude)

Carboniferous of North America The six investigations (data from Cox and Doell[6], p 704 Nos 38, 40, 43, 45, 47, 49) are distributed over three sampling areas (Table 1 and Fig 1) The great circles intersect at one point, from where the distances p to the three sampling sites are measured. This yields three values for $R_{\text{Carboniferous}}$ (Table 1 and Fig 3)

Triassic of North America Twelve palæomagnetic investigations are confined to two sampling areas, they are (Cox and Doell[6], pp 713–718) Nos 22, 39, 79, 91, 105, belonging to sampling site 4 on Fig 1 The Nos 93, 96, 9o, 100 and 102 from Cox and Doell[6] and the investigations by Irving and Banks[7] and by Opdyke[8] are all situated in the eastern United States, the sampling site 5 of Fig 1

Permian of Siberia. The data have been taken from Irving[9] and from Popova[10] Both calculations of R_{Permian} produce about the same value (Figs 2 and 3)

Cretaceous of North America Five palæomagnetic investigations are distributed over three sampling localities (Table 1). Location 10 Cox and Doell[6], p 725, No 4, and the work by Currie *et al* [11]; Location 11· the observations of Larochelle[12]; Location 12 the results of Larochelle and Black[13] The three intersecting great circles form a triangle, which offers six estimates for the ancient polar distance p and the R_a/R ratio From their average value a Cretaceous Earth's radius of 6,027 km results

Permian of western Europe A qualitative selection was made from the numerous Permian data of western Europe Five studies were chosen because of the greater reliability of their results, due to laboratory tests in a c -fields or thermal methods, they are the palæomagnetic researches by Van Everdingen[14] (Oslo volcanics), Nijenhuis[15] (Nahe effusives, Germany), Roche *et al* [16] (Vosges volcanics), De Magnée and Nairn[17] (Poudingue de Malmédy, Belgium), and Kruseman[18] (Lodève sediments, Southern France) These authors demonstrated that a certain amount of postdepositional or recent magnetization may be present in the majority of the rocks investigated, by which especially the inclination is affected The earlier analysis of European data by Cox and Doell[5] included a high percentage of 'not-cleaned' rocks Furthermore, they envisaged also the volcanic series of the Estérel (southern France) an area which probably[19,20] should not be considered with the stable part of Europe By forming couples of the two extreme sampling sites (Oslo and Lodève) with the mid-European ones, and with one another, in total seven estimates of R_{Permian} can be made For the value of d in equation (2) the difference between the distances of the sampling sites from their average ancient pole position was taken The arithmetical mean of the seven calculations yields a Permian Earth's radius of 5,612 km (Fig 3)

Utterly inconsistent with the foregoing evidence for an increase in the Earth's radius during geological history is the coincidence of the virtual pole positions derived from western Europe and Russia[21], though their sampling sites are well on different palæolongitudes (Fig 2) Advocates of global expansion might attribute this inconsistency to the 'orange-peel' effect half an orange, squeezed to fit the flat bottom of the fruit-squeezer, will produce a number of radial tears in its outer rim Something analogous may be expected to occur at the borders of a continental slab adjusting itself to a less-curved surface, though, taking into account the slow rate of the process and the somewhat different materials involved, the tears may present themselves rather as zones of extension The more coherent blocks beside these zones perform tangential rotations, each unit moving away from its neighbour In Fig 2 some zones of extension (hatched) have tentatively been drawn between Europe–Russia–Siberia Their positions have been chosen in order to fit the geological outlines of those regions the western zone is thought to coincide with the Pechora depression[22], the eastern one with the Ob Valley, they are both areas of thick Mesozoic and Tertiary sedimentation The activity of these zones of extension during the post-Permian expansion of the globe might have torn the Eurasian poles apart, by which the expected configuration of intersecting great circles was destroyed

To summarize, it may be stated that palæomagnetic evidence (compiled in Fig. 3) seems to indicate a noteworthy increase in the Earth's radius since the Carboniferous, the rate of which agrees roughly with the hypotheses of Carey[1] and Heezen[3]

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SEAWARD EXTENSION AND ORIGIN OF THE FREETOWN LAYERED BASIC COMPLEX OF SIERRA LEONE

By PROF. D. C. KRAUSE

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island

GEOLOGICAL and geophysical surveys of the Freetown layered basic complex of the Sierra Leone Peninsula suggest that the mass is shaped like an inverted cone or saucer of 30 km radius and that the western half is beneath the Atlantic Ocean[1] This hypothesis was tested with the echo sounder and magnetometer traverses with the R V. *Trident* of the University of Rhode Island on May 16, 1963 (Figs 1 and 2) The dense complex is characterized by four repeated sequences of troctolitic, gabbroic and anorthositic rocks considered to be igneous intrusives in which there is a generalized upward sequence from olivine-rich to plagioclase-rich rocks, but in which there is no progressive variation in the composition of the minerals The floor is concordant to the layering and dips toward the centre at 20°–30°, where examined[1] Baker and Bott[2] found that the eastern margin of the basic complex is marked by a very intense, narrow, negative anomaly of up to 2,000 gammas

Both echo sounder profiles show a submarine channel (points *C*, *F*) where the magnetic anomalies begin and show rough bottom over the zone of the anomalies The channel of profile 1 is the Pleistocene channel marking the sub-aerial course of the Sierra Leone River during the glacial lowering of sea-level based on soundings of U S N H O Chart 2344 The other channel of similar origin trends toward Sherbro River and Yawri Bay The irregular bottom over the anomalies must mark the outcrop of the basic complex or the rock overlying that body The channels were incised along the contact between the basic body or its overlying rock, and the bordering softer rock

In order to interpret the intense anomaly of profile 2, theoretical anomalies were calculated for a series of horizontal plates the thicknesses of which equalled the depth to their upper surface The plates calculated were for depths of $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4 and 8 km These calculated anomalies were used to compare a vertically sided body and a stepped, wedge-shaped body The principle of superposition was used wherein the calculated anomalies of the plates were added to one another after the relative position of plates was determined The magnetic field due to the body is assumed to be induced by the present Earth's field which here dips $2\frac{1}{2}$° N towards N 17° W The calculations are based on the method of Gulatee[3]

The vertically sided body did not fit the situation because of the high narrow negative anomaly at the contact The wedge-shaped body gives an anomaly that is much closer to that of the observed feature The interpretation indicates that the floor of the magnetic body dips 40° or less to the south along profile 1 and that the magnetized body does not extend below 4 km depth The body may be overlain by at least 100 m of relatively non-magnetic rock. The apparent magnetic susceptibility required of the rock for such an anomaly is 0 02 if all the magnetism is induced This is far too large for a natural feature Probably one half of the anomaly is due to a remnant field imposed when the igneous body cooled The assumption of direction of the magnetism in the body appears to be justified

The anomaly of the second crossing is not so great as that of the first crossing though still large A partial explanation of this probably is that the second crossing is almost at right angles to the Earth's field which should yield, theoretically, a much reduced and differently shaped anomaly The large size of this anomaly indicates structural and perhaps chemical variations within the body

An upper contact of the Freetown complex as indicated by the magnetic anomalies (points *B*, *E*) falls on circle *M*1 the centre of which is point *M* This is the same centre as derived by Wells[1] for the arcuate gravity anomalies of the complex However, circle *M*2 about this centre only poorly outlines the outcrop of the complex If circle *N*1 is drawn about a new centre *N*, a better agreement is attained for the outer eastern margin of the outcropping body as indicated by the gravity anomaly The latter circle also passes close to the submarine channels (points *C*, *F*) Points *C* and *F* are also near the actual edge of the smaller magnetic anomalies

On the basis of the foregoing observations, the Freetown layered basic complex seems to consist of two parts (1) an outcropping rock best defined by circle *N*1 with relatively local magnetic anomalies compatible with the type of rock exposed on land, (2) a highly magnetic rock defined by the intense magnetic anomalies

The intense magnetic anomalies of zones *A–B* and *A–E* of the profiles are caused by rocks which seem to have no outcropping counterparts on the Peninsula The correspondence of loci of the intense magnetic and the gravity anomalies suggest that a very large, saucer-shaped,

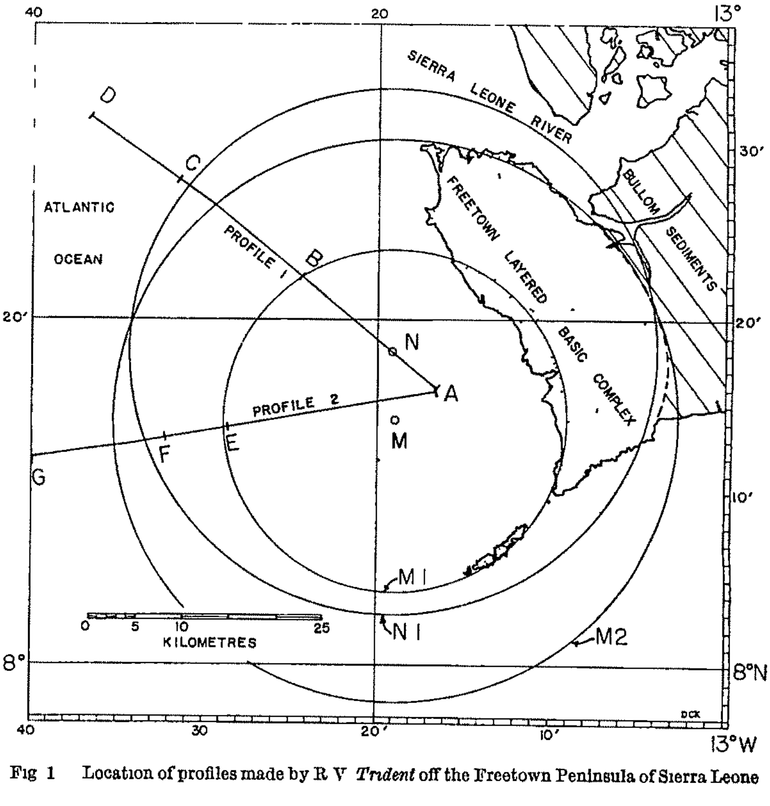

Fig 1 Location of profiles made by R V *Trident* off the Freetown Peninsula of Sierra Leone

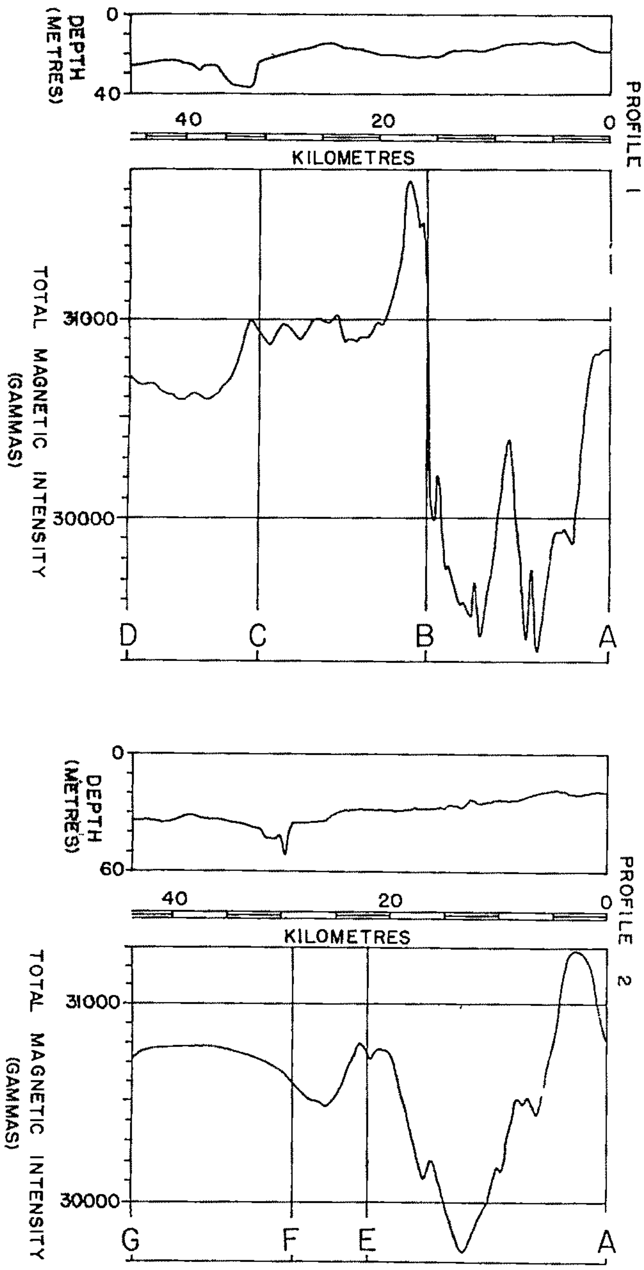

Fig. 2 Echo soundings and magnetometer profiles The vertical exaggeration of the sounding profiles is roughly 333 times The magnetic profiles consist both of the local magnetic anomalies due to nearby rock and of the regional magnetic field due to the Earth's core

magnetic body of rock lies unexposed and central below point *M*

The geometry of the central magnetic body to the layered basic complex of the Peninsula must be one of the following (1) the magnetic rock overlies the basic layers which are truly basal, (2) the magnetic rock is sandwiched between basic layers above and below, (3) the magnetic rock underlies the basic layers, (4) the outcropping basic layers interfinger at depth with the magnetic rock. The magnetic interpretation favours cases one or two because of the apparent 4-km limitation to the base of the body

The lack of correspondence between points *M* and *N* and circles *M*1 and *N*1 suggests that the body is asymmetrical with the southern basal contact rather steeper than the northern basal contact although the body is still relatively saucer-shaped

Identification of meteorite crates by shatter cones[4] and by coesite[5] recently has shown that these are widespread features All circular, enigmatic geological features must now be examined in light of this new evidence and such as the Freetown layered basic complex

As an alternative to Wells's strictly igneous intrusion, I therefore propose that the Freetown basic complex is meteoric in origin, in regard to the location and/or the parent rock If the complex is assumed to be 30 km in radius and cone-shaped with an average dip of 25°, then the apex of the cone is 14 km below sea-level, almost half of the expected crustal thickness The body is so large that the meteorite must have been exceedingly large The asymmetry of the rock body with its more steeply dipping southern wall indicates that the supposed asteroid was moving in a north–south line. A sequence of events is envisaged much like that described by Dietz[6-8] for the Vredefort dome and the Sudbury nickel deposit The forces involved in this impact would be very large and probably disturbed the Earth's crust clear to the mantle Two alternatives exist as to the origin of the basic rock itself under this hypothesis

(1) The crust was fractured and a portion of the mantle was melted The basic rock then intruded the crust and was extruded into the crater as a molten pool which afterwards differentiated and solidified in the manner described by Wells The feature would be funnel-shaped

(2) The basic rock is the asteroid itself which fused on impact The asteroid would have been roughly 9 km in radius and 'stony' in character The mass lay in its crater and slowly differentiated, giving a saucer-shaped body

The latter alternative appears to be of low probability on the basis of the composition of meteorites[9] and on the probable dynamics of meteoritic impact of this size[6] The former alternative is therefore preferred Tests can be made to confirm this hypothesis

I thank the scientists and crew aboard the R V *Trident* for their assistance This work was largely supported by grants from the U S Navy Office of Naval Research

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KINETICS OF GRAPHITIZATION OF A PETROLEUM COKE

By Dr. D. B. FISCHBACH

Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California

GRAPHITE has a hexagonal crystal structure with an ordered *ABABAB* basal plane stacking sequence and a unit cell height, *c*, of 6 708 Å. Pregraphitic synthetic carbons have a similar structure, except that the stacking sequence is randomly disordered (turbostratic structure) with *c* values of 6 85–6 90 Å When such turbostratic carbons are heated to high temperatures graphitization occurs, the layer-stacking evolving toward the ordered sequence of graphite with a consequent decrease in c. Crystallite growth in both the basal plane and c-axis directions also occurs.

The process by which graphitization occurs is complex and is not well understood despite its fundamental importance to the science and technology of carbon and graphite. Information on the kinetics of the process should be very helpful in determining the detailed mechanism. However, until recently, there has been a notable lack of information on the subject. Kinetic studies by Fair and Collins[1] on a conventional composite pitch-coke carbon, and by Mazza, Marchand and Pacault[2] on a coked pitch indicated that the rate of graphitization decreased rapidly with increasing heat treatment time and the degree of graphitization appeared to approach a limiting value at any given heat treatment temperature which increased with heat treatment temperature. However, in general, a zero rate of change was not attained in the total treatment times used, and Mizushima[3] found that the electrical and thermal conductivity changed approximately linearly with the logarithm of treatment time. A recent investigation of pyrolytic carbons[4] has shown that in these materials graphitization occurs by a sequence of two simple first-order rate stages. These results suggested that a further investigation of the kinetic behaviour of conventional carbons would be worth while.

The material chosen for investigation was a petroleum coke which had been baked approximately 15 min at 2,000° C. It had a turbostatic structure with a unit cell height, c, ~ 6.86 Å and a crystallite diameter, L_a, of ~150 Å. Additional information on this coke has been published elsewhere[5]. The progress of the graphitization was followed by X-ray diffraction measurements at room temperature as a function of isothermal heat treatment time. The c value was determined from the position of the (004) diffraction peak using the Debye–Scherrer technique with copper $K\alpha$-radiation. Powder samples were treated in graphite crucibles in a graphite tube resistance furnace at 2,300, 2,500 and 2,700° C in helium at one atmosphere.

The results are shown in Fig. 1 (solid lines) along with curves for a pyrolytic carbon treated at 2,500 and 2,700° C (broken lines). There is a qualitative similarity between the results for the two materials. However, it is evident that the initial rate of graphitization of the coke is much faster than that of the pyrolytic carbon. Furthermore, the kinetic behaviour of the coke cannot conveniently be described by a simple sequence of resolvable first-order stages. The kinetics of the coke can be represented instead by a superposition of many first-order processes with a broad distribution of rate constants. The distribution is so broad that at any one temperature only a portion of the total graphitization process is observed in the time range utilized here. This makes it difficult to determine even an average or effective rate constant at each temperature.

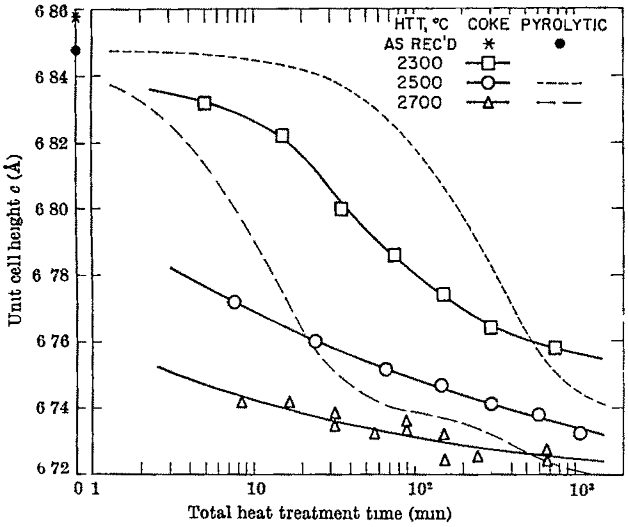

Fig. 1. The unit cell height, c, as a function of heat treatment time at various heat treatment temperatures (HTT) for a petroleum coke (solid lines) and a pyrolytic carbon (broken lines)

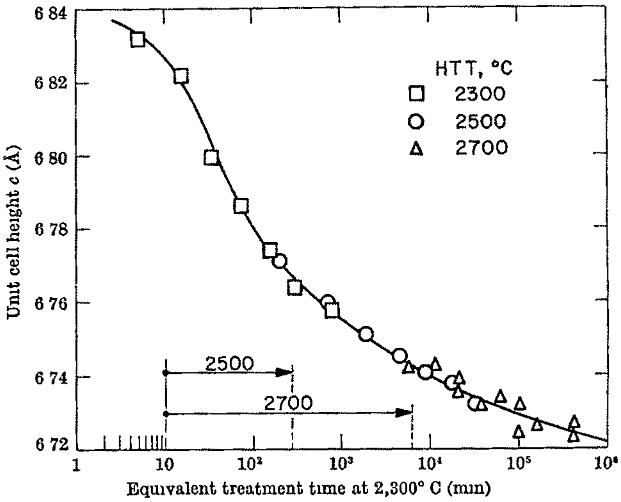

Fig. 2. Composite curve for petroleum coke obtained by shifting the 2,500 and 2,700° C curves to join smoothly with the 2,300° C curve from Fig. 1. The arrows indicate the amount the higher temperature curves were translated along the abscissa

An approach which is commonly used in the analysis of relaxation phenomena is useful here. An effective activation energy can be computed from the amount a kinetic curve corresponding to temperature T_1 must be translated along the time axis to superimpose smoothly on a portion of the curve corresponding to another temperature T_2. It was found that the curves in Fig. 1 could be superimposed in this manner. Fig. 2 shows the composite curve obtained, referred to a temperature of 2,300° C. The arrows show the amount that the 2,500 and 2,700° C curves were displaced. This composite curve may be interpreted as representing the behaviour that would have been observed at 2,300° C if treatment times could have been extended to ~ 10^6 min (about 2 years). This suggests that there is in fact no temperature dependent limit to the degree of graphitization which can be realized at a given heat treatment temperature if the treatment time is long enough.

The value of the effective activation energy obtained from the foregoing analysis is ~ 250 kcal/mole, in excellent agreement with the ~ 260 kcal/mole value observed for pyrolytic carbons. These values are nearly identical to the theoretical value for vacancy diffusion calculated by Dienes[6], as corrected by Kanter[7] (263 kcal/mole), but this agreement is probably fortuitous. On the assumption that graphitization is governed by a broad distribution of activation energies, Mizushima[3] found apparent energy values which increased from ~ 100 kcal/mole at 1,400° C to ~ 200 kcal/mole at 2,200° C, and Fair and Collins[1] deduced somewhat higher values as a function of the degree of graphitization. On the other hand, energy values of 170 kcal/mole for the heat of sublimation[8], 163 kcal/mole for self-diffusion[7] and 90 kcal/mole for crystallite growth[9] have been reported. Evidently, further work is required to establish the nature of the thermally activated processes in carbon and graphite.

The results of the work recorded here indicate that it may be more appropriate to analyse the graphitization process in terms of a distribution of pre-exponential ('frequency') factors than in terms of a distribution of activation energies. The identity of the observed activation energies for pyrolytic carbons and for coke indicates that the thermally activated process is the same for both types of carbon and the activation energy is constant, at least over the range 2,300°–3,000° C. The broad distribution of rate constants in the coke and the different rates in different pyrolytic carbons[4] must then result from large differences in the pre-exponential factor in the rate equation While it is premature to explore this possibility in any detail, such a situation could arise if different numbers of thermally activated steps (for example, diffusion jumps) are required to produce the observed property change in different microscopic environments in the carbons Such differences are presumably associated with differences in the detailed microstructure of the graphitizing carbons

In summary, the graphitization kinetics of a petroleum coke at temperatures in the range 2,300°–2,700° C can be represented by a superposition of first-order rate processes with a broad distribution of rate constants There does not appear to be any temperature-dependent limit to the degree of graphitization attainable at a given heat treatment temperature An effective activation energy of $\sim$ 250 kcal/mole was found, in excellent agreement with the value observed for pyrolytic carbons This indicates that the graphitization process is the same in the two types of carbon and the activation energy is constant The different kinetic behaviour of different carbons is attributed to a broad range of pre-exponential factors which is probably related to the detailed microstructure of the material

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MEASUREMENT OF LIQUID SCINTILLATION COUNTING EFFICIENCIES

By HORACE E. DOBBS

U K Atomic Energy Research Establishment, Wantage, Berkshire

LIQUID scintillation techniques for measuring weak β-emitting isotopes are widely used The dissolution or suspension of the substances to be counted in the liquid phosphor often result in reduction of counting efficiency. Such substances, known as quenching agents, can be divided into two broad categories Colourless substances that lower the efficiency of the energy-transfer processes prior to photon emission by the phosphor are known as primary quenching agents Coloured substances that absorb the photons produced by the phosphor are known as secondary quenching agents Some substances exhibit both primary and secondary quenching properties

As most substances exhibit some degree of quenching it is necessary to determine the efficiency of the phosphor solution counted if quantitative radioactivity measurements are required Several methods of achieving this have been described

The most widely used method, known as the internal standard method, is to add a known weight, or volume, of a non-quenching substance of known counting rate[1–4] This method is subject to the personal error of measuring the aliquot of standard The sample is no longer in its original form after 'spiking', and recounting of the original specimen is therefore precluded

A method that overcomes these difficulties, described by Baillie[5] and expanded by Bush[6], utilizes the change in pulse height distribution brought about by the addition of quenching agents, and involves measuring the count rates in two different channels The disadvantage of this method is that when the amount of activity present is small, the time required for the counts to reach a level of sufficient statistical accuracy may be considerable

A method described by Higashimura *et al* [7] utilizes an external γ-source This method relies on the production of electrons within the liquid phosphor by Compton interactions It is therefore subject to the selection of a suitable γ-source and is open to error due to changes in electron density of the counting sample

A method advocated by Peng involves measuring the count rate at different quencher concentrations and extrapolating to zero concentration[8–10] This requires a number of samples to be counted and is valid only if the quenching is a simple function of the sample concentration

If the concentration and composition of the quenching agent are accurately known, the counting efficiency can be determined by reference to prepared quenching curves[11]

Several authors[12–14] have used the extinction coefficient of the solution at 400 mμ (near the optimum sensitivity of the photocathode) to measure the degree of quenching from coloured solutions This method has limited application, and is unsuitable when primary quenching agents are present

A method is described here which has the advantages of the internal standard method, but eliminates the two disadvantages of the method—in that the sample is in its original form after the measurement, and the personal error is eliminated as the amount of activity added for each determination does not vary In this method, a glass rod carrying a fused coating of a suitable long-lived isotope is inserted into the liquid scintillator, and the resulting rise in counting rate is used to measure the counting efficiency of the solution

An 'efficiency stick' was constructed from a piece of glass rod mounted with 'Araldite' into a phial cap (Fig 1) The end of the glass rod was heated to dull red heat and immediately rolled in carbon-14 labelled barium carbonate powder (specific activity 80 μc /mg), located in a channel in a carbon block The glass rod was afterwards reheated to a dull red colour for a short period to allow the barium carbonate to fuse into the glass When cool the

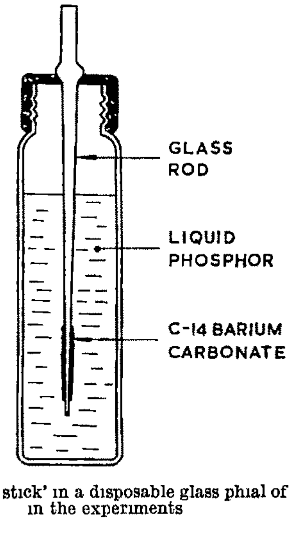

Fig 1. The 'efficiency stick' in a disposable glass phial of the type used in the experiments

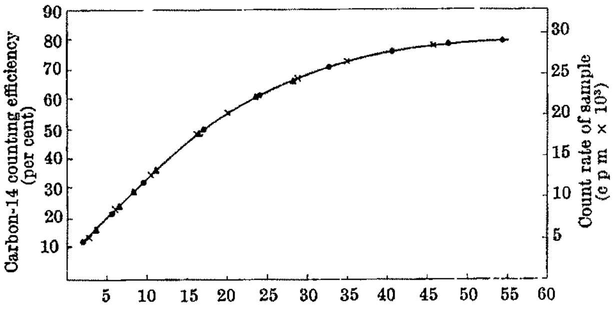

Fig 2 A calibration curve showing the variation of count rate of the stick with the counting efficiency for carbon-14 in a liquid phosphor ×, Primary quenching agent (quinoline) in standard toluene phosphor, ●, secondary quenching agent (scarlet *R*) in standard toluene phosphor, ▲, secondary quenching agent in liquid scintillator composed of toluene, methyl alcohol, 2-phenylethylamine, naphthalene, POPOP and PPO

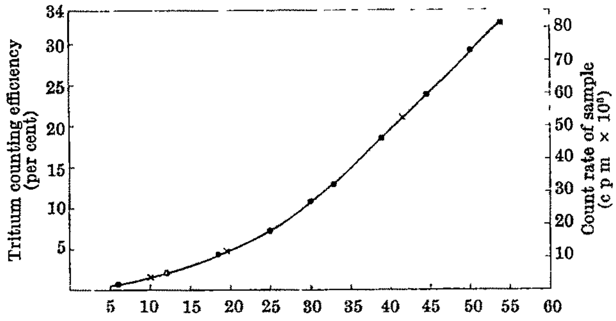

Fig 3 A calibration curve, indicating the variation of the count rate of the same stick, for different tritium counting efficiencies in a toluene-based phosphor ×, Primary quenching agent (quinoline), ●, secondary quenching agent (scarlet *R*)

stick was washed in toluene and wiped vigorously with a paper tissue to remove any loosely bound activity

Experiments were conducted in a co-incidence system[15] in which 9 0 ml counting samples were placed in disposable glass phials The discriminators were set such that all pulses greater than 5 V were recorded A suitable working voltage for the photomultipliers was selected by plotting the counting rate of the phosphors against high-voltage supply both with, and without, the efficiency stick It was necessary to leave the stick in the solution for several minutes before counting to allow the count rate to reach a constant level

The calibration curve for the efficiency stick was prepared as follows 0 1 ml of carbon-14 labelled toluene of known count rate was added to 9 0 ml of liquid phosphor The composition of the phosphor was 5 g PPO (2,5-diphenyl oxazole) and 0 05 g POPOP (1,4-*bis*(2-[5-phenyloxazole]) benzene) per litre of toluene Successive quantities of a primary quenching agent (quinoline) were added, and the count rate measured before and after insertion of the stick for each addition The count rate of the solution (which is directly proportional to the counting efficiency) was plotted against the additional count rate produced by inserting the stick The results are shown in Fig 2

The procedure was repeated with a secondary quenching agent (a solution of scarlet *R* in toluene) in phosphor of the same composition A liquid phosphor of different composition, composed of equal parts of toluene, methyl alcohol, and 2-phenylethylamine, containing 5 g/l of PPO, 0 05 g/l POPOP, and 100 g/l naphthalene[16] was also investigated using scarlet *R* as a quenching agent The results are shown in Fig 2

It can be seen from Fig 2 that the results from all three experiments fall on the same curve The additional counts produced by inserting the efficiency stick can therefore be used to measure the counting efficiency of a liquid scintillator containing an unknown quantity of a primary and/or secondary quenching agent

The same efficiency stick has been calibrated for measuring the efficiency of a phosphor (toluene containing 5 g/l PPO and 0 05 g/l POPOP) containing tritium activity The results (Fig 3) indicate that both primary and secondary quenching agents give points that lie on one curve

The stick is stored in liquid scintillator solution and has shown no detectable loss of activity over a period of 3 months It has frequently been used without ill effect to measure the efficiency of a phosphor containing a strong base[16] used to absorb carbon dioxide produced by the combustion of carbon-14 labelled compounds

The use of the method described has the advantage that results can easily be cross-checked by recounting the solution after the introduction of another efficiency stick containing more, or less, activity.

Caution should obviously be exercised when using the stick to measure the efficiency of acid solutions, as there is a possibility that some of the activity on the stick would be liberated as carbon dioxide The method does depend on the day-to-day stability of the counting equipment This can easily be checked by counting a sealed phial containing a standard solution, or by counting the stick in unquenched phosphor of known efficiency before use

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A NEW CONFORMATION OF METHACRYLIC ACID RADICALS IN SOLID-STATE POLYMERIZATION

By PROF. C. H. BAMFORD, DR. G. C EASTMOND and Y SAKAI
Department of Inorganic, Physical and Industrial Chemistry, University of Liverpool

WE have previously shown[1,2] that the solid-state polymerization of methacrylic acid initiated by ultra-violet radiation proceeds through radical intermediates and that during the reaction radicals become trapped in the matrix Further, it was shown that radical formation appears to have an unusually large temperature coefficient for a photochemical process In order to obtain more information on this point we have investigated the polymerization at temperatures below 0° C in more detail, using electron spin resonance spectroscopy to examine the radicals The results described here showed an unexpected variation of the radical concentration with temperature of irradiation and also revealed a new electron spin resonance spectrum of methacrylate-type radicals, containing thirteen lines instead of the usual nine

Methacrylic acid was purified as described previously[2] and the polycrystalline specimens, formed by freezing at 77° K in vitreosil electron spin resonance sample tubes, were irradiated for various periods by light from a high-pressure mercury arc at predetermined temperatures in the range +10 to −40° C These temperatures were maintained by passing cold nitrogen around the sample tubes[2] A Varian *V* 4500 spectrometer was used for recording the electron spin resonance spectra with the specimens maintained at 77° K

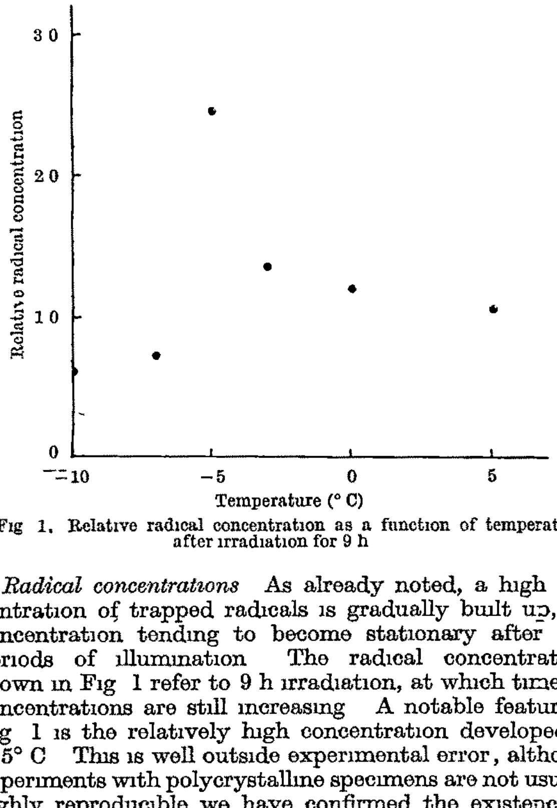


Fig 1. Relative radical concentration as a function of temperature after irradiation for 9 h

Radical concentrations As already noted, a high concentration of trapped radicals is gradually built up, the concentration tending to become stationary after long periods of illumination The radical concentrations shown in Fig 1 refer to 9 h irradiation, at which time the concentrations are still increasing A notable feature of Fig 1 is the relatively high concentration developed at −5° C This is well outside experimental error, although experiments with polycrystalline specimens are not usually highly reproducible we have confirmed the existence of this effect many times Initiation is probably controlled by the freedom of movement of the monomer molecul[illegible] in the lattice, radical formation may require int[illegible] between an excited and a normal molecule [illegible] event, it must be accompanied by conf[illegible] At −5° C there may be a trans[illegible] that at this temperature the [illegible] enhanced, together with [illegible]

Table 1

No	Temperature of irradiation (° C)	Time of irradiation (h)	$\frac{x}{y}$	Time of annealing (min)	$\frac{x}{y}$ after annealing
1	−30	9	0 35		
2	−20	9	0 25		
3	−5	3	0 11		
4	−5	18	0 04		
5	−5	40	0		
6	−10	3	0 28		
7	−10	6	0 20	20	0 13
8	−10	40	0 05		
9	−10	6	0 11	40	0 033
10	−10	6	0 11	60	0 026

comparison with Fig 2 The ratio x/y is used as a measure of the importance of the splitting It will be seen that the splitting becomes less noticeable as the period of irradiation at a given temperature is increased (Exps 3–5, 6–8)

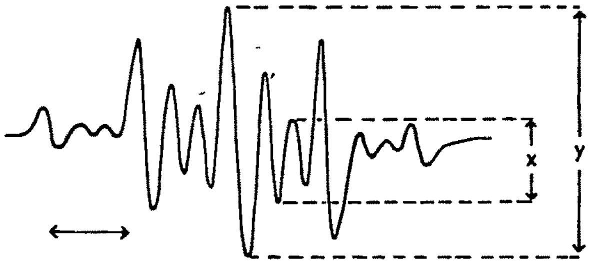
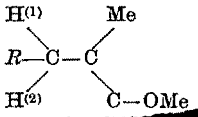


Fig 2 Electron spin resonance spectrum of radicals formed in crystalline methacrylic acid at −30° C Scale=20 gauss

Annealing experiments were also carried out in which specimens irradiated at lower temperatures were maintained at 0° C The electron spin resonance spectra obtained from such specimens show a reversion to the conventional 9-line spectrum (Exps 7, 9, 10)

We believe that the observations can be discussed most profitably in terms of radical conformation, and indicate that below −5° C radicals are formed photochemically and have a conformation different from that of the familiar methacrylate radical (obtained, for example, by trapping during the polymerization of methyl methacrylate at room temperatures and above) Symons[3,4] has recently discussed the evidence that the 9-line spectrum of this radical arises from a single radical species I, and an extension of his arguments is capable of accounting for our 13-line spectrum



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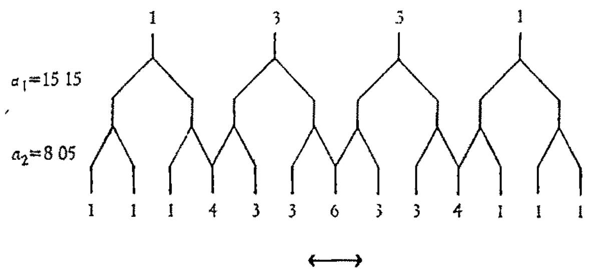

Fig 3 Origin of 13-line spectrum and relative intensities of lines Scale = 10 gauss

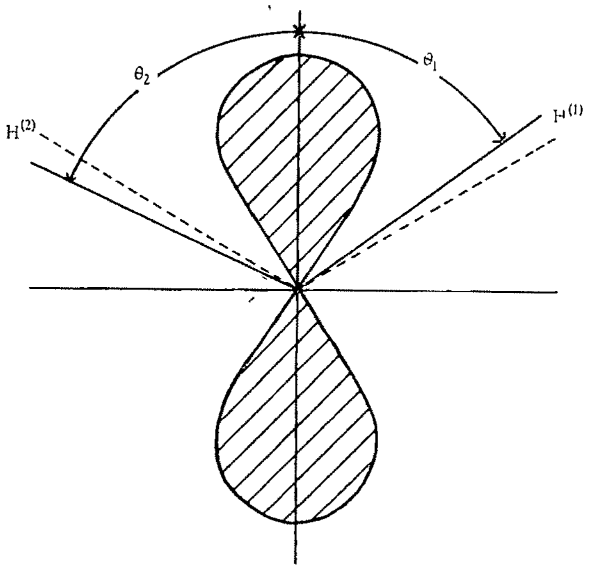

Fig 4 Positions of methylene hydrogen atoms in methacrylic acid radical, looking along C—C bond ---, Symmetrical conformation, $\theta_1 = \theta_2 = 60°$, ——, new conformation, $\theta_1 = 55°$, $\theta_2 = 65°$

Experimentally we find that $\Delta = 7\ 1$ gauss, so that $a_1 = 15\ 15$, $a_2 = 8\ 05$ gauss The manner in which the 13-line spectrum arises with these parameters is shown in Fig 3, from which it appears that the relative intensities are 1, 1, 1, 4, 3, 3, 6, 3, 3, 4, 1, 1, 1

If we assume that the non-equivalence of the methylene hydrogen atoms arises from displacements from the symmetrical conformation $\theta_1 = \theta_2 = 60°$ (Fig 4) we may calculate θ_1, θ_2 by the use of Symons's relation[4]

$$a = 46 \cos^2 \theta \tag{2}$$

It turns out that $\theta_1 = 55°$, $\theta_2 = 65°$ There is, therefore, no distortion of the $H^{(1)}CH^{(2)}$ angle, but the whole group is rotated about the C—C bond through 5° (Fig 4)

We believe that, during the solid-state polymerization below −5° C, geometrical constraints within the crystal force the radicals (or some of them) to adopt this conformation, which is maintained at these temperatures on account of the reduced mobility below the 'transition point'

On warming to 0° C, however, relaxation occurs and the usual conformations appear If irradiation is prolonged, extensive disorganization of the crystals is caused by the polymerization, with resultant weakening of the constraints responsible for the new conformation Over most of the narrow range of irradiation temperatures −5° to −30° C the spectrum corresponds to a mixture of the conventional and new conformations, with the proportion of the latter increasing as the temperature falls After irradiation at −30° C the observed relative intensities are not very different from those predicted (1, 1, 1, 4, 3, 3, 6, 3, 3, 4, 1, 1, 1) so that under these conditions substantially all the radicals have the new conformation So far as we are aware this is the most definite evidence that a methacrylic (or methacrylate) radical spectrum can be related to a single conformation without the need for special assumptions

The conformational hypothesis is not the only one capable of accounting for the difference between a_1 and a_2 Even in the symmetrical conformation the methylene hydrogen atoms may not be equivalent since their environments differ[4] However, since methacrylic acid and methyl methacrylate radicals normally give similar electron spin resonance spectra we do not think this alternative is very likely

The results described do not seem capable of interpretation in terms of the formation of a chemically different radical species at the lower temperatures

We thank Dr L H Sutcliffe for discussion and Dr A Bullock for help in recording the electron spin resonance spectra

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[illegible] TINUOUS DETERMINATION OF ANÆSTHETIC [illegible] TENTIAL *IN VIVO*

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[illegible] compartments as fatty tissue are [illegible] be considered divided into equilibrium with the compartment entered. A number of physical properties of materials vary quantitatively with the concentration of an introduced volatile substance, but it is thought that the easiest quantity to measure with a small probe is the rate of permeation of the substance itself through a thin plastic membrane.

The permeation of plastic membranes by organic vapours has been investigated quantitatively and theoretically[6,7]. For polymers which are either in their rubber-like state or, for other reasons, do not have high viscosities, the process is describable in terms of solution at one surface, diffusion through the material according to Fick's law, and evaporation from the other surface.

For simple gases and substances chemically different from the membrane material, the rate of permeation is strictly proportional to the difference in chemical potentials across the membrane. Organic vapours permeate many times faster than simple gases, the highest figures being given by substances chemically similar to the membrane material and showing the greatest tendency to dissolve it. The rate of permeation of these solvent-like substances increases disproportionately fast with rising chemical potential, probably owing to swelling of the polymer by the agent. At the low chemical potentials used in anæsthesia (in the range 0.02–0.05) the curvature is not great and may be accurately described by an equation of the form

$$R = \frac{PA\mu p_s e^{\alpha\mu}}{d}$$

where R = rate of transfer (ml at S.T.P./sec) across a membrane of area A (cm²) and thickness d (cm) exposed on one side to an anæsthetic at chemical potential μ, the concentration on the other side being negligible. P is the permeability coefficient (ml at S.T.P. cm/cm²/sec/mm mercury) at $\mu \to 0$, p_s is the saturation partial pressure of the anæsthetic (mm mercury) and α is a constant which must be determined for each combination of anæsthetic and membrane material.

The permeability coefficient P typically shows a large increase with temperature defined by the equation

$$P = P_0 e^{-E_p/RT}$$

where P_0 is the value of P at infinite temperature, T is the absolute temperature, R the gas constant, and E_p the activation energy of permeation, which proves close to the activation energy of solution of the membrane material. Changes in P of 5 per cent per deg. C are not uncommon when E_p is high and may need correction even within the narrow range of temperatures encountered in normal anæsthesia.

The rate of permeation through the test membrane can be determined conveniently by measuring concentration in a constant flow of gas passing over its free surface. In tissue with poor circulation it would be necessary to keep the rate of removal of anæsthetic to a minimum. In order to estimate the permissible withdrawal, assume the probe to be in tissue with a long half-equilibration time of 50 min, let the equilibrium anæsthetic concentration be 1 mg/ml, and assume withdrawal at equilibrium of 10 ng/min. This would give a detector concentration of 10^{-9} g/ml at 10 ml/min gas flow. If the probe is in equilibrium with 0.1 ml of tissue, then at half saturation of this compartment anæsthetic will be entering at an instantaneous rate of 0.7 μg/min. The rate of withdrawal will depend on the instantaneous chemical potential and at this moment would be 5 ng/min. The value under observation would be lowered less than 1 per cent and this would be true at all stages of equilibration. For measurements in tissue with good circulation and in blood and cerebrospinal fluid the permissible withdrawal would be several orders of magnitude higher.

The argon gas chromatograph detector[10] will operate at 10^{-12} g/ml of organic vapour, and measurements at 10^{-10} g/ml are within the quantitative range. It can now be constructed with a volume of 0.3 ml or less. Unfortunately, many of the heavily halogenated compounds used in anæsthesia will not produce a positive response in the argon detector, though a modification of this to detect them has been suggested[11]. They can, however, be measured at about the same level of sensitivity by the electron capture detector[12], and it might be possible to design a detector cell which could be used on either principle, the essential practical difference being the voltage applied to the electrodes and the sensitivity of the arrangements for current measurement.

Possible interfering substances which may also cross the membrane include oxygen, nitrogen, carbon dioxide and water vapour. Lovelock[13] has shown that the sensitivity of the argon detector is reduced only at concentrations higher than 3×10^{-4} (by volume) of water vapour and 1×10^{-4} of carbon dioxide. The saturated vapour pressure of water at 37° C is 47 mm, the physiological partial pressure of carbon dioxide is about 38 mm and the effective partial pressure of a typical anæsthetic is only 4 mm. Taking permeabilities for the interferants from Table 1, assuming a permeability to anæsthetic equal to that for water and a hypothetical molecular weight of 100, then with a membrane thin or large enough to give the limiting concentration for water, carbon dioxide will be only at 1/10 of its interference-level and the detector can receive 1×10^{-7} g/ml of anæsthetic, which is 10^3 times higher than the lowest quantitative range. Using a polythene membrane 0.001 in. thick and a detector gas flow of 10 ml/min, a probe area of about 3 cm² would be needed to achieve this concentration. With a detector sensitivity of 10^{-9} g/ml for full-scale deflexion this could be reduced to 0.03 cm² and very small probes should be possible.

Table 1. PERMEABILITY COEFFICIENTS AT 30° C FOR LOW-DENSITY POLYTHENE
(ml at S.T.P. cm/cm²/sec/mm mercury)

Permeant	$P \times 10^{11}$	Ref
Oxygen	5.5	8
Nitrogen	1.9	8
Carbon dioxide	35	8
Water (liquid or vapour)	100	9
Methyl bromide (μ=0.12)	99	7
n-Hexane (μ=0.23)	180	7

The ideal membrane should have the highest possible permeability to organic vapours relative to water and should therefore be non-polar. Low-density polythene was used in an experimental probe, but it is possible that polyvinylidene chloride (Saran) or PTFE might have even more favourable properties.

A simple probe was constructed by fastening polythene film 0.001 in. thick over the end of two concentric glass

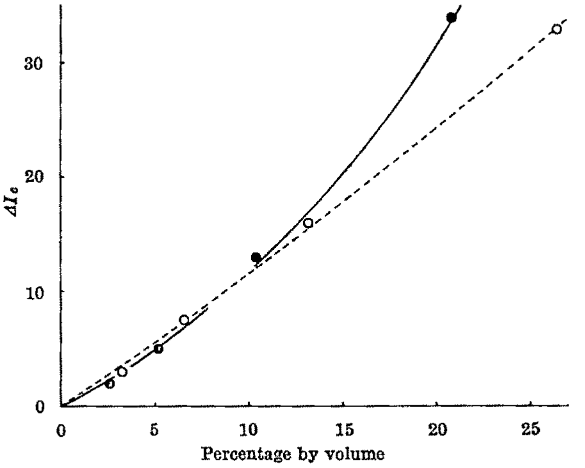

Fig. 1. Arbitrary units of increase in detector cell current plotted against percentage by volume of anæsthetic in air (calculated at S.T.P.). ●, Ether; ○, ethyl chloride

tubes, the inner fractionally shorter than the outer The free membrane area was 0 55 cm^2 Argon was passed into the outer tube at 10 ml /min and led from the inner through glass tubing and an empty column at 50° C to the detector of a Pye argon chromatograph, type 12001 (W G Pye and Co , Ltd , Cambridge), used at 2,000 volts and maximum sensitivity This method of connexion entailed a very large dead volume, but was necessary for reasons of safety as the instrument has a strontium-90 rather than a tritium source

The probe was immersed in water at room temperature in a small flask through which bubbled a stream of air at 2 l /min The air stream came from an anæsthetic vaporizer of the type described by Burn, Epstein and Goodford[14] and passed through a large mixing chamber (5 l) When a baseline was established, known concentrations of anæsthetic were added to the stream The increase in cell current was determined at equilibrium after each stepwise increase in concentration Only anæsthetics suitable for the argon detector could be used Fig 1 shows scale units of current rise plotted against the composition of the known mixtures It was confirmed that replacement of the air stream by oxygen with 5 per cent carbon dioxide had no detectable effect on the reading The points for 6 6 and 13 per cent by volume of ethyl chloride were repeated with citrated human blood in place of water, and it was confirmed that readings agreed with those in water to an accuracy within the noise-level of the recording instrument (average one scale unit) Foaming of the blood was checked by use of Midland Silicones 'Anti-foam *A*'

It is concluded that continuous measurement of anæsthetic potential in blood or cerebrospinal fluid by a membrane probe is a practicable proposition and that it should be possible to develop the instrument to a sensitivity high enough to make measurements in tissue with poor circulation

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ADDITIVE EFFECT OF SUB-THRESHOLD CONCENTRATIONS OF SOME ORGANIC COMPOUNDS ASSOCIATED WITH FOOD AROMAS

By D. G GUADAGNI, RON G. BUTTERY, S. OKANO and H. K BURR

Western Regional Research Laboratory, U S Department of Agriculture,
Albany 10, California

THE marked advance of gas chromatographic techniques in recent years has revealed the presence of large numbers of organic volatile compounds in fruits and vegetables[1]. Since many of these volatiles are found in extremely small quantities, the organoleptic significance of mixtures of trace amounts becomes of interest

Taste and olfactory thresholds of some organic compounds associated with food flavours have been reported[2,3], and the possible additivity of sub-threshold concentrations of mixtures of organic compounds have recently appeared in the literature[4,5] The purpose of these experiments was to determine which of the following possibilities is characteristic of the odour properties of mixtures (1) The mixtures exhibit an independent property, in which case no odour would be perceived unless at least one compound in the mixture was at or above its threshold (2) They counteract one another, in which case a mixture of the threshold concentrations of two compounds would no longer give an odour sensation (3) They exhibit an additive effect, in which case half the threshold concentration of each of two compounds mixed together would give a perceptible odour (4) They exhibit a synergistic effect or enhancement in excess of addition

Purity and thresholds of individual compounds and mixtures of compounds were determined by methods that will be published elsewhere[6] Very briefly, compounds were purified by gas liquid chromatographic separation and thresholds were determined as follows The panel of judges consisted of 20–25 persons who consistently demonstrated sensitivity and reproducibility in perception of odour There were 16 males and 9 females selected from a group of more than 60 laboratory employees Odour measurements were made in a room maintained at 22° C and equipped with individual booths which were constantly flushed with fresh air which had been scrubbed through activated charcoal filters The rate of air movement was such that the room was under slight positive pressure to prevent entrance of odours through booth openings and doors Pure compounds were dissolved in triple-distilled odour-free water at 22° C just before the panel convened Thresholds were determined by evaluating two or more series of 3–6 concentrations each Each concentration was paired with samples of triple distilled odour-free water, and the entire panel was asked to check the samples that contained the odorous material Position of the water sample was randomized in each pair, and order of presentation of the different concentrations was also randomized. The solutions (4 fl oz) were presented in boiled odour-free 8-oz 'Teflon' bottles Sufficient time (2–3 min) was allowed for equilibrium to be established between solution and vapour The judges gently squeezed the bottles to force the vapour above the solution directly into the nasal cavity The percentage of correct responses was plotted against concentration The threshold is arbitrarily defined as the concentration at which a highly significant ($P = 0\ 01$) percentage of the judges detected the aroma

The threshold of a mixture was usually determined by mixing the compounds in a 1 1 ratio according to their individual thresholds The mixtures were judged in concentrations above and below their expected thresholds, usually at levels of 50–300 per cent of the expected threshold. assuming condition 3 (additive effect) to be operative With a mixture of 3 compounds, for example, each compound was present at 16 66 per cent of its threshold for the low concentration (50 per cent) and at 100 per cent of its threshold for the high concentration (300 per cent) The theoretical threshold of a mixture was calculated from the individual threshold values of the compounds of the mixture, assuming an additive effect The percentage of the theoretical threshold actually found in the mixture was then calculated, and the percentage or fraction of this value contributed by each compound was also calculated Thresholds of the purified individual compounds were verified both before and after threshold determinations of the various mixtures

For the initial experiment on effect of sub-threshold concentrations, 3-methylbutanal and heptanal were combined in several different proportions, and the thresholds of the mixtures determined Even though the range of concentration ratios of the pure compounds varied from 0 1 1 to 4 95 1 the thresholds of the various mixtures were always near 100 per cent of the theoretical level (Table 1) This experiment demonstrates an additive effect for sub-threshold concentrations of these two compounds over a wide range of combinations

Table 1 ADDITIVEE FFECT OF THE ODOURS FROM *n*-HEPTANAL AND 3-METHYLBUTANAL MIXED IN VARIOUS RATIOS

Ratio of *n*-heptanal to 3-methylbutanal*	Ratio of threshold concentrations of *n*-heptanal to 3-methylbutanal	Threshold of mixture as a percentage of theoretical threshold
2 1	0 10 1	102
4 1	0 20 1	101
9 1	0 45 1	100
19 1	0 95 1	87
29 1	1 45 1	103
39 1	1 95 1	99
99 1	4 95 1	107

* Thresholds of *n*-heptanal and 3-methylbutanal were 3 0 and 0 15 parts per billion parts of water (p p b) respectively

To investigate other mixtures of interest, various compounds were combined in a 1 1 ratio of their respective thresholds, and the thresholds of these mixtures were determined Again, an additive effect was demonstrated (Table 2) The only exception is the mixture of dimethyl sulphide and 3-methylbutanal which gave a threshold of 137 per cent of the theoretical value All other values are close to the theoretically calculated thresholds

Members of a single class of compounds, such as the saturated aldehydes, might be expected to exhibit an additive effect, but members of different classes with different structures would not necessarily be expected to behave in the same way Only a limited number of mixtures containing different structures or classes were tested, and it is not implied that the effect observed would hold for all combinations that could be made However, the mixture of saturated, unsaturated, and branched-chain aldehydes and the mixture of these aldehydes with dimethyl sulphide indicate that a wide range of combinations do give an additive effect Furthermore, the mixture of an alcohol, an acid, a sulphide, an aldehyde and an amine also demonstrated the effect The possibility of chemical interaction is considered negligible because of the extremely low concentrations and short time interval between mixing the chemicals and smelling Therefore, additivity of sub-threshold concentrations appears to be widespread and could be of importance in determining the characteristic flavour of various food products Consider the mixture of 10 saturated aldehydes (Table 2) Each compound in the mixture is present at only about 10 per cent of its threshold concentration, but when combined with the 9 other compounds, odour was perceived In food products, which often contain large numbers of compounds, it is not difficult to imagine the importance of such complicated mixtures, even if each component is present in sub-threshold amount

Table 2 ADDITIVE EFFECT OF ODOUR OF COMPOUNDS IN MIXTURE

	1 . 1 Threshold ratios of	Threshold of mixture as a percentage of theoretical threshold	Average % contributed by each compound
	Saturated Aldehydes		
A	3-Methylbutanal + heptanal	100	50
B	*A* + propanal	102	34
C	*B* + butanal	94	24
D	*C* + pentanal	105	21
E	*D* + octanal	114	19
F	*E* + methylpropanal + decanal	97	12
G	*F* + hexanal + nonanal	94	9
	Unsaturated Aldehydes		
H	2-Butenal + 2-hexenal	114	57
I	*H* + 2-nonenal	95	32
	Saturated + Unsaturated Aldehydes		
J	2-Butenal + butanal	100	50
K	2-Butenal + 3-methylbutanal	100	50
L	Dimethyl sulphide + 2-nonenal	96	48
M	,, ,, + 2-butenal	108	54
N	,, ,, + butanal	109	55
O	,, ,, + 2-methylpropanal	107	54
P	,, ,, + 3-methylbutanal	137	68
Q	Octanol + butyric acid	90	45
R	*Q* + dimethyl sulphide	102	34
S	*R* + 2-nonenal	109	27
T	*S* + butylamine	100	20

In a recent report, Nawar and Fagerson[7] pointed out the potential significance of sub-threshold amounts of some methyl ketones They concluded that a synergistic effect was obtained by combining the compounds. With the compounds and mixtures used in our experiments, the effect appears to be additive rather than synergistic, subtractive, or independent In any event, the observation that mixtures of sub-threshold amounts of odorous materials are capable of inducing olfactory response is of both practical and theoretical interest

This work was carried out in co-operation with the Instant Potato Granule Manufacturers Association

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CHEMICAL INDUCTION OF MAMMARY CANCER IN GERM-FREE RATS

By DR. MORRIS POLLARD

Lobund Laboratory, University of Notre Dame, Indiana

NEOPLASMS can be induced in experimental animals by chemical, by physical, and by biological agents[1] Experiments on carcinogenesis have been performed predominantly in conventional animals which carry a microbial flora of great variety and of uncertain significance While viruses have been clearly implicated in the genesis of neoplastic cells[2], they are not necessarily exclusive agents in that effect The role of micro-organisms in chemical carcinogenesis needs clarification, particularly from the point of view of the chemical agents unmasking (activating) latent oncogenic viruses in the host The unmasking of viral leukæmia by X-rays in low-leukæmia mouse strains has been abundantly documented[3], and this may be relevant to a similar effect by chemical agents Virus-like particles have been described in or around cells of spontaneous tumours in man and animals, thereby leading to an interpretation of causal relationship, but this may actually represent a fortuitous encounter with an unrelated viral agent Viruses initiate tumour formation and then may disappear as distinct entities, while their product flourishes[4] This suggests that perpetuation of the oncogenic effect is not necessarily dependent on overt viral activity, once the cell transformation has occurred

If germ-free animals are free of virus, then they should serve to clarify the role of microbial flora in chemical carcinogenesis Several strains of rats and mice have been

Table 1 RESPONSE OF CONVENTIONAL AND GERM-FREE RATS TO ORAL ADMINISTRATION OF 20 MG DMBA

	Conventional			Germ-free		
Rat strain	Number fed	DMBA toxicity	Tumours	Number fed	DMBA toxicity	Tumours
Sprague–Dawley	29	11/29*	16/18 (104)†	11	2/11	9/9 (82)*
Wistar	12	0/12	0/12	8	3/8	1/5 (111)*
Fischer	9	0/9	0/9	15	15/15	

* Deaths associated with DMBA toxicity/number fed

† Number of animals developing tumours/number fed (average latency period in days)

reared, maintained, and propagated through numerous generations in the germ-free environment, by a method developed in this Laboratory[5]. At term, Wistar rats were transferred *in utero* through a germicidal trap into the germ-free isolator. The baby rats were then removed from the uterus and fed individually a sterile diet by stomach tube until weaning time. The germ-free rat strain, which was started in this way, has now been propagated by natural means through 21 successive generations in the germ-free state. Sprague–Dawley and Fischer rats were similarly transferred *in utero* into the germ-free isolator. They were foster-nursed on germ-free Wistar rats, and thereafter propagated up to the sixth generation by natural means. Some spontaneous breast tumours and occasional thymomas have appeared in Wistar rats[6], whereas none has been observed in germ-free Fischer and Sprague–Dawley rats, which are being propagated here. The incidence of tumours in Wistar rats could not be determined since animals were being removed frequently from the colony for unrelated experiments. Examinations of such germ-free animals in this laboratory for parasites, fungi, bacteria, pleuropneumonia-like organisms and for viruses have thus far been negative[7].

In preliminary experiments several strains of germ-free Swiss–Webster mice and Fischer rats were inoculated subcutaneously with sterile 3-methylcholanthrene (3-MC) in olive oil; and all animals so inoculated developed transplantable fibrosarcomas in the inoculated area. Germ-free and conventional Swiss–Webster mice, inoculated subcutaneously at birth with 3-MC, developed pulmonary adenomas within 8 weeks[8]. All the 3-MC-induced germ-free and conventional tumours, although indistinguishable histologically, appeared in the germ-free animals in more uniform fashion. Examinations of spontaneous and of 3-MC-induced tumours from germ-free animals, by standard virological procedures and by electron microscopy, have failed to reveal evidence of viral agents.

Huggins *et al.* have described the rapid induction of breast tumours in conventional rats, following a single oral administration of 20 mg 7,12-dimethylbenz(*a*)anthracene (DMBA) in sesame oil[9]. The rat most responsive to this treatment is the Sprague–Dawley female, fed DMBA between 50 and 60 days of age. Examinations of such animals and their tumours for viral agents have not been reported. Susceptibility to breast cancer by this procedure was not altered by foster-nursing new-born Sprague–Dawley rats on mothers of other genetic strains, or *vice versa*, indicating that susceptibility may be related to a genetic trait[10]. Strain differences may thus be due to an inherited variation in their endocrine constitution with varying sensitivity of the mammary gland to the carcinogenic effect of the hydrocarbon.

DMBA was dissolved by heat in sesame oil (20 mg/ml.), sealed in glass ampoules, and sterilized in the autoclave (15 lb./20 min). Female Wistar, Fischer and Sprague–Dawley rats, germ-free and conventional counterparts, were each fed 20 mg DMBA by stomach tube, on a single occasion, at 50–60 days of age. The germ-free animals were thereafter maintained in the germ-free state. When a palpable tumour was detected, the affected animal was removed from the isolator, or from the animal house, to the laboratory for autopsy. The animal was killed by cervical section, tumour and other tissues (lung, liver, spleen, lymph nodes, lachrimal gland) were collected aseptically for determination of viral content, for examination in paraffin- and in epon-embedded sections—the latter for examination by electron microscopy. Thirty-eight Sprague–Dawley rats were fed the DMBA preparation, and 13 (34 per cent) of them died within 30 days (Table 1). The cause of DMBA-associated deaths, in germ-free and in conventional rats, was not determined. The remaining rats (9 germ-free and 18 conventional) were observed for 180 days. Three of 8 germ-free Wistar rats died within 20 days following DMBA feeding, and they showed no evidence of tumours at autopsy. All of 15 germ-free Fischer rats, fed 20 mg DMBA, died in 15 days without showing evidence of tumour formation. All germ-free rats showed the enlarged cæcum which is usually observed in such animals.

The 9 germ-free Sprague–Dawley rats which survived the treatment with DMBA developed breast tumours (one to eight per animal) on average at 82 days. Of 18 conventional Sprague–Dawley counterparts, 16 developed mammary tumours on average at 103 days; the number and distribution of tumorous glands were essentially the same as among the germ-free animals. A mammary tumour was detected in one of 5 germ-free Wistar rats at 111 days following administration of DMBA. Tumours appeared only in the mammary glands, and all were solid, circumscribed adenocarcinomas. Metastatic neoplastic foci were not observed.

The fate of cancerous animals was not determined since each animal was killed and autopsied soon after a tumour was detected. The difference in latent periods, 82 days for germ-free and 103 for conventional, may be significant since palpation of tumours in the former through heavy rubber gloves was not so sensitive as with the latter for which the bare hand was used. Possibly, therefore, the latent period in germ-free rats may be shorter.

Tissue cultures were prepared as cell monolayers in bottles and on coverslips in Leighton tubes from trypsin-dispersed germ-free rat fœtuses. The nutrient fluid contained Mixture 199 with 0.5 per cent lactalbumin hydrolysate and 5 per cent heat-inactivated calf serum. The tissue cultures were inoculated with (*a*) cell-free extracts of tumours or of organ tissues in Mixture 199; (*b*) explants prepared from minced tumour tissue; (*c*) cell suspensions prepared by trypsinization of the minced tumour tissue. Nutrient fluids were changed every 4 days over a period of 60 days. Tissue cultures were examined microscopically at frequent intervals for evidence of cytopathogenic effect. If cytopathogenic effect was observed, the whole culture was frozen and thawed twice, and passaged to fresh tissue cultures. The cells on coverslips were stained by May–Grunwald–Giemsa technique and examined microscopically at intervals after onset of cultivation. Extracts of the tissue cultures were examined for hæmagglutinins with mouse, sheep, guinea pig, and human type O erythrocytes at room temperature and at 4° C. No viral agent has as yet been detected in the tumours or in the tissues of the germ-free rats. Tumour cells, examined by electron microscopy, have not revealed evidence of virus-like particles in or around the cells.

Mammary carcinomas were induced by orally administered DMBA in germ-free Sprague–Dawley rats, in which neither spontaneous tumours nor viral agents have as yet been detected by us. Spontaneous tumours might appear among older germ-free rats of the Sprague–Dawley strain, however, a sufficient number has not yet been accumulated on which to base such information. The findings reported here add affirmation to the concept that there are undoubtedly mutiple initiators of the same cellular alterations which lead to a neoplastic transformation , and that they may be evoked by chemical, physical, or biological agents

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BIOSYNTHESIS OF MESSENGER AND RIBOSOMAL RIBONUCLEIC ACIDS IN THE NUCLEOLOCHROMOSOMAL APPARATUS OF ANIMAL CELLS

By G. P GEORGIEV, O. P. SAMARINA, M I. LERMAN, M N. SMIRNOV
and A. N. SEVERTZOV

Institute of Animal Morphology, Institute of Radiation and Physico-Chemical Biology,
Academy of Sciences and Institution of Biological and Medical Chemistry,
Academy of Medical Sciences, Moscow, U.S S R.

DURING recent years we have been investigating the problem of biosynthesis of RNA in the nucleus of mammalian cells The main method used was phenol fractionation of cellular RNA which permits the separation of the rapidly labelled RNA of the cell and isolation of its fractions This article presents some new results concerning the properties of nuclear RNA's and their transport from the nucleus into the cytoplasm. The terminology adopted here for designation of different types of high-molecular weight RNA is as follows. R–RNA–RNA with base composition similar to ribosomal RNA; D–RNA–RNA with DNA-like base composition (it corresponds to messenger RNA)

Present state of phenol method of cellular RNA fractionation The first approach to phenol fractionation of cellular RNA was made independently by Sibatani *et al*[1] and by Georgiev and Mantieva[2] It was shown that a small part of RNA was not deproteinized by phenol at neutral *p*H and that this RNA possessed high specific activity This RNA was shown to be bound in the cell nuclei which survived the treatment with phenol *p*H 6–0 14 M sodium chloride in the cold The nuclei are shown on the boundary of saline and phenol as an intermediate layer ('interphase') The procedure for purification of these 'phenolic nuclei' was described[2]

The RNA of phenolic nuclei was roughly identified as RNA of nucleoli and chromosomes or RNA of 'nucleolochromosomal apparatus' (NC-apparatus) The cytoplasmic RNA and RNA of nuclear sap were extracted under standard conditions After incubation of cells with labelled precursors for 0 5–1 h nearly 90 per cent of total radioactivity incorporated into high-molecular weight RNA of the cell was recovered in NC–RNA Therefore, NC–RNA appears to be a 'rapidly labelled' RNA of the animal cell[1–3]

Further progress was achieved when the procedure for isolation of RNA from phenolic nuclei was carried out[3] If phenolic nuclei are treated by phenol *p*H 6–0 14 M sodium chloride at 65° C, all their RNA is liberated It was demonstrated that NC–RNA contained both ribosomal RNA identified by typical profile in analytical ultracentrifuge and RNA with different base composition[4] We succeeded in separating these RNA's and in obtaining for the first time from animal tissues RNA fractions similar in base composition to DNA (D–RNA, messenger RNA) and also RNA fraction similar to ribosomal RNA (R–RNA)[3] Sibatani *et al*[4] have proposed another procedure for isolation of D–RNA, but only a part of total NC–D–RNA (nucleolar D–RNA) was recovered by their method In the course of further examination of the fractionation of RNA of phenolic nuclei we observed that at temperatures below 50° C it is R–RNA which is predominantly released, D–RNA being bound with phenolic nuclei On this observation the method for preparation of R–RNA and D–RNA of NC-apparatus was based[5] After some modifications the following procedure was finally adopted

(1) *Isolation of phenolic nuclei.* Cell suspension or tissue homogenate in 0 14 M sodium chloride was treated with an equal volume of phenol, *p*H 6, at 4°–10° C After 15 min shaking and after centrifugation, high-molecular weight RNA of cytoplasm and nuclear sap and total low-molecular weight RNA were revealed in the aqueous layer Interphase was collected and repeatedly treated (twice or three times) with phenol *p*H 6–0 14 M sodium chloride (1 1) in the cold The interphase washed contained non-purified phenolic nuclei that may be used as such or purified further by a procedure earlier described[2] In the case of Ehrlich ascites carcinoma cells, the cytoplasmic contaminations in the interphase were negligible

(2) *Thermal fractionation* Phenolic nuclei (purified nuclei or washed interphase) were treated by shaking in phenol *p*H 6–0 14 M sodium chloride at 40°–45° C for 20 min, and the main part of R–RNA of NC-apparatus was released The same procedure was repeated at 55° C, the RNA liberated being a mixture of R–RNA and D–RNA Interphase was washed once in the same system in the cold and again treated with phenol, *p*H 6–0 14 M sodium chloride at 60°–65° C Under these conditions D–RNA passed into the aqueous layer

The fractions were referred according to temperature intervals in which they were obtained Thus, 10° C fraction possessed cytoplasmic RNA, 10°–40° C or 10°–45° C fractions—R–RNA (with coefficient $K = G + C/A = U =$ 1 5–1 6) and 55°–60° C or 55°–65° C fractions —D–RNA ($K =$ 0 75–0 85)

(3) *Purification of RNA fractions* For the inhibition of RNase traces, if present, sodium dodecyl sulphate is added to all fractions, and solutions are repeatedly deproteinized with phenol and chloroform RNA was

Table 1. BASE COMPOSITION OF NC–RNA FRACTIONS

Tissue	Type of fractionation	RNA fraction	Base composition of RNA fraction in the whole: Mol per cent of base *G*	*C*	*U*	*A*	$K=\frac{G+C}{A+U}$	Base composition of newly formed RNA in fraction: Mol per cent of base *G*	*C*	*U*	*A*	$K=\frac{G+C}{A+U}$
Ehrlich ascites carcinoma	Phenol	NC–R–RNA (10°–40° C)	32.6	29.9	18.8	19.3	1.67	30.8	30.3	22.6	16.3	1.57
		NC–D–RNA (55°–60° C)	24.9	20.1	27.1	27.8	0.82	20.7	19.7	29.2	30.3	0.68
	Salt	Nucleolar RNA	32.4	29.3	21.3	16.9	1.62	32.5	31.0	20.0	16.6	1.73
Rat liver	Phenol	NC–R–RNA (10°–40° C)	31.2	29.5	18.2	21.4	1.53	34.8	27.6	18.2	19.4	1.65
		NC–D–RNA (55°–65° C)	22.4	24.0	24.7	28.8	0.86	22.4	22.8	23.2	31.6	0.83
	Salt	Nucleolar RNA	33.0	29.9	20.7	16.3	1.70	31.7	29.7	20.3	18.3	1.59

Mice with Ehrlich ascites carcinoma and rats were injected intraperitoneally with ^{32}P (200 and 50 µg/animal respectively). 30–60 min later the animals were killed and RNA fractions were isolated from carcinoma cells of mice and from rat liver by the phenol method or by salt fractionation of isolated nuclei (ref. 8). RNA fractions were washed with 5 per cent TCA on 'Millipore' filters (Rufs), the material of filter was removed by acetone, and RNA was hydrolysed by 0.5 *N* potassium hydroxide (37° C 18 h) and 2′,3′-nucleotides were separated by paper chromatography into four spots (ref. 9). Spots were excised, counted, eluted and measured spectrophotometrically.

precipitated once by ethanol and once by 2.5 M sodium chloride at 0°–4° C and treated with DNase (Worthington). The DNase was removed by shaking with phenol and RNA was reprecipitated 2–3 times by ethanol in the presence of 0.005 M versene. It was stored under the 70° ethanol at −20° C. The yield from 100 ml. of ascites was about 2 mg of R–RNA and 1 mg of D–RNA.

The specific activities of both RNAs were much higher than that of 10° C-fraction (RNA of cytoplasm + nuclear sap). Thus, this fractionation yielded two types of rapidly labelled RNA of the cell.

It is important that the base composition of these preparations, estimated on the basis of ultra-violet absorption of nucleotides (composition of the whole fraction) and estimated on the basis of radioactivity of 2′,3′-nucleotides after short labelling with phosphorus-32 (composition of the newly formed RNA), coincide (Table 1).

Therefore (1) Rapidly labelled nascent RNA of the animal cell is a mixture of D–RNA and R–RNA that may be separated experimentally, (2) our method is suitable for the separation of both total NC–RNA and newly formed NC–RNA into D- and R-fractions.

Some properties of R–RNA and D–RNA of nucleolo-chromosomal apparatus. During recent years many papers have been published concerning total rapidly labelled RNA[8]. It was shown to differ from ribosomal RNA with respect to molecular weight. Some authors claimed it as 'messenger RNA'. However, as shown here, this RNA consists of two types of molecules. Therefore, experiments were carried out in which separated D–RNA and R–RNA were ultracentrifuged in sucrose-density gradient[9]. The results are presented in Fig. 1.

In accordance with our previous results[4], R–RNA contains two components with sedimentation constants equal to 18 and 28 *S*, respectively. Thus, the bulk of R–RNA of phenolic nuclei is a typical ribosomal RNA. However, some material of this fraction sediments at higher velocity. Distribution of radioactivity depends on the time of incubation. In short impulses, all radioactivity is found in heavy 45–35 *S* components. In longer impulses it is also revealed in ribosomal RNA. The total newly formed RNA of R–RNA fraction and newly formed RNA isolated from 18–28 *S* and 35–45 *S* components have nucleotide composition of strong *GC*-type.

From these results the conclusions can be drawn that (1) our fractionation method makes possible the isolation of non-degraded R–RNA of NC-apparatus: the heavy components (45–35 *S*) are not affected by this method, (2) the heavy 45–35 *S*′-component is R–RNA which seems to be a precursor of ribosomal R–RNA[10], (3) the transformation of non-ribosomal R–RNA (45–35 *S*) to ribosomal R–RNA (18 and 28 *S*) occurs in the NC-apparatus.

In order to determine the site of the R–RNA synthesis in NC-apparatus we analysed the base composition of RNA fractions obtained by the salt fractionation of cell nuclei, isolated in aqueous media[6].

It was shown earlier that residue after extraction of nuclei with dilute and concentrated salt solution contained only nucleoli and 'residual chromosomes'. These structures are built of nucleonemata—the filaments 100 Å in diameter with ribosome-like granules attached to filaments[11]. The RNA of this material is the most active fraction among other nuclear RNA's[6]. Table 1 presents the base composition of this 'nucleolar' RNA. The base composition of the fraction as the whole and that of newly formed RNA are of strong *GC*-type. At sucrose gradient ultracentrifugation two radioactive components of ribosomal RNA type are revealed. Thus the total and newly formed RNA of nucleonemata of nucleoli and residual chromosomes is a typical ribosomal RNA. Nucleonemata and their granules appear to be newly formed ribosomes or ribosomal precursors.

In Fig. 1 the results of ultracentrifugation in sucrose gradient of D–RNA are also presented. D–RNA is a heterogeneous population of molecules with the distribution maximum in the zone of light ribosomal RNA component (about 18 *S*). Radioactivity follows ultra-violet absorption although there is no full correlation between these curves. There are also no significant differences in distribution of radioactivity among preparations of different labelling time. Probably chains of different length with molecular weights of the order of 0.3–1.5 × 10⁶ are synthesized without subsequent division into chromosomes.

Fate of D–RNA and R–RNA synthesized in nucleolo-chromosomal apparatus. Many papers have recently appeared demonstrating that RNA's formed in NC-apparatus were further transferred into the cytoplasm (for reviews, see ref. 12). We shall not discuss this problem in detail, but would like to mention one of the

Table 2. RESULTS OF ACTINOMYCIN EXPERIMENTS

Tissue	RNA fractions	Total activity of fraction (c.p.m.): U–^{14}C or ^{32}P + 30 min	U–^{14}C or ^{32}P + 30 min + actinomycin + 90 min	The change of total activity: c.p.m.	Per cent
Ehrlich ascites carcinoma	D–RNA (50°–65° C fraction)	7,250	5,250	−2,000	−28
	R–RNA (10°–50° C fraction)	18,700	16,200	−2,500	−13
	Cytoplasmic RNA (10° C fraction)	2,240	1,680	−560	−25
Rat liver	D–RNA (50°–65° C fraction)	12,600	6,900	−5,700	−45
	R–RNA (10°–50° C fraction)	6,600	2,175	−4,425	−75
	Cytoplasmic RNA (10° C fraction)	7,650	18,750	+11,100	+145

Ehrlich carcinoma cells were labelled with uridine-^{14}C 30 min *in vitro*, rat liver cells with ^{32}P 30 min *in vivo*. The actinomycin *D* (10 µg/ml. of ascites) or 'Aurantin' (1.5 mg per rat intraperitoneally) were administered. The activity of fractions was studied 30 min and 120 min after adding of precursor. Phenol fractionation was slightly modified for quantitative recovery of RNA fractions.

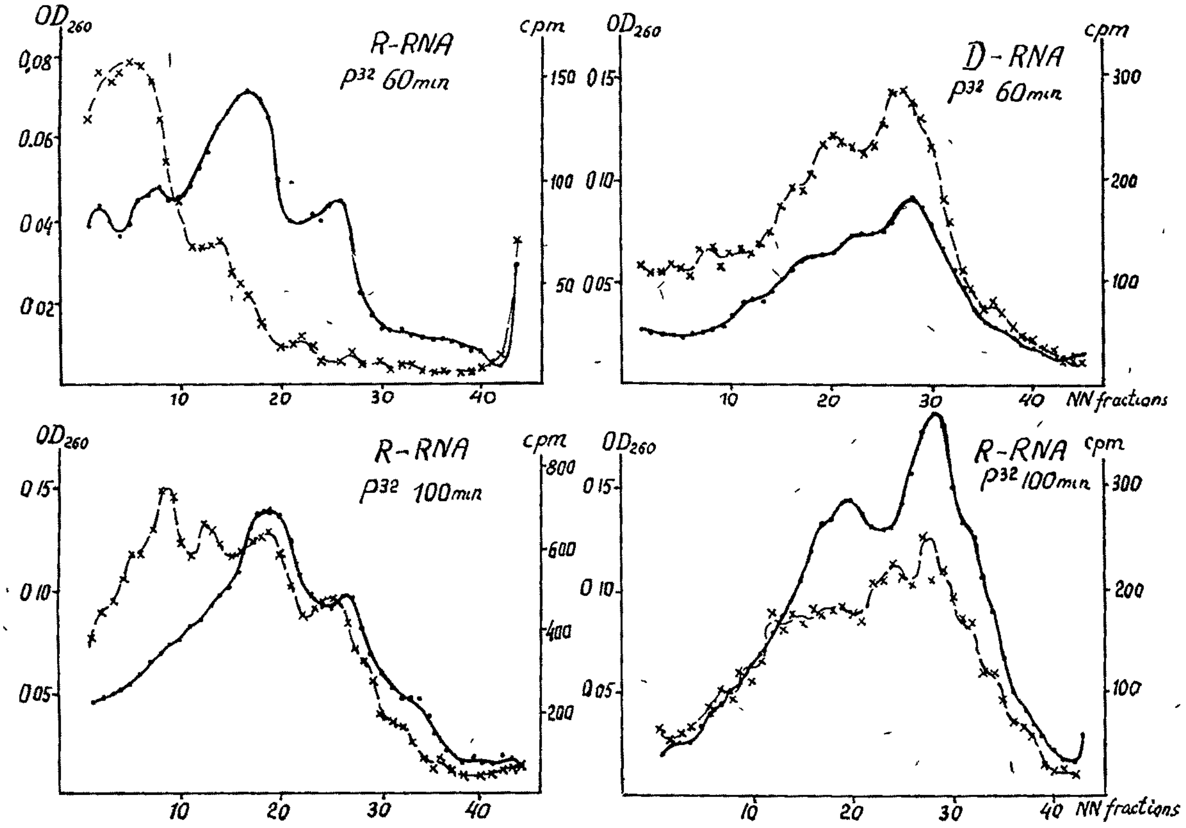

Fig. 1. Sedimentation properties of the NC–RNA fractions. Ehrlich ascites carcinoma cells were incubated with phosphorus-32 *in vivo* (50–100 μc./animal). 1–2 mg of each NC–RNA was ultracentrifuged in *SW*-25 rotor of Spinco-*L* in 5–20 per cent sucrose gradient at 25,000 r.p.m. for 10 h. ——, Ultra-violet absorption; – – –, radioactivity

experimental approaches. We tried to use actinomycin blockage of total RNA synthesis[13] for demonstration of nucleo-cytoplasmic RNA transport. After impulse labelling of cells the actinomycin (actinomycin *D* or 'Aurantin') was administered and redistribution of radioactivity among RNA fractions was investigated.

In two systems tested (Ehrlich ascites carcinoma and culture of human amnion) actinomycin inhibited not only synthesis of RNA but also liberation of RNA from NC-apparatus of the cell. Neither transport nor considerable breakdown (as in Harris's experiments[14]) of rapidly labelled RNA was observed. About 80 per cent of the latter was detained in NC-apparatus (Table 2). However, in the case of rat liver the liberation of RNA is less affected, and actinomycin blockage leads to a rapid and significant decrease in the activity of NC–RNA, especially of R–RNA, and to a corresponding increase in the activity of cytoplasmic RNA. The total activity loss by NC-apparatus roughly equals the activity rise revealed in the cytoplasm. Although some part of NC–RNA was also arrested in the case of rat liver, these results indicate the transport of RNA from the NC-apparatus to the cytoplasm.

But these experiments do not give any insight into the nature of RNA moving to the cytoplasm. As was shown by Hiatt and Monier[8], all labelled cytoplasmic RNA was connected with ribosomal components and 4*S*-component. Base composition of newly formed cytoplasmic RNA was of strong *GC*-type, D–RNA seems to be absent in the cytoplasm. To demonstrate the presence of D–RNA in the cytoplasm of animal cells and thus to prove the possibility of its nucleo-cytoplasmic movement the following experiments were carried out. The biosynthesis of R–RNA was shown to be rather sensitive to actinomycin, as compared with D–RNA formation[15]. It seems possible to inhibit selectively the biosynthesis

Table 3. BASE COMPOSITION OF RNA'S FORMED DURING INCUBATION FOR 6 H WITH ^{32}P IN THE PRESENCE OF 'AURANTIN'

No. exp.	RNA studied	Dose of 'Aurantin' (μg per animal)	Sp. activity c.p.m./mg RNA	Base composition of newly formed RNA: Mol per cent *G*	*C*	*U*	*A*	*K* = G+C/A+U	D–RNA synthesized per cent of total RNA synthesized
1	Total RNA	—	28,300	31.0	29.1	19.6	20.4	1.50	16
		10	14,400	29.6	27.1	21.8	21.5	1.31	32
		40	8,000	25.3	22.0	23.8	28.9	0.90	75
		160	4,350	22.0	23.5	24.5	30.0	0.82	86
		600	1,470	—	—	—	—	—	—
		2,500	650	—	—	—	—	—	—
2	Cytoplasmic RNA (10° C fraction)	—	32,600	33.9	28.6	18.0	19.5	1.67	4
		40	11,300	28.3	27.1	20.2	24.5	1.23	39
		160	6,100	21.6	26.5	25.2	26.7	0.93	72
	NC–RNA (10–65° C fraction)	—	—	28.0	25.8	22.6	23.7	1.16	46
		40	—	20.6	25.2	27.6	26.8	0.83	85
		160	—	20.4	22.6	27.1	29.6	0.76	95

The rats were injected intraperitoneally with 'Aurantin' and 40 min later with ^{32}P (0.5–1.0 mc./animal). 6 h later animals were killed and liver RNA fractions were isolated: total RNA—by the treatment with phenol *p*H 6—0.5 per cent sodium dodecyl sulphate at 60° C, cytoplasmic RNA and NC–RNA—as described here. All fractions were reprecipitated by 1.5 M sodium chloride. The method of determination of newly formed RNA is the same as in Table 1. Content of D–RNA synthesized was calculated assuming $K_{D\text{-}RNA}$ = 0.73 and $K_{R\text{-}RNA}$ = 1.73

of R–RNA without affecting that of D–RNA if using small doses of this antibiotic The results are presented in Table 3 In normal rat liver cell nucleotide composition of RNA formed during incubation for 6 h with phosphorus-32 is typical for ribosomal RNA and no signs of the presence of D–RNA are revealed But in the case of partial inhibition of RNA synthesis $K = G + C/A + U$ is lowered and reaches 0 8 It means that about 90 per cent of RNA synthesized in these conditions represents D–RNA If the RNA from these cells is divided into NC–RNA and cytoplasmic RNA it can clearly be observed that D–RNA is present not only in nuclei but also in the cytoplasm (Table 3) Preliminary results of the fractionation of the cytoplasmic RNA on methylated serum albumin support this view In the cytoplasm of a normal cell non-ribosomal RNA cannot be detected after labelling for 6 h, but in a partially inhibited cell a distinct non-ribosomal RNA–component with some lower molecular weight can be observed

When this work was completed the report of Penman *et al* [16] appeared demonstrating the presence of D–RNA in polysomes These results indicate that D–RNA formed in the cell nucleus transits into the cytoplasm, where it can be detected Further work on this transport and transformations of D–RNA in the nucleus and the cytoplasm is in progress.

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FUNCTION OF THE THYMUS AND GROWTH OF TUMOUR HOMOGRAFT

By Dr G. C. PERRI, M FAULK, E SHAPIRO, J. MELLORS
and Dr W. L. MONEY

Divisions of Experimental Chemotherapy and of Clinical Investigation,
Sloan-Kettering Institute for Cancer Research and Sloan-Kettering
Division of Cornell University Medical College, New York 21

THE development of a normal immune system has been shown to be dependent on the presence of the thymus gland[1–4] Thymectomy performed at birth in experimental animals is followed by a severe depletion of the lymphocyte population in both the spleen and lymph nodes, and animals so treated show serious immunological defects; homograft acceptance by such animals is significantly increased[5–9] Thus, available data support the concept that the absence of the thymus gland during the post-natal period produces a depression of the immunological reaction sufficient to suppress the skin homograft rejection mechanism Recent reports suggest that in the thymus two factors are present, one accelerates the growth of neoplastic tissue and another slows neoplastic growth[10,11] It is also possible that the thymus regulation of the maturation of immunologically competent cells[12–14] may be of humoral nature

The thymus and its function could be of particular importance in the field of oncology since it might participate, along with other glands, in the regulation of tissue homœostasis and be of paramount importance in repressing the activity of cells which potentially may develop into neoplasms

If the thymus is involved in the immune reaction, it might be expected that the growth pattern of the tumour would be changed in thymectomized animals It seemed, therefore, of interest to examine the effect of thymectomy on the growth of a transplantable tumour For this investigation on rats we have used Jensen sarcoma which, on the basis of its origin and host relationship, can be considered a homograft

More than 300 male and female rats of the Sprague–Dawley strain (Charles River) were used in this work, and each experiment repeated several times with consistent results (Table 1) The general pattern of the experiments was as follows Pregnant rats were maintained under normal laboratory conditions until the young were delivered Litters were pooled and animals thymectomized on a random basis regardless of sex within 48 h after birth under light ether anæsthesia through an incision in the neck and the upper sternal cartilage which was closed with skin clips and covered with collodion to ensure maternal care Jensen sarcoma was implanted by trocar

Table 1 COMPARISON BETWEEN JENSEN SARCOMA GROWTH IN THYMECTOMIZED AND CONTROL RATS (LITTER-MATES) WEIGHTS (G)

Control			Thymectomized		
Body	Spleen	Tumour	Body	Spleen	Tumour
103	0 425	0 02	92	0·465	21 20
96	0 740	21 00	81	0 436	19 00
99	0 485	0 37	112	0 786	18 40
100	0 877	28 00	79	0 755	3 00
86	0 983	2 50	91	0 610	16 00
104	0 565	0 18	90	0 517	14 00
87	0 613	12 50	79	0 411	15 00
86	0 366	0 15	96	0 429	18 40
83	0 308	0 13	94	0 793	4 10
78	0 356	0 14	69	0 598	8 20
70	0 332	no tumour	80	0 433	no tumour
72	0 238	,, ,,	77	0 307	,, ,,
63	0 282	0 30	66	0 294	16 55
68	0 281	1 40	61	0 962	4 18
60	0 426	10 00	60	0 440	no tumour
68	0 286	no tumour	50	0 460	19 60
64	0 282	,, ,,	58	0 321	9 10
61	0 251	,, ,,	57	0 306	1 20
66	1 035	15 75	50	0 478	9 10
55	0 536	23 00	54	0 566	7 90
54	0 722	20 29	50	0 439	16 00
56	0 237	no tumour	48	0 625	13 73
47	0 287	0 28	49	0 549	21 62
49	0 851	21 00	44	0 466	16 15
42	0 629	23 20	42	0 497	17 60
$\bar{x}$=72 68	$\bar{x}$=0 496	$\bar{x}$= 7 21	$\bar{x}$= 69 16	$\bar{x}$=0 518	$\bar{x}$=11 60
76% takes			88% takes		
64% < 5 g			24% < 5 g		
36% >5 g			76% >5 g		

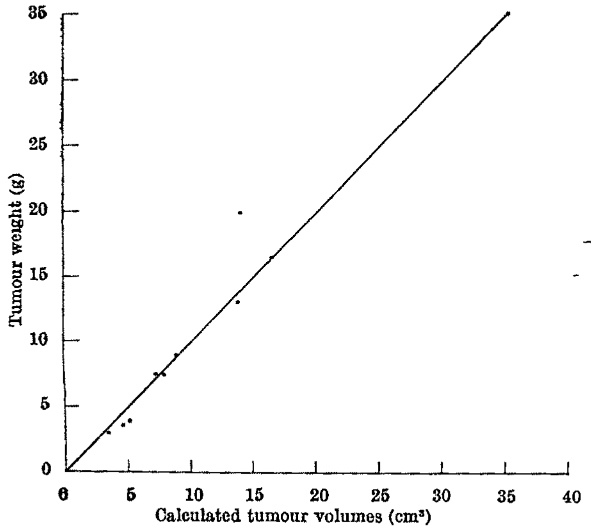

Fig. 1. Correlation between tumour volumes (cm³) calculated and tumour weight at autopsy, $r = 0\ 93$

or as a minced suspension into animals of different ages When implants were made beyond 20 days of age the animals were weaned and transferred to separate cages, otherwise the young were left with the mother until completion of the experiment Body weight and tumour diameters were recorded daily Loss of weight was observed in animals at weaning or on transfer to single cages; otherwise the growth curve was quite regular About 12–14 days after implant of the tumour the animals were killed and those showing signs of incomplete thymectomy or infection were discarded.

Several ways of calculating tumour volumes from the daily measurements were tried. Although theoretically the tumour volume should be better expressed as that of an ellipsoid of revolution, neither the formula for oblate nor for prolate spheroid is applicable Values obtained by the former were higher and for the latter lower than the weight obtained at autopsy However, a good correlation was found between the calculated volume (cm³) and weight (g) when the tumour volume was considered equal to a solid having the length of the major axis *a*, and the height and width equal to the minor axis *b* In fact, when the formula $V = ab^2$ was used the correlation coefficient obtained by plotting the weights of the tumour against the calculated volume was equal to 0 93 It can be seen (Fig 1) that the discrepancy between the two sets of values increases with tumour weights above 20 g

Thymectomy in non-tumour-bearing new-born rats did not produce any significant change in the growth rate of the animals during the time of these experiments, the body weights of the thymectomized and control animals were comparable within normal variations. Furthermore, the subsequent mortality of animals thymectomized at birth was less than 5 per cent, and those that died or were discarded because of extreme loss of weight showed substantial infection, usually in the thoracic cavity

Fig 2 shows the rate of growth of Jansen sarcoma in thymectomized and control animals Although the tumours in both groups became measurable at approximately 7 days after implant, the growth curves diverged markedly around the tenth day By the fourteenth day the tumours in thymectomized animals were 2–3 times greater than in the controls, and showed striking macroscopic differences in appearance In control tumours the tissue showed the usual appearance of necrosis in the central portion and peripheral proliferating tumour of transplants of Jensen sarcoma at this stage of growth, whereas tumours in thymectomized animals showed little or no evidence of necrosis, the entire mass consisted of typical Jensen sarcoma tissue

In the normal control rats the percentage of successful transplants was the 75 per cent usually observed with Jensen sarcoma In the thymectomized groups, on the other hand, transplants were 100 per cent successful with the exception of one group with 88 per cent success. Table 1 summarizes the results of this experimental group of 25 thymectomized rats and 25 unoperated littermates implanted with the same pooled tumour tissue from 14-day-old Jensen sarcoma, and shows that 24 per cent of the tumour implants in the normal animals were rejected with no trace of the implant on autopsy Of the remaining implants, 36 per cent grew but were regressing (weight less than 5 g at 14 days) and 40 per cent were fully growing In the thymectomized rats 12 per cent of the implants were rejected, 12 per cent were considered regressing, and 76 per cent had become fully growing tumours (more than 5 g).

Our results show clearly that Jensen sarcoma grows in rats thymectomized at birth at a significantly faster rate than in normal animals Implantation of the tumour in splenectomized new-born animals (unpublished data) gave results identical to normal rats and implants in splenectomized and thymectomized animals did not differ significantly from results obtained in animals thymectomized only Thus the normal aggressive growth of this type of tumour is definitely enhanced by thymectomy That thymectomy significantly modifies the behaviour of Jensen sarcoma is clearly shown by the slope of the growth-curve, by the increased percentage of successful transplants, and by the weight of the neoplastic mass at autopsy, and is further supported by the macroscopic appearance of the tumour masses in thymectomized rats, masses practically free of necrotic areas in contrast to Jensen sarcoma of the same age grown in normal animals The present data are consistent with the results reported by Martinez *et al*[6] obtained with tumour homografts in thymectomized mice

The Jensen sarcoma homograft in normal animals is capable of overcoming the histo-incompatibility barrier of the host Sprague–Dawley rat in 75 per cent of the cases, but the host immune reaction makes possible the regression and outright rejection of some of the implants with time. Those implants which grow show extensive hæmorrhagic and necrotic areas In thymectomized animals the number of rejections and regressions of the tumour implants is significantly lower and the hæmorrhagic and necrotic component is virtually absent It is rather unlikely that the necrotic areas of the tumour growing in normal rats are caused by circulatory insufficiency or accumulation of catabolites since such areas do not appear in tumour

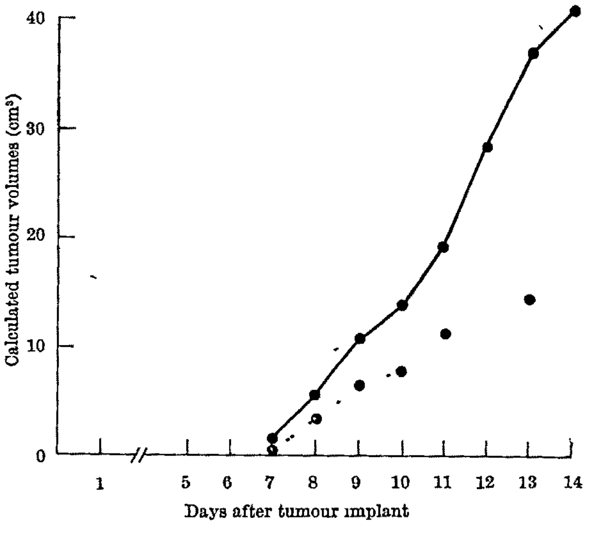

Fig 2. ——, Thymectomized tumour-bearing rats, – – –, control tumour-bearing rats

masses growing more rapidly, and to larger volumes, in thymectomized animals. Therefore, it seems plausible to consider all these signs as significant indication that thymectomy has abolished, or greatly depressed, some of the immune reactions of the host. Jensen sarcoma seems to grow in thymectomized animals in a more uncontrolled and unchallenged fashion as if a repressing factor had been removed. From the slope of the growth curve obtained in 76 thymectomized and 61 control animals it can be calculated that the daily growth rate of Jensen sarcoma in thymectomized animals is 1.8 times higher than in normal animals.

The present evidence adds additional support to the concept that the thymus gland in the post-natal period is important for normal development of the immune reaction, but the mechanism of its action is not clear. While the lymphocyte migration theory has not been directly demonstrated in the development of the immune system, the humoral theory[12] has received support[13,14]. Although additional evidence such as the demonstration that spleen tissue does not have such an effect is needed, other results strongly support the humoral theory. For example, histological examination of the thymus shows epithelial cellular formations containing granules localized around capillaries and with a characteristic relationship to reticular fibres, that can be considered capable of a secretory function. Such formations, easily seen in the thymus of reptiles after hibernation[15,16], when the thymus is depleted of lymphocytes, and also described in the calf[17,18], are evidence in favour of a secretory function of the thymus. This might imply that the thymus gland has not only the postulated function of responsibility for the development of the immune system in post-natal life, but possibly also a more general and complex function yet to be defined. Recent reports[10,11] suggesting that the thymus gland contains substances which affect the rate of growth of cells may ultimately help to resolve these questions.

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EXCRETION AND THE ACCRUAL OF DRUG METABOLITES

By A. J. CUMMINGS and DR. B. K. MARTIN
Nicholas Research Institute, Ltd., Slough, Bucks

THE relationship of the rate of decline of a drug in the plasma to the rate of excretion of unchanged drug in the urine has been established in respect of a number of drugs, for example, sulphaethylthiadiazole[1] and tetracycline[2]. A similar study of the rate of excretion of metabolites when a drug is excreted predominantly as metabolites is more complex, for this involves the specific rate constants for metabolite formation (k_f) and for metabolite excretion (k_e).

When a drug is rapidly metabolized and the metabolite is slowly excreted, that is $k_f > k_e$, the concentration of the metabolite may eventually exceed that of the parent drug to become the only form of the drug in the body, and there will ultimately be a period when only the metabolite is excreted. The urinary excretion data may then permit the calculation of k_e. When, however, a drug is slowly metabolized and the metabolite is rapidly excreted, that is when $k_f < k_e$, the drug and the metabolite will always be excreted together and the observed rate of metabolite excretion is a function of k_f and k_e.

This article relates to k_e under the conditions when $k_f < k_e$ and points to its significance in the explanation of certain observations.

Little consideration appears to have been given to those instances where the metabolite is believed to be 'rapidly excreted'. It has sometimes been assumed that when excretion is rapid, the rate of excretion of the metabolite can be equated to its rate of formation. The two processes may ultimately decline in parallel, but this is not so in the early phase of excretion and they are equal only at one particular time.

Butler[3] has shown that in theory the half-life ($t_{0.5}$) of a substance excreted by the kidney can be related to its renal clearance (C) and its apparent volume of distribution (V_d), by the expression

$$t_{0.5} = \frac{V_d}{C} \ln 2$$

Metabolite formation is considered to be an intracellular reaction and, while this may be limited to certain cells, the probability must be considered that the metabolite may then enter other cells. The apparent volume of distribution of a metabolite will therefore exceed the extracellular water volume and may well approach that of total body water. A metabolite exhibiting the maximum renal clearance ($C = 700$ ml./min.) but having a high apparent volume of distribution ($V_d = 50$ l.) would have a half-life of 50 min, that is, only 56 per cent would be excreted each hour.

After the administration of salicylic acid, the major metabolite in human urine is salicyluric acid. The oral administration of salicyluric acid (0.5 g), and calculation of $t_{0.5}$ from the excretion data, gives a value for salicyluric acid of about 50 min. This metabolite is 'rapidly excreted', yet of that present at the beginning of an hour little more than 50 per cent will be excreted during the hour, while of that formed in the course of the hour the percentage excreted will be even smaller.

The rate of formation of a metabolite may be calculated from the rate of decline of the concentration of drug in the plasma, after allowing for the decline due to excretion of the unchanged drug. In the first hour after drug administration the quantity of metabolite formed will be greater than the amount excreted and some will remain in the body to be added to that formed in the next hour. Present concepts imply that metabolite formation will usually reach its maximum when the concentration of drug is at its maximum, thereafter, it will decrease. The amount of metabolite which remains from the previous hour may, however, be more than sufficient to compensate for the

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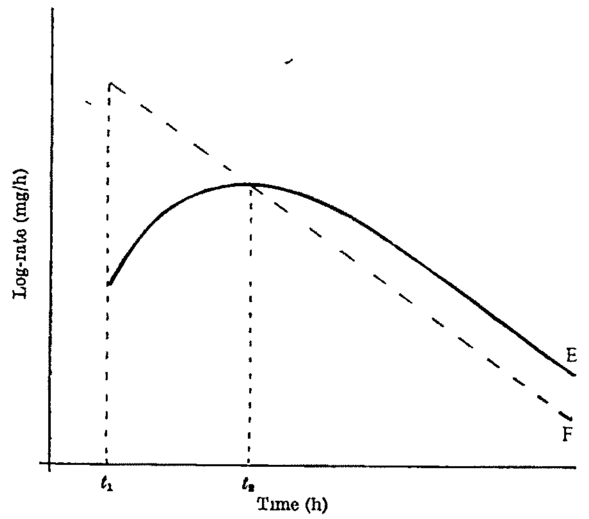

Fig. 1. Rate of metabolite excretion is depicted by the line *E* and rate of metabolite formation by line *F*. Maximum plasma concentration of drug occurs at time t_1, maximum rate of excretion of the metabolite at time t_2

decrease in the amount formed, and, if so, the accrual of metabolite will continue. The maximum rate of excretion of the metabolite will occur when the plasma metabolite concentration reaches its peak and this may therefore occur much later than the peak drug concentration. The magnitude of metabolite accrual depends not only on the relationship of k_e to k_f, but also on the rate of absorption, for three consecutive processes are involved. After the maximum rate of excretion has been attained, the quantity of metabolite excreted per hour exceeds the amount formed per hour.

A plot of log amount of metabolite excreted per hour against time ultimately approximates to a straight line (Fig. 1). The slope of this line can then be closely identified with that of the log rate of metabolite formation and it provides an accepted basis for the calculation of k_f. If the excretion of metabolite reaches its maximum when its concentration in plasma is maximum, this will occur when its rate of formation and excretion is equal. The plots of log rate of metabolite formation and log rate of metabolite excretion will therefore intersect at the point where the latter is maximum. On the basis of these considerations the line representing the log decline of metabolite formation may be positioned approximately (Fig. 1).

In a previous communication[4], we reported that whereas peak plasma salicylate-levels may be attained about 1 h after the oral administration of aspirin, the maximum rate of 'total salicylate' excretion does not occur until the third hour or later. Elimination of salicylic acid from the body is slow and depends almost entirely on the formation of metabolites. The apparent delay in their excretion may be attributed to metabolite accrual.

A consideration of excretion rate constants may also provide an explanation of the urinary findings when a drug gives rise to more than one metabolite. Each metabolite has a specific excretion rate constant and each will consequently attain its maximum rate of excretion at different times. A plot of log rate of excretion of each metabolite would be expected ultimately to provide a set of parallel lines. Similarly, a log plot of the rate of excretion of total metabolites will only become linear when the foregoing state has been realized and will then parallel the log rate of decline of the drug in plasma. The excretion of total metabolites is a composite function and for this reason may show a number of variations until the individual metabolites are excreted in constant ratio.

The foregoing considerations can be related to the observed excretion of salicylate metabolites in urine. Schachter[5] and Schachter and Manis[6] have shown that after the administration of sodium salicylate the ratio of the metabolites in the urine varied with time. This might suggest that the rate of formation of one or more of the metabolites varies with time or with plasma salicylate concentration. Cummings[7] has suggested that salicyluric acid shows an apparent change in its rate of formation as the plasma salicylate concentration increases. This will cause a change in the ratio in which the metabolites are formed and excreted. An additional explanation is, however, necessary, for a plot of log rate of excretion of 'total salicylate' against time does not become linear for several hours after a small dose (0.3 g) of aspirin. This dose provides a plasma salicylate concentration below that at which the rate of formation of salicyluric acid shows an apparent change. The observed excretion pattern can be explained on the basis that the metabolites are initially excreted in varying ratios in accordance with the foregoing theory. It is not necessary to postulate the delayed formation of one or more metabolites, for the uniform decline in log plasma salicylate during this period indicates that the metabolites are formed at uniform rates.

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DETERMINATION OF CEREBRAL BLOOD FLOW BY A KRYPTON-85 DESATURATION METHOD

By Captain LAWRENCE C. McHENRY, jun.
Department of Neurophysiology, Walter Reed Army Institute of Research, Washington, D.C.

SINCE the development of the nitrous oxide technique by Kety[1] in 1945 for the quantitative determination of cerebral blood flow there have been a variety of new methods proposed for the examination of the cerebral circulation. Except for the krypton saturation technique of Lassen and Munck[2] most newer methods do not give specific quantitative data or are technically difficult to perform.

A desaturation method based on the Fick principle for determination of cerebral blood flow was originally considered feasible by Kety[3], but was not utilized until reported by Lewis *et al.*[4] In 1960, Lewis *et al.*[4] described both nitrous oxide and krypton-79 saturation and desaturation methods. In 1961, Kempinsky[5] reported using a nitrous oxide desaturation method, but did not give normal values for the method.

The krypton-85 desaturation method for cerebral blood flow determination was developed for three reasons First, when the saturation methods of Kety or Lassen are used, smooth curves are obtained only by maintaining a constant concentration of inert gas during inhalation. This is easily done when using nitrous oxide, but when using krypton-85 or krypton-79 special equipment is needed to maintain a precise concentration of the tracer In the desaturation method the maintenance of precise concentration of inert gas during saturation is unnecessary. When a desaturation investigation is carried out, mask leaks during saturation are unimportant, and no special equipment is needed to administer the tracer

Secondly, since there is no mask on the patient's face during the actual examination of cerebral blood flow, respiration is unimpeded and any influence of anxiety on cerebral hæmodynamics is reduced Thirdly, by using a desaturation technique the effect of extra-cerebral contamination of measurement of cerebral blood flow is minimized. This is the most important reason for using the desaturation technique, in addition to the ability to do away with the face mask[6]

The method is based on the Fick principle and is essentially a modification of the Kety–Schmidt technique In order to carry out the desaturation procedure, the brain is first saturated with approximately 8 μc of krypton-85 by re-breathing it for 7 min in the closed circuit of a BMR machine or portable anæsthesia machine

During the last minute of saturation, prior to removal of the face mask, continuous internal jugular venous and arterial blood samples are obtained to estimate the quality of krypton in the brain at the end of saturation After removal of the face mask, continuous 1-min jugular venous and arterial blood samples (3 c c each) are drawn during the first 2 min of desaturation Individual 3 c c blood samples are then obtained during the remaining desaturation period at the end of the 2nd, 3rd and 4th min and thereafter every 2 min to the 12th min A 12-min desaturation period was chosen to provide a slightly longer period of examination than in the original 10 min of the nitrous oxide method This may be of particular value in patients with a lowered cerebral blood flow

Blood samples are obtained in 10 c c oiled-heparinized syringes via 5-stopcock manifolds After removal of the syringes from the manifolds, 7 c c of air are drawn into each syringe to a total of 10 c c (3 c c blood–7 c c air) Since krypton is 25 times more soluble in air than in blood, more than 90 per cent of the krypton is released into the air-phase The air-phase is then injected into a glass-jacketed Geiger–Muller tube by the displacement of mercury and counted on a standard scaler

The concentration of krypton or the counts per min of each sample is plotted against time as shown in Fig 1 The cerebral blood flow (CBF) is calculated using the Fick equation*:

$$\mathrm{CBF} = \frac{(C_t/W) \times \lambda \times 100}{\int_0^{12} (C_v - C_a)\,dt}$$

The (C_t/W) is assumed to be equal to the concentration of krypton in the venous blood at the end of saturation (C_{v_s}) minus the concentration at the end of desaturation (C_{v_d}):

$$C_t/W = C_{v_s} - C_{v_d}$$

The blood brain partition coefficient for krypton, λ, has been calculated by Lassen[2] It is constant from individual to individual with a normal hæmoglobin level and is only slightly above unity, that is, 1 06 A value for λ of 1 00 was used in these investigations In patients with anæmia or polycythæmia the partition coefficient must be taken into consideration when calculating the cerebral blood flow

Table 1

	Mean	*S D*
Cerebral blood flow (CBF), c c blood/100 g brain/min	56 5	7 7
Cerebral vascular resistance (CVR), mm Hg/c c blood/100 g brain/min	1 52	0 89
Cerebral metabolic rate ($CMRO_2$), c c O_2/100 g brain/min	3 37	0 5

$$\mathrm{CBF} = \frac{(C_{v_s} - C_{v_d}) \times 100}{\int_0^{12} (C_v - C_a)\,dt}$$

Using the krypton desaturation method, investigations of the cerebral circulation were performed on 25 normal males (mean age 27 years) with the results shown in Table 1 The mean arterial carbon dioxide tension during these investigations was 45·93 volumes per cent with *S D.* 2 94 A typical normal desaturation curve is shown in Fig 1

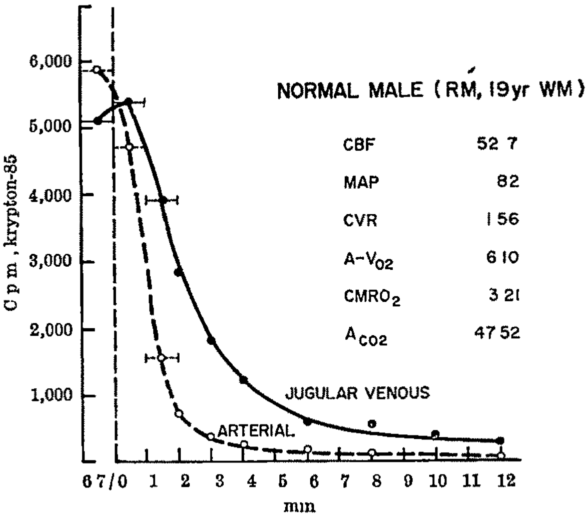

Fig 1 A krypton-85 desaturation curve in a normal male CBF, cerebral blood flow, c c blood/100 g brain/min, MAP, mean arterial blood pressure, mm mercury, CVR, cerebral vascular resistance, mm mercury/c c blood/100 g brain/min, A-V_{O_2}, arteriovenous oxygen difference, volume per cent, $CMRO_2$, cerebral metabolic rate, c c O_2/100 g brain/min, A_{CO_2}, arterial carbon dioxide content, volumes per cent

In order to test the reproducibility of the method, two cerebral blood flow investigations (o) were performed on the same individual, one after the other at an interval of 10 min The mean values for five cases were (1) 55 3 c c and (2) 56 2 c c The individual values (o) are plotted in Fig 2 and indicate a high degree of reproducibility

In order to evaluate the period of time required for brain saturation prior to the desaturation investigations, two cerebral blood flow investigations (×) were performed on six patients The first was done after 7 min saturation and the second after 15 min saturation. The mean value for the first (1) cerebral blood flow was 45 4, and for the second (2) 44 2 The results (×) of this work are also shown in Fig 2 Although the mean CBF values obtained are quite close, in a patient with low CBF there was a decrease of 15 per cent between the first, 17 3 c c, and second, 14 7 c c, CBF value obtained after longer saturation This demonstrates that in patients with diminished cerebral circulation or metabolism a longer period of saturation is necessary prior to the examination of cerebral blood flow

This method, based on the Fick principle, is essentially a modification of the inert gas method of Kety and Schmidt Instead of measuring the arteriovenous difference during saturation of the brain, arteriovenous

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* C_t = concentration of krypton-85 given off by the whole brain during 12 min desaturation measured from the end of saturation C_v = concentration of krypton-85 in the cerebral venous blood during 12 min desaturation C_a = concentration of krypton-85 in the arterial blood during 12 min desaturation C_{v_s} = concentration of krypton-85 in the cerebral venous blood at the end of saturation C_{v_d} = concentration of krypton-85 in the cerebral venous blood at the end of desaturation W = weight of brain λ = blood brain partition coefficient for krypton

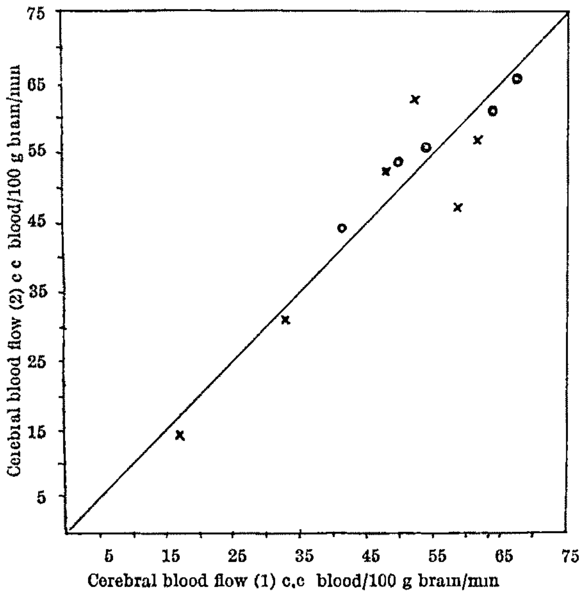

Fig 2 Values (O) obtained from two cerebral blood flow investigations performed on the same normal individual (1) after the other (2) at a 10-min interval preceded by 7 min saturation Values (×) obtained from two cerebral blood flow investigations on the same patient at a 10-min interval preceded by (1) 7 min saturation and by (2) 15 min saturation

differences during desaturation are measured The denominator of the Fick equation is the integral of the veno-arterial krypton difference during desaturation. The numerator of the equation is the concentration of krypton in the venous blood at the end of saturation minus the amount remaining in the venous blood at the end of desaturation

The value for cerebral blood flow, 56 5 c c , obtained by this method is similar to values reported by Lewis *et al* [4] Using krypton-79 desaturation, they obtained a mean CBF value of 57 3 c c A simultaneous nitrous oxide desaturation mean CBF value was 57 5 c c These values are slightly higher than that, 54 c c , obtained originally by Kety and Schmidt[1] using nitrous oxide saturation, but are less than the mean CBF value, 65 c c , obtained by the Scheinberg and Stead[7] modification. The mean values for cerebral metabolic rate, 3 37 c c oxygen, and for cerebral vascular resistance, 1 52 mm mercury, obtained by this krypton-85 desaturation method are similar to accepted values

The validity of the desaturation method will primarily depend on the length of time of saturation prior to the desaturation investigation Since inert gas equilibrium between blood and brain actually takes less than 10 min (about 7 min) to occur[8], a 7-min period was chosen as the minimal saturation time for normal individuals Although only based on six determinations, the actual comparison of mean cerebral blood flow values after 7 and 15 min saturation would indicate that 7 min is probably adequate for normal individuals On the basis of theoretical and empirical evidence outside of these results[6], however, a period of 10–15 min would be preferable, particularly when abnormalities of the cerebral circulation are being investigated

I thank Drs Niels Lassen, Louis Sokoloff and S. S Kety for their advice

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EFFECT OF KINETIN ON SENESCENCE AND ABSCISSION IN EXPLANTS OF *PHASEOLUS VULGARIS*

By DAPHNE J OSBORNE and SUSAN E. MOSS
Agricultural Research Council Unit of Experimental Agronomy, Department of Agriculture,
University of Oxford

MUCH of our knowledge concerning the effects of plant hormones and related substances on abscission has been derived from observations on the abscission of leaves and other parts of whole plants, and from examination of the control of separation in excised segments of petioles or stems which contain abscission zones These segments are usually referred to as 'explants'

Depending on the concentration, and the position of application relative to the abscission zone, the auxins indolyl-3-acetic acid or 2,4-dichlorophenoxyacetic acid will either accelerate or retard separation at the abscission zone both in intact plants of *Gossypium* or *Phaseolus* and in their explants[1] The effect of gibberellin on *Gossypium* explants is similarly dependent on the concentration and position of application[2] A low concentration of ethylene in the surrounding air normally accelerates the abscission of leaves in intact plants[3,4], as well as accelerating separation in abscission zones of explants[5] A number of naturally occurring, non-volatile substances isolated from plant tissues will also accelerate abscission in *Phaseolus*[6] or *Gossypium* explants[7]

From a consideration of the effects of these different kinds of substances on abscission it would seem that there are certain features of cellular physiology which are common to the processes of separation, and that abscission occurs only when these conditions are fulfilled Many workers have already pointed out that abscission of an organ is associated with senescence of that organ, and that separation is dependent on senescence in the abscission zone itself[8] Provided that the senescent condition is attained, the nature of the agent which brings about senescence is not critical to the actual processes of separation For example, it has been shown[5] that separation of abscission zones in leaves or explants of *Phaseolus* does not occur so long as there is a gradient of pectinmethylesterase activity from the pulvinar to the petiolar side of the zone, but during abscission this gradient changes, and at separation the pectinmethylesterase activity on either side of the zone is the same These changes can be induced by ethylene, by a number of different chemical defoliants or by complete removal of the leaf blade

It seems unlikely that abscission can be directly controlled by such different causal agents, it is more likely that each of these treatments results in similar kinds of biochemical changes in the cells of the abscission zone A decline in the total protein content and a loss of chlorophyll, both symptoms of senescence, have been demonstrated during abscission in *Phaseolus* explants[5], particularly in the parts distal to the zone Evidence of this kind suggests that separation of the cells at an abscission zone should be examined in the wider physiological context of cellular senescence

This article records some experiments with the plant growth regulator kinetin, and the results support the view that abscission in explants of *Phaseolus* is primarily a problem of cellular senescence at the abscission zone

One segment, 1 cm long, was cut from the leaf-stalk of each of the two fully expanded primary leaves of 14-day-old plants of *Phaseolus vulgaris* Each segment included the whole of the distal pulvinus, the abscission zone and part of the petiole below the zone The segments were arranged, groove side up, on notched 'Perspex' racks embedded in 3 per cent agar in 9-cm Petri dishes[5] The segment from one primary leaf of each plant was treated and the segment from the other leaf was a control Twelve treated and twelve corresponding control explants were arranged in each Petri dish, and at least two dishes were used for each treatment in each experiment The individual experiments were repeated many times

Kinetin was made up in aqueous solution at 10, 25 and 50 mg/l and measured drops of this solution were applied to either the pulvinar, the petiolar or to both ends of the explants with an Agla micro-syringe fitted with a Vernier attachment Similar volumes of distilled water were added to each control segment The dishes were kept

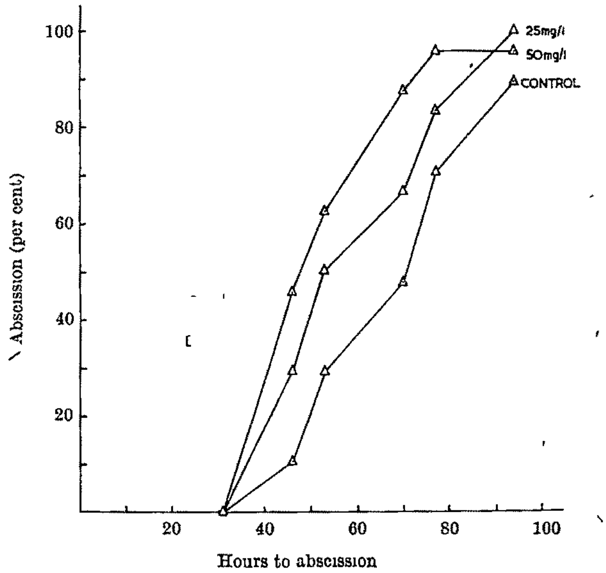

Fig 1 Acceleration of abscission by application of 0 002 ml of aqueous kinetin solution (50 mg/l and 25 mg/l) to the pulvinar ends of explants of *Phaseolus* Controls treated with water

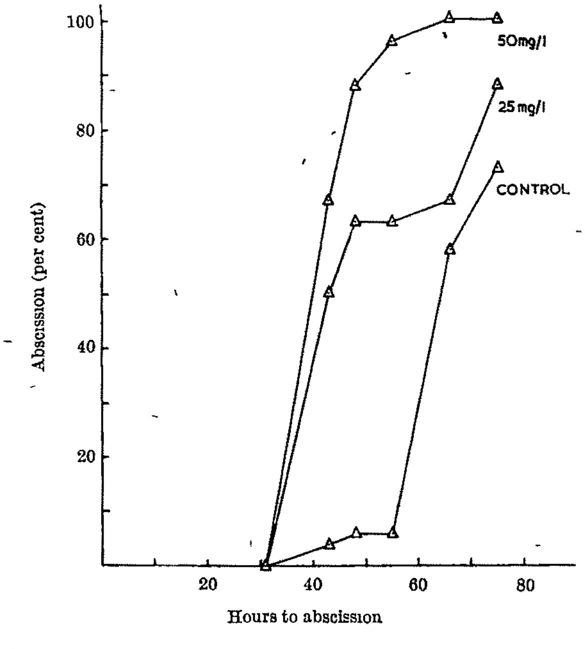

Fig 2. Acceleration of abscission by application of 0 002 ml of aqueous kinetin solution (50 mg/l and 25 mg/l) to the petiolar ends of explants of *Phaseolus* Controls treated with water

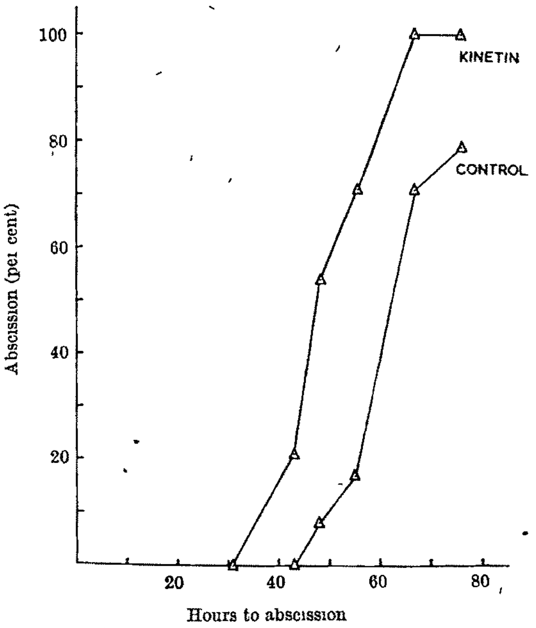

Fig 3 Acceleration of abscission by application of 0 001 ml of aqueous kinetin solution (50 mg/l) to both pulvinar and petiolar ends of explants of *Phaseolus* Controls treated at both ends with water

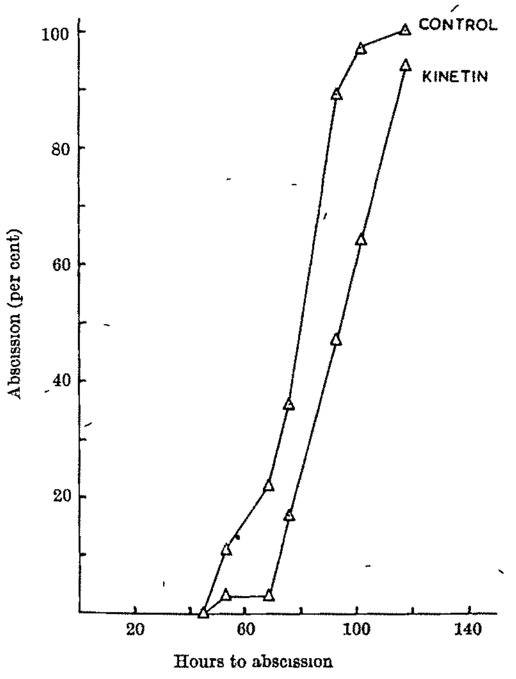

Fig 4 Retardation of abscission by application of 1 per cent kinetin in lanolin directly to the abscission zone of explants of *Phaseolus* Controls treated with plain lanolin

under humid conditions in a dark room controlled at 24·5° C The number of explants which had separated into two parts at the zone of abscission was recorded at intervals, and the results are expressed in Figs. 1–4 as percentage abscission against the time in hours from the start of the experiment

When kinetin is supplied to the ends of the explant, abscission is accelerated Applications of 0 002 ml drops containing 25 or 50 mg/l kinetin to the pulvinar (Fig 1) or petiolar (Fig 2) ends, or of 0 001 ml drops to both ends (Fig 3), cause more rapid abscission At the lowest concentration (10 mg/l), kinetin did not affect the time of separation

Applications of kinetin directly to the abscission zone, however, retard separation Here, a narrow ring of lanolin containing 1 per cent kinetin, encircling the line of separation between pulvinus and petiole, effectively delays abscission compared with controls treated with plain lanolin (Fig 4)

These results show that, dependent on the position of the application, kinetin can either accelerate or retard abscission The observed dual role of kinetin is therefore not unlike that of auxins and gibberellin, both of which accelerate or delay abscission depending on their position of application and the concentration supplied

In view of the differences in the chemical constitution of these different growth regulators and their known differences in physiological activity in plant tissues, their role in abscission processes is sought in some function which is common to them all.

The retardation of senescence in leaf tissue by treatment with auxins[9], kinins[10] and gibberellins[11] has been reported for a number of different species For auxins and kinetin, this effect has been associated with a maintenance of protein and nucleic acid synthesis[12,13] The treated areas have also been shown to act as loci for the accumulation of both organic and inorganic substances For example, endogenous metabolites accumulate in the parts of individual leaves treated with an auxin[14] or kinetin[15], while exogenously applied substances have been shown to move more readily into, or to be retained more readily within, those parts that have been treated with an auxin[16] or kinetin[17].

Very low doses of an auxin may well become immobilized at the cut ends of the explant to which they are supplied[1,18], or, particularly for proximal applications, the auxin may fail to be transported the length of the segment[19] Under these conditions the ends which are treated with auxins might then function as loci of accumulation Any accumulation of essential metabolites at, or near, these cut ends may well involve the depletion of other parts of the segment Since abscission zone cells will frequently separate before complete senescence of a leaf blade it is possible that the abscission zone is particularly sensitive to the withdrawal of some essential metabolites, which results in an accelerated senescence and separation When higher doses of an auxin are supplied[1,18], all the cells of the explant may receive a sufficient supply of the auxin to prevent movement of metabolites from any one part to another, and therefore premature senescence in the abscission zone should not occur In fact, abscission is then retarded This can be associated with the known effect of auxins in retarding senescence in excised leaves[9] and with the more recent findings[12] that auxins maintain protein synthesis in leaves under these conditions

It is proposed that the effects of kinetin on abscission in *Phaseolus* explants are, like those of auxins, mediated through a control of senescence processes, and are dependent on the content of kinetin in the cells of the abscission zone, compared with those in other parts of the segment When kinetin is applied directly to the zone in lanolin, and the local concentration is presumably relatively high, this region may act as a locus of accumulation and also of high synthetic activity, with the result that senescence and abscission are retarded If kinetin is supplied elsewhere to the explant, then the cells of the zone become depleted of essential metabolites and undergo a premature senescence which results in accelerated abscission

We wish to thank Prof G E Blackman for his interest and encouragement

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INDUCED TEMPERATURE TOLERANCE OF PLANT TISSUE *IN VITRO*

By PROF. C. A SCHROEDER

University of California, Los Angeles

THE concept of temperature lesions in plant tissues which may be corrected by exogenous metabolites of several types[1] suggests that heat tolerance of such tissues might be increased by appropriate manipulation of the environment During a series of investigations of the effects of temperature on tissue explants from avocado fruit pericarp grown under sterile conditions on agar media[2], it was noted that survival to extremely high temperatures could be induced with appropriate preconditioning treatment Empirical observations indicated that tissue taken from firm, mature fruit will tolerate a temperature of 50° C for 10 min with no apparent impairment of subsequent growth potential whereas exposure to 55° C for 10 min resulted in killing of the tissues Incubation of tissue at 25° C allows for optimum growth of the segments by cell proliferation If the tissue was pretreated by exposure to 50° C for 10 min, incubated at 25° C for 3 days and then subjected to 55° C for 10 min and again incubated at 25° C for a period of 4 weeks, there appears to be survival of the pretreated tissues whereas nonpretreated control tissue fails to grow when similarly exposed Survival and subsequent growth in this case are determined by evidence of mitotic activity which can be detected under the microscope and by increase in fresh and dry weight following a standard 4-week final incubation period

The basic technique involved is that used for tissue culture under sterile conditions[3] Six-dram screw cap vials are used, each containing 10 ml of Nitsch's[4] media in which Geigy-138 iron-chelate is substituted for the iron citrate and with the addition of 10 p p m of indolyl-3-acetic acid Pericarp disks 2 mm thick and 8 mm diam are prepared from fresh, firm fruits under sterile conditions The disks are placed on the nutrient agar in the vials which are then immersed in a temperature-controlled water bath for appropriate periods Incubation is accomplished in temperature-controlled cabinets at 25° C Mitotic activity is observed in those sections which survive and show growth.

A recent review of responses of plant materials and bacteria to high temperatures[5] indicates that this behaviour can be explained on the basis of differential reaction rates of synchronous metabolic systems competing for substrate materials at successively higher temperatures resulting in a condition of 'temperature unbalance' Conditioning of a tissue by thermal shock could inactivate heat-sensitive enzyme systems allowing the more heat-labile enzymes to build up products or by-products which can then be made readily available for further reactions at higher temperatures The results in Fig. 1 indicate a progressive increase in survival percentage of individual tissue disks with increase in time following initial pretreatment Thus in the work recorded here a maximum protective response is attained approximately two or three days following initial sub-lethal exposure This high-level survival response is maintained for a period of two or three days and is again lost as the pretreated tissue develops a state of equilibrium and afterwards begins a period of mitotic activity Control tissues untreated by thermal shock show positive evidence of mitotic activity well under way three days after planting on agar nutrient media The non-pretreated control tissues in active mitotic condition are killed on exposure to high temperature at the 3-day period and show no further growth Pretreated tissues tested under comparable conditions (55° C, 10 min) 3 days after pretreatment show mitotic activity and growth at the termination of the final incubation period

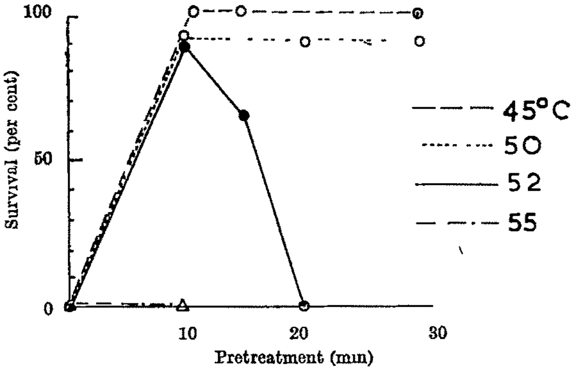

Fig 1 Survival of avocado pericarp tissue to high temperature (55° C) *in vitro* in response to pretreatment temperatures

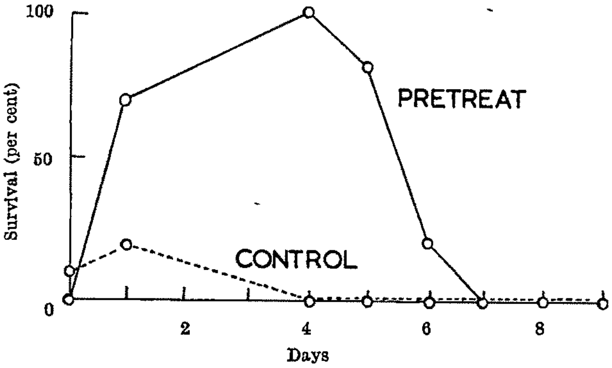

Fig 2 Survival of avocado pericarp tissue (at 55° C) in relation to time (in days) following pretreatment at 50° C Control not pretreated

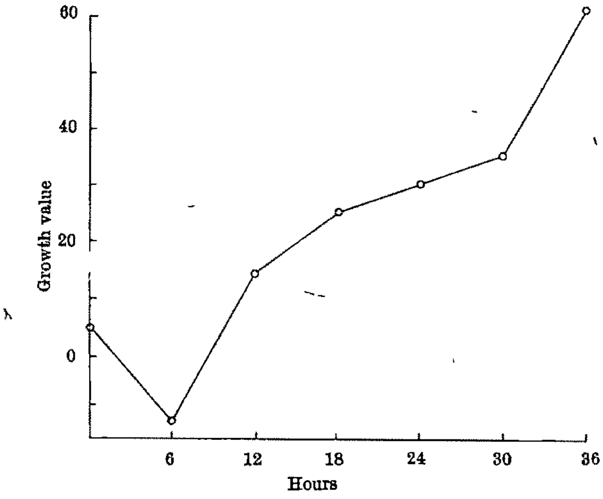

Fig 3 Growth value (difference between initial and final weights) of pretreated avocado pericarp tissue disks *in vitro* exposed to 55° C at various times (h) following pretreatment at 50° C

Pretreatment at 45°, 50° and 52° for 10 min respectively (Fig 1) provides essentially equal conditioning of tissue for subsequent high-temperature exposure, but as pretreatment temperature time is increased, there is apparent injury to the tissue and a failure to survive at the longer pretreatment exposures of 20 min at 52° C Some preliminary experiments have suggested that exposure time of 2–3 min at 50° C can provide sufficient thermal shock for the tissue to tolerate the standard test of 55° C, 10 min after 3 days' incubation

The thermal shock response is depicted in Fig. 3 in which the growth value (difference between initial and final weights) of pretreated disks minus the growth value of control disks is plotted against time (in hours) after pretreatment at which high-temperature test exposure is made These results suggest an accumulation of a substance or the development of a condition which provides protection against extreme temperature This condition reaches a maximum about 4 days after pretreatment and then declines in effectiveness (Fig 2).

The relationship between auxins and other exogenous materials to the alteration of protein coagulability as affected by heat has been demonstrated by Galston *et al* [6]. The several theories concerned primarily with protein and other organic molecules and their relationship with heat and cold tolerance of intact leaf segments to heat have been demonstrated[8]

It appears that the phenomenon of heat tolerance can be induced in plant tissue *in vivo* and *in vitro* and that tentative explanations are available concerning this response[7] The use of tissue culture techniques appears to offer a refined approach to the testing of the phenomenon and to aid in possible analysis concerning some of the biochemical reactions which may be involved

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LETTERS TO THE EDITOR

PHYSICS

Observations of the *Zeta* Spectrum in the Wave-length Range 16 Å–400 Å

SPECTROSCOPIC studies of *Zeta*[1] are now being carried out in the important wave-length region below 400 Å. Previous investigations in the vacuum ultra-violet have been mainly confined to the region between 400 Å and 2000 Å using a variety of vacuum grating spectrometers with both photographic and photoelectric detectors. Thus, spectral line identifications have been made by Butt *et al.*[1], and Fawcett *et al.*[2]. Measurement and interpretation of the temporal variation of spectral line intensities have been made by Burton and Wilson[3], and Hobbs *et al.*[4], and measurement of the Doppler profiles of spectral lines has been made by Jones and Wilson[5]. More recently, observations have been made by Heroux[6,7], who measured the relative intensities of spectral lines in order to determine the electron temperature, using a grazing incidence rocket monochromator[8].

Recent observations using grazing incidence spectrometers designed and built at Culham have extended the range of measurement down to 16 Å, and it is the purpose of this letter to give an early survey of these results. Two instruments were used in this work. The first is a 1-m instrument[9] with both photographic and photoelectric recording, using a 1,080 line/mm original grating ruled at the Nobel Institute, Stockholm, and operated at 5° grazing angle. This is a laboratory instrument designed to examine high-temperature plasmas, and has been used for the photoelectric studies and for accurate wave-length calibration of strong lines in higher orders. The second is a rocket instrument with photographic recording designed to study the solar spectrum from above the atmosphere. It has a Bausch and Lomb 600-line/mm blazed replica grating of 1 metre radius, and can operate at grazing angles between 1° and 4°. Fitted with a vacuum tank it has been used on *Zeta* for photographic survey work and has recorded the *Zeta* spectra reproduced in this letter.

For most of this work the *Zeta* discharge was operated in deuterium or hydrogen at a pressure of 0·5 m torr, sometimes with a small addition of other gases. The electron temperature was not measured during these observations, but can be inferred from the work of Heroux[7] to vary in the range 20 eV–40 eV. Two spectral ranges are of particular interest and have been selected for discussion.

(*a*) 16 Å–50 Å. This region contains the important resonance lines of the common impurity elements carbon, nitrogen and oxygen in their helium-like and hydrogen-like ionic species. These are shown in a section of *Zeta* spectrum reproduced in Fig. 1. This was obtained with a 1° grazing angle and recorded on Kodak *S.W.R.* film. The filling gas was deuterium at 0·5 m torr pressure to which 2 per cent of air and a trace of argon were added. The gas current reached a peak of 630 k.amp, and an axial stabilizing magnetic field of 1,040 gauss was applied. Identification of the stronger lines is indicated in Fig. 1 and details are listed in Table 1. The shortest wave-length so far observed is at 16·0 Å and is tentatively identified as the Lyman β line of OVIII.

(*b*) 170 Å–212 Å. Above 50 Å the spectral lines are generally formed by more complex systems but identifications are proceeding satisfactorily. An important exception occurs in the wave-length range 170 Å–212 Å. A very intense system of lines occurs in this region, the wave-lengths of which do not coincide with any known lines. A group of intense lines is also present in this region of the solar spectrum as observed by Tousey *et al.*[10], Behring *et al.*[11], and Hinteregger[12], and these lines also remain unidentified. The *Zeta* spectrum in this region is reproduced in Fig. 2, together with the solar spectrum of Tousey *et al.*[10]. The wave-lengths of the more intense *Zeta* lines have been measured in the fourth and fifth order using first order neon reference lines[2,13,14] and the weaker members in the first order using oxygen standards[15]. These wave-lengths and their approximate relative intensities have been listed in Table 2 together with the wave-lengths and relative intensities of some of the solar lines. The latter have been derived by Allen[16] from published[10–12] and circulated data.

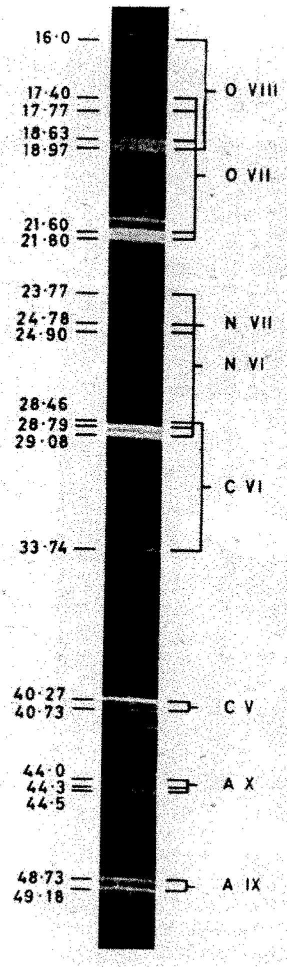
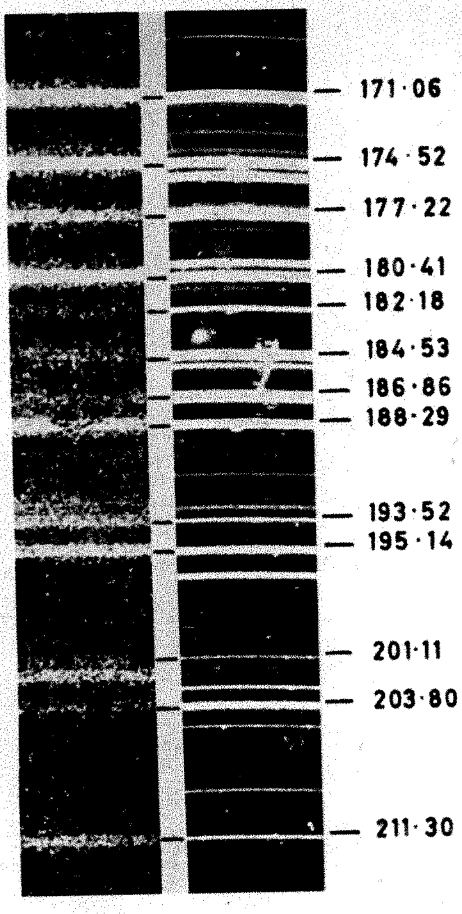


Fig. 1

Table 1. SOME OF THE IDENTIFIED SHORT WAVE-LENGTH *Zeta* LINES

Ion	λ (Å)	Transition
CV	40·27	$1^1S - 2^1P^o$
	40·73	$1^1S - 2^3P^o$
CVI	33·73	$1^2S - 2^2P^o$
	28·46	$1^2S - 3^2P^o$
NVI	28·79	$1^1S - 2^1P^o$
	29·08	$1^1S - 2^3P^o$
NVII	24·79	$1^2S - 2^2P^o$
OVII	21·60	$1^1S - 2^1P^o$
	21·80	$1^1S - 2^3P^o$
	18·63	$1^1S - 3^1P^o$
OVIII	18·97	$1^2S - 2^2P^o$
AIX	48·73	$2p^6\ ^1S - 3s\ [\frac{1}{2}]^o$
	49·18	$2p^6\ ^1S - 3s\ [1\frac{1}{2}]^o$
AX	44·5 44·3 44·0 43·3 43·0	$2p^5\ ^2P^o - 2p^4\ 3s\ (^4P, ^2P)$



Sun *Zeta*

Fig. 2

It is clear from Fig. 2 and Table 2 that, with a few exceptions (notably the solar line at 201·9 Å), these lines match in wave-length within the errors of measurement and have similar relative intensities. There can be little doubt that many of these lines arise from the same transitions in the two sources. This is of some importance since the production of the lines in the laboratory strengthens considerably the chances of identification.

These lines from *Zeta* cannot be identified as being due to known impurities present or introduced in the filling gas, that is, carbon, nitrogen, oxygen, neon and argon, as the addition of controlled quantities of these elements failed to produce the expected changes in their intensities. Furthermore, the lines are still present when the discharge is operated in a mixture of neon and argon (that is, without hydrogen present). Photoelectric studies of these lines showed that their peak intensity is reached at the time of peak current and increases with increasing current and with decreasing stabilizing magnetic field. These observations are consistent with the unknown lines being due to material liberated from the surface of the stainless steel wall during the discharge-cycle. Work to extend the identification of the *Zeta* spectrum is proceeding and includes investigation of the unknown lines between 170 Å and 212 Å.

Table 2. Unidentified Lines between 170 Å and 212 Å

Zeta lines			Solar lines		
λ (Å)	± (Å)	Relative intensity	λ (Å)	± (Å)	Relative intensity
171·06	0·02	11	171·06	0·1	11
174·52	0·02	10	174·48	0·1	10
175·26	0·02	8	175·26	0·1	1
177·22	0·02	8	177·23	0·1	8
179·76	0·02	7			
180·41	0·02	9	180·39	0·1	10
182·18	0·02	6	182·19	0·1	3
184·53	0·02	6	184·57	0·1	4
184·80	0·02	6			
185·22	0·02	6	185·26	0·1	1
186·86	0·02	8	186·86	0·1	8
188·29	0·02	8	188·25	0·1	8
193·54	0·05	5	193·43	0·1	5
195·14	0·05	8	195·03	0·1	7
196·64	0·02	6	196·73	0·1	1
201·11	0·05	3			
201·72	0·05	2			
			201·88	0·1	14
202·00	0·05	2			
202·81	0·02	5			
203·80	0·02	9	203·62	0·1	2
205·02	0·02	3			
211·30	0·05	4	211·22	0·1	4

This work represents the initial phase of a general programme at Culham Laboratory to develop grazing incidence techniques for the study of high-temperature plasmas. This includes launching the rocket spectrograph described, together with a number of other instruments designed to investigate solar radiation, in the first British stabilized rocket during the coming year.

We thank several colleagues for valuable assistance, Mr. R. S. Pease for useful discussions, and Dr. R. Tousey for permission to reproduce a print of his solar spectrum. Finally, we thank Prof. C. W. Allen for valuable advice and for providing us with his data on the solar spectrum.

B. C. Fawcett
A. H. Gabriel
W. G. Griffin
B. B. Jones
R. Wilson

U.K. Atomic Energy Authority,
Culham Laboratory,
Abingdon, Berks.

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Magnetic Effects in the Far Infra-red Spectrum of Nitrogen Dioxide (NO_2)

In this communication we report the first observation of magnetic splittings in the far infra-red spectrum of a simple molecule. These effects can be predicted qualitatively from the free radical nature of the molecule and quantitatively from the analysis of the microwave spectrum[1]. An earlier search in this region[2] failed to exhibit these effects, due to lack of adequate resolving power. With the attainment of resolution of 0·10 cm^{-1} by interferometric methods[3], the phenomenon can now be observed.

The magnetic interactions which produce the splittings observed in the spectrum of nitrogen dioxide (NO_2) involve a coupling of the odd electron spin to the magnetic moment generated by rotation of the molecule. The principal term involved is the indirect coupling term, which can be treated as a mixing of the excited electronic states of the molecule with the $^2\Sigma$ ground-state. This coupling has been treated by several authors, and Curl and Kinsey[4] have reduced all these treatments to a common basis. Lin's formulation of the theory is the most convenient for

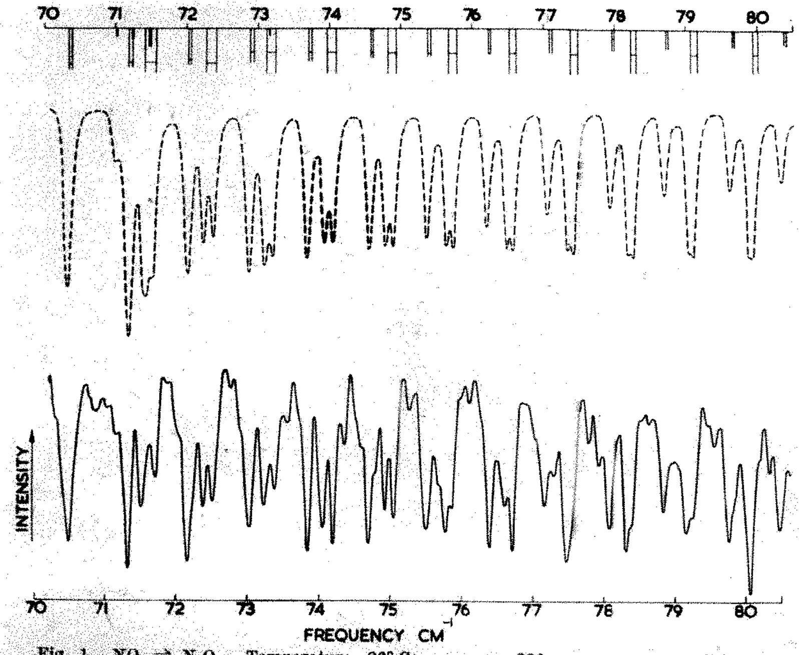

Fig. 1. $NO_2 \rightleftharpoons N_2O_4$. Temperature, 26° C; pressure, 226 mm mercury; cell-length, 44 cm; resolution, 0·1 cm^{-1}

the present problem[5]. This theory has been extended to the case of finite asymmetry[6]; but the symmetric top approximation ($b_p = 0$) is adequate for present purposes. The complete equations for the energies of the two magnetic levels $J = N + \frac{1}{2}$ and $J = N - \frac{1}{2}$ are given by equations (1*a*) and (1*b*), with the coupling constants shown in equation (2):

$$W(N, K_{-1}K_{+1}, J = N + \tfrac{1}{2}) = W^\circ(N, K_{-1}K_{+1}) + \frac{N}{2}\,\varkappa_{NK}$$

$$W(N, K_{-1}K_{+1}, J = N - \tfrac{1}{2}) = W^\circ(N, K_{-1}K_{+1}) - \frac{(N+1)}{2}\,\varkappa_{NK} \qquad (1a)$$

$$\varkappa_{NK} = \left[\frac{\varepsilon_B + \varepsilon_C}{2}\right] + \frac{K_{-1}{}^2}{N(N+1)}\left[\varepsilon_A - \frac{\varepsilon_B + \varepsilon_C}{2}\right] + \left\{\frac{(-1)^N}{4}\,\delta_{1K_{-1}} - \frac{b_p F^2(N, K+2)}{16N(N+1)(K+1)} + \frac{b_p F^2(N, K-2)}{16N(N+1)(K-1)}\right\}[\varepsilon_B - \varepsilon_C] \qquad (1b)$$

where: N, K_{-1}, K_{+1} are the usual asymmetric top quantum numbers:

$J = N \pm \frac{1}{2}$ is the sum of electron spin plus rotational angular momentum

ε_i is the spin-rotation coupling constant for rotation, about the i_{th} axis.

$\varkappa_{NK}$ is simply a convenient intermediate computational term, constant for a given rotational state.

$\delta_{1K_{-1}} = 1$ when $K_{-1} = 1$, 0, otherwise

$$b_p = \frac{C - B}{2\left[A - \dfrac{B+C}{2}\right]}$$: the Wang asymmetry parameter.

$$F^2(N, K \pm 2) = \{N(N+1) - K(K \pm 1)\}\,\{N(N+1) - (K \pm 1)(K \pm 2)\}$$

This term vanishes for $F^2(N, 1)$, $F^2(N, 0)$ and for $F^2(N, 2)$ associated with the state $N_{2,N-1}$:

$$\varepsilon_A = 2\zeta A \sum_l \frac{|(O|L_A|l)|^2}{E_0 - E_l} + a_A \qquad (2)$$

ζ = spin-orbit coupling constant.

A = reciprocal moment of inertia.

l = summation over excited electronic states.

$|0(|L_A|)|^2$ = squared transition element to excited state l.

a_A = direct-coupling term usually $\ll \varepsilon_A$.

It is found in the microwave analysis that the *B*- and *C*-axis coupling constants are relatively small, and may be ignored in treating the far infra-red data at this level of resolution, except for levels with $K_{-1} = 1$.

Some early interferometric spectra of NO_2 were taken while the microwave analysis was still in progress, but the present spectra at high resolution were not obtained until the microwave analysis was substantially complete. A theoretical spectrum was calculated from the preliminary microwave parameters as shown in Table 1, using Dr. R. H. Schwendeman's values of calculated Wang expansion coefficients for the energies of a slightly asymmetric top to 5th order in asymmetry. The rotational transitions were calculated on the IBM 7090 computer at the Massachusetts Institute of Technology, and the output cards from this programme were then carried through a simple splitting programme to give the final spectrum complete with relative intensities. A contour matching the observed spectrum in resolution has been produced from the theoretical results and compared directly with the observed spectrum as shown in Fig. 1. Magnetic effects are present throughout the spectrum, but in most cases the well-split lines are overlapped by other strong lines. The region 70–80 cm^{-1} happens to be free of this sort of overlap, and the regular progression of doublets is a set of related lines showing splitting greater than 0·10 cm^{-1}. In Fig. 1, the theoretical profile has been displaced towards high frequency by 0·1 cm^{-1}, to match the observed curve. Otherwise the observed curve is in agreement with the predictions from the microwave spectrum.

Table 1. MICROWAVE PARAMETERS OF NO_2*

$\frac{B+C}{2}$ = 0·422004 cm^{-1}

$A - \frac{B+C}{2}$ = 7·578805 cm^{-1}

b_p = − 0·001532786

D_J = 2·795 × 10^{-7} cm^{-1}

D_{JK} = − 1·910 × 10^{-5} cm^{-1}

$\varepsilon_A(1)$ = + 0·1518 cm^{-1}

D_K = 2·532 × 10^{-3} cm^{-1}

* A more refined set of parameters will appear in ref. 1; differences from the present set will have trivial effects on the far infra-red spectrum. Centrifugal distortion has been calculated on a symmetrical top basis—a procedure adequate except for K = 0, 1, 2.

$\varepsilon_A(1)$ is the *a*-axis spin rotation parameter obtained from first-order calculation. The more accurate value derived from second-order corrections is $\varepsilon_A(2) = 0{\cdot}1804_4$ cm^{-1}. The calculations given here are consistent with the use of $\varepsilon_A(1)$.

There is one very important distinction between a microwave spectrum and a high-resolution far infra-red spectrum, and this lies in the ease of analysis. In the microwave region one sees data which are at once too detailed and too narrowly selected from the entire rotational spectrum. Possession of the far infra-red spectrum allows one to look first at broad patterns and to proceed smoothly through increasing degrees of complexity in the analysis. Two of the writers of this paper have been concerned with both types of spectra and can state from experience that the ratio of times expended in analysing spectra in these two regions is about one month of work in the microwave spectrum per hour of work in the far infra-red. Whenever the spectrum of some new polyatomic free radical is obtained in both the microwave and far infra-red regions, we would predict that the analysis will proceed most easily through initial treatment of the far infra-red spectrum followed by detailed analysis of the microwave spectrum.

H. A. GEBBIE
N. W. B. STONE

Basic Physics Division,
National Physical Laboratory,
Teddington, Middlesex.

GEORGE R. BIRD

Research Laboratories,
Polaroid Corporation,
Cambridge, Mass.

GRAHAM R. HUNT

Spectroscopy Laboratory,
Massachusetts Institute of Technology,
Cambridge, Mass.

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Sedimentation of a Large Number of Particles as a Cluster in Air

IN an attempt to explain the distribution produced on the ground by the sedimentation of particles from an "instantaneous point source" under the influence of the wind, Davies[1] assumed that the particles fell with their Stokes velocity and had no influence on the rate of fall of neighbouring particles. The complicating factors of the differing settling velocities, resulting from an extended range of particle sizes, and turbulent diffusion, from the influence of the wind, make it difficult to test this assumption under his experimental conditions. It would, however, be remarkable if particles did not affect each other when falling through air as a cluster, as they do in other fluids[2].

An examination has been made of the sedimentation of clusters 25–90 cm diameter, composed of small spherical particles of glass and also of 'Diakon', through still air. In each case the particles were exhaustively sieved through sieves which complied with B.S.S. 410 and dispersed as a roughly spherical cluster, the initial distribution along the horizontal axes being Gaussian. An attempt was made to obtain a Gaussian distribution vertically, but this was abandoned when it was found that the behaviour of the cluster was independent of the distribution of the particles for a given size of cloud. Three different types of behaviour have been observed all of which indicate mutual interference in the sedimentation of the particles. In all cases the rate of fall of the cluster exceeded the terminal velocity of the individual particles.

With 'Diakon' particles less than 64μ diameter, clusters approximately 50 cm diameter which contained approximately 2 g of material fell initially as roughly spherical vortices with vertical axes. The circulation of the particles in these clusters was downwards in the centre. This observation was in accord with the behaviour of suspensions in liquids and similar, though of opposite sense, to the rise of masses of hot moist air to form cumulus clouds[3].

When the particles were larger (>150μ) the behaviour of the cluster changed. Immediately on release a circulation of the same nature as above was set up; this was transient and the particles became distributed in the form of a bowl (Fig. 1). These bowl-shaped clusters were formed in all cases when the terminal velocity of the individual particles was greater than about a third of that of the sedimentation rate of the cluster as a whole. It would appear that this distribution of particles resulted from the inability of the individuals to rise at the edges of the vortex at a sufficient velocity to maintain the vortex.

When the weight of material dispersed was increased the cluster fell in a turbulent manner. The mass at which turbulent behaviour set in increased with increasing particle size and increasing density of the disperse phase.

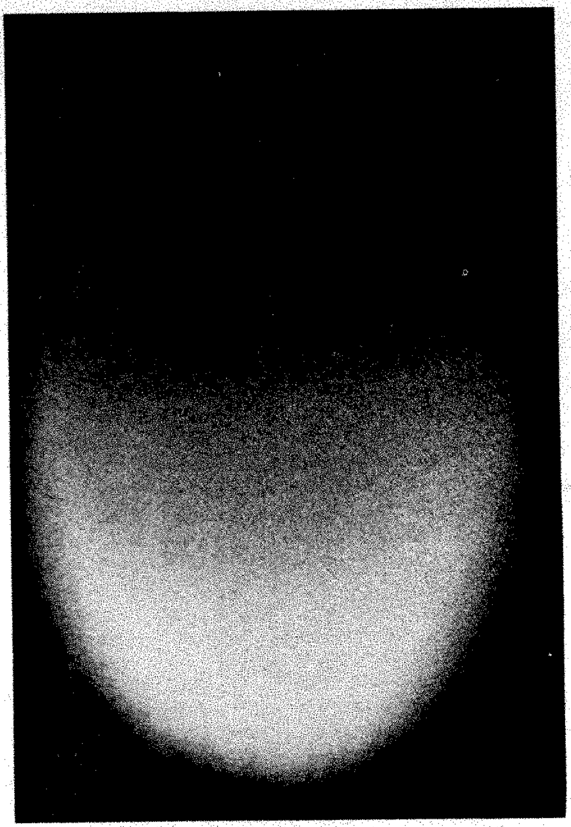

Fig. 1. Bowl-shaped cluster of falling particles. (*Courtesy* H.M.S.O.)

G. W. SLACK

War Department,
Chemical Defence Experimental Establishment,
Porton Down,
Salisbury, Wilts.

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GEOPHYSICS

A Solar Anisotropy in Cosmic Radiation, viewed from a Terrestrial Frame of Reference

IT is generally recognized that a solar anisotropy in the primary cosmic radiation must be responsible for at least part of the observed terrestrial daily variation of intensity, although it seems that the exact nature of this contribution is not yet clear. However, Rao, McCracken and Venkatesan[1] have recently presented a method of determining the characteristics of a daily variation of this type. Their method takes into consideration the influence of the Earth's magnetic field and in particular they emphasize the part played by the asymptotic cones of acceptance of cosmic ray detectors. Consequently, using experimental values of the daily variation of neutron intensity averaged over the year 1958, they have been able to postulate a type of anisotropy which appears to account for the complex manner in which the amplitude and phase of the first harmonic of the daily variation depends on the geographical location of the detector. However, it would perhaps be truer to say that they predict that if the Earth's atmosphere and its magnetic field were removed the annual mean diurnal variation of intensity observed at the Earth's surface would have the following characteristics: (1) the diurnal variation would be independent of rigidity in the range 1–200 GV, (2) the amplitude would vary as the cosine of geographic latitude and the time of maximum would be in the direction 85° to the east of the Earth–Sun line, (3) the maximum amplitude, observed with narrow-angle detectors at the equator, would be 0 4 per cent of the average primary flux It is proposed to describe here the co-ordinate transformations which relate these important characteristics to those of a solar anisotropy specified in the frame of reference in which its orientation is fixed

Following Rao *et al*, let us suppose that the intensity anisotropy is fixed in the co-ordinate system the reference planes of which are the ecliptic and the plane normal to it through the Earth–Sun line In this framework let us specify the anisotropic component of the total intensity as $AI_0(R) \cos \Lambda \cos (\psi - \psi_0)$, where A is the amplitude constant, $I_0(R)$, the average cosmic ray differential rigidity spectrum, Λ, the ecliptic latitude of the detector, and ψ, its direction of viewing in the plane of the ecliptic, measured eastward of the Earth–Sun line, with ψ_0 the direction of maximum intensity In what follows it will be more convenient to refer the direction of viewing in the plane of the ecliptic to the direction of maximum intensity and express it as $\psi - \psi_0$ We now have to transform to the terrestrial co-ordinate system the reference planes of which are the equator and the plane normal to it through the Earth–Sun line

Transforming first to the conventional ecliptic system, specified by the plane of the ecliptic and the equinox, the spherical co-ordinates of the direction of viewing become Λ and $\lambda_a + \psi - \psi_0$, where λ_a is the ecliptic longitude in the direction of maximum intensity The corresponding co-ordinates in the terrestrial equatorial system, specified by the equator and the equinox, are the geographic declination δ and the right ascension $\alpha_a + \varphi - \varphi_0$, where α_a is the right ascension of the direction of maximum intensity and $\varphi - \varphi_0$ are the directions of viewing and of maximum intensity in the plane of the equator, both measured eastward of the Earth–Sun line We can now transform simply from one set of co-ordinates to the other by rotation of the axes of one of the co-ordinate systems through the angle ε without change of origin, ε being the obliquity of the ecliptic Projections on the appropriate axes yield

$$\cos \Lambda \cos (\lambda_a + \psi - \psi_0) = \cos \delta \cos (\alpha_a + \varphi - \varphi_0) \tag{1}$$

and

$$\cos \Lambda \sin (\lambda_a + \psi - \psi_0) = \cos \delta \sin (\alpha_a + \varphi - \varphi_0) + \sin \delta \sin \varepsilon \tag{2}$$

Since the maximum difference between ecliptic longitude λ_a and right ascension α_a never exceeds about 2 5 degrees (cf values for the Sun in the *Nautical Almanac*) α_a can be substituted for λ_a in equations 1 and 2 Expanding and combining equations 1 and 2 to eliminate $\sin \psi$, we obtain·

$$\begin{aligned}\cos \Lambda \cos (\psi - \psi_0) = {} & [1 - (1 - \cos \varepsilon) \sin^2 \alpha_a] \cos \delta \cos (\varphi - \varphi_0) \\ & - \frac{1 - \cos \varepsilon}{2} \cos \delta \sin 2\alpha_a \sin (\varphi - \varphi_0) \\ & + \sin \varepsilon \sin \delta \sin \alpha_a \end{aligned} \tag{3}$$

The right ascension of the anisotropy, α_a, is $\alpha_a = \alpha_s + \varphi_0$, where α_s is the Sun's right ascension We can replace φ_0 by ψ_0 since, like λ_a and α_a, the two constants never differ by more than about 2 5 degrees Therefore making these substitutions in equation 3 and multiplying through by $AI_0(R)$ we finally have

$$\begin{aligned} AI_0(R) \cos \Lambda \cos (\psi - \psi_0) = {} & AI_0(R) \{[1 - (1 - \cos \varepsilon) \sin^2 (\alpha_s + \psi_0)] \cos \delta \cos (\varphi - \psi_0) - \left(\frac{1 - \cos \varepsilon}{2}\right) \cos \delta \sin 2(\alpha_s + \psi_0) \sin \\ & (\varphi - \psi_0) + \sin \varepsilon \sin \delta \sin (\alpha_s + \psi_0)\} \qquad (4) \\ = {} & AI_0(R) \{[1 - 0\ 0826 \sin^2 (\alpha_s + \psi_0)] \cos \delta \cos (\varphi - \psi_0) \\ & - 0\ 0413 \cos \delta \sin 2 (\alpha_s + \psi_0) \sin (\varphi - \psi_0) \\ & + 0\ 3979 \sin \delta \sin (\alpha_s + \psi_0)\} \text{ for } \varepsilon = 23^\circ\ 27' \end{aligned}$$

On the right-hand side of equation 4 the anisotropic component of primary intensity is expressed as a function of geographic latitude, time of day relative to local noon, and day of the year as specified by α_s, which, for practical purposes, can be regarded as the right ascension of the Sun at transit at Greenwich The co-ordinates δ and φ refer, of course, to the asymptotic direction of viewing of a cosmic ray detector with respect to primaries of rigidity R

It may be of interest to mention an application of equation 4 to observations of the intensity of sunlight falling on unit area of the Earth's surface In this case, $\psi_0 = 0$ and the conditions for sunrise and sunset are that $\psi = \pm \pi/2$ The solutions for φ then give the approximate sundial times of sunrise and sunset at the latitude δ at the time of the year specified by α_s The co-ordinates δ and φ may be likened to asymptotic directions of viewing since the atmosphere, and therefore refraction, has been disregarded

We note that $AI_0(R) \cos \Lambda \cos (\psi - \psi_0)$ transforms to a solar diurnal variation and a total intensity term $AI_0(R) \sin \varepsilon \sin \delta \sin (\alpha_s + \psi_0)$ to which it reduces at the geographic poles That is to say, at the poles the anisotropy manifests itself as a small seasonal variation of the total intensity The expression for the diurnal variation at other latitudes allows the seasonal variations of amplitude and phase to be worked out For example, at the latitude of Hobart (43° S) these amount to a small semi-annual variation of amplitude, the maximum departure from the mean being less than 10 per cent, and an almost negligible (less than 5 degrees) semi-annual variation of phase Spurious sidereal and anti-sidereal effects are absent

The annual mean expression for the anisotropy is obtained by integrating equation 4 with respect to α_s, giving

$$\begin{aligned} AI_0(R) \cos \Lambda \cos (\psi - \psi_0) & = AI_0(R) \left(\frac{1 + \cos \varepsilon}{2}\right) \cos \delta \cos (\varphi - \psi_0) \\ & = 0\ 9587\ A \cos \delta \cos (\varphi - \psi_0) \text{ for } \varepsilon = 23^\circ\ 27' \end{aligned} \tag{5}$$

Therefore it happens that on the yearly average the anisotropy postulated by Rao *et al* would exhibit the same characteristics, except for a slight difference in amplitude, whether viewed from the terrestrial frame of reference or from that in which its orientation was fixed As might be expected a similar result would be obtained if the orientation were fixed in the plane of the Sun's equator instead of the plane of the ecliptic However, seasonal variations of amplitude and phase would be more pronounced and small apparent sidereal and anti-sidereal effects would be generated

If the latitude dependence differs from $\cos \Lambda$, the characteristics of a solar anisotropy may become greatly modified on transformation to the terrestrial frame of reference The cases $A \cos \psi$, $A \cos^2 \Lambda \cos \psi$, $A \cos^3 \Lambda \cos \psi$ and $A \cos^5 \Lambda \cos \psi$ will be discussed elsewhere Although transformation procedures for a simple type of sidereal anisotropy will also be mentioned a more detailed treatment may be found in a paper by L Davis[2]

In conclusion, it is hoped that the method outlined here may have indicated the need for correct transformations (*a*) when the characteristics of the anisotropy in its rest framework are to be deduced from the annual mean daily variation observed in the terrestrial frame of reference, after accounting for the influence of the atmosphere and the Earth's magnetic field, (*b*) when the day-by-day contributions which the specified anisotropy makes to the daily variation and to the total intensity of cosmic radiation in the terrestrial frame of reference are to be determined and the seasonal effects calculated

R M JACKLYN

Antarctic Division,
Department of External Affairs,
568 St Kilda Road, Melbourne, S C 3,
and Department of Physics,
University of Tasmania, Hobart

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GEOLOGY

Dissolved Gases in East African Lakes

ACCORDING to the knowledge we have concerning the volcanic gases of Nyiragongo, the nearest volcano to the Lake Kivu of all the volcanoes of the Virunga Range, which constitutes the natural dam behind which Lake Kivu has formed, I cannot agree completely with Dr Kevin Burke's and Prof G Mueller's views regarding the origin of the dissolved gases of this Lake (*Nature*, 198, 568, 1963)

In 1959, we were able to collect gases close to, and even on, the permanent lava lake of Nyiragongo volcano[1] The sampling conditions were exceptionally good, in fact the best available so far with similar ones encountered by Shepherd[2] and Jaggar[3] in 1919 at the Halemaumau lava lake of Kilauea (Hawaii) Our own analyses, like those of Shepherd, showed no trace of methane But all of them disclosed relatively high contents of unoxidized gases carbon monoxide (average amount) 1 7 per cent of the 'active gases' (other than water, nitrogen + argon, oxygen) at Kilauea, 5 2 per cent at Nyiragongo and hydrogen (1 9 per cent for both Kilauea and Nyiragongo), moreover, Kilauea had also a sulphur content attaining 3 6 per cent of the 'active gases'

The nitrogen/oxygen average ratio in our four best Nyiragongo analysis is 10/1 5, and the nitrogen content (1 65–14 5 per cent) is quite different from that which should be expected should atmospheric oxygen have been consumed in oxidation of methane It seems difficult to accept the idea of all 'juvenile' methane having been oxidized prior to its escaping from vents situated very close to the molten lava lakes, while sulphur, carbon monoxide and hydrogen, on the contrary, are still present in significant proportions

On the other hand, methane has been found only in those volcanic gases—collected relatively far from the molten lava—on volcanoes situated in a geologically quite different situation, namely, in orogenic belts: Ambrym volcano (New Hebrides)[4], Guadeloupe[5], Santorin[6], Novarupta and the Valley of Ten Thousand Smokes[7], Kliuchevskii[8], Bezymiannyi[9], Asama Yama[10], etc

The analyses of the occluded gases in the lava (melilite basanite) of Nyiragongo[11] show no methane but 23 per cent carbon monoxide and 13 per cent hydrogen for 60 per cent carbon dioxide and 4 per cent nitrogen. This very high content of unoxidized gases (36 per cent), the low content of nitrogen (4 per cent) and the total absence of methane, all argue against a juvenile origin hypothesis of methane in the waters of the close-by Lake Kivu On the contrary, methane has been found occluded in the lavas from orogenic belts Tuscany ignimbrites[12], Mont Pelée (Martinique)[13], Novarupta[14]

All these facts show, I think, that the absence of methane in the volcanic gases of Nyiragongo is original, and is not due to atmospheric oxidation

Therefore, the high methane content of Lake Kivu should not be attributed to volcanism, and most probably, as Kufferath and Schmitz have supposed, is of biogenic origin; while carbon dioxide, the main constituent of the Nyiragongo 'active gases', would be of a juvenile origin

H TAZIEFF

15 Quai de Bourbon,
Paris, 4

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TAZIEFF's conclusion that the presence of substantial amounts of carbon monoxide and hydrogen in gas from Nyiragongo rules out the possibility of juvenile methane existing at depth is not justified since oxidation of methane by steam according to the reaction $CH_4 + H_2O = CO + 3H_2$ would yield just the gases that appear in his analyses This reaction has been investigated at one atmosphere[1,2] at which pressure equilibrium lies well to the right My library facilities are limited and I have been unable to find an account of variation with pressure, but clearly high pressure will shift the equilibrium towards the left Oxidation of methane by steam is not, of course, atmospheric oxidation and I regret the omission of a reference to alternative means of oxidation in my original communication

If methane is a widespread constituent of magmatic gas at depth its occurrence in surface gas from orogenic belt volcanoes may be correlated with their generally explosive character Slow upwelling of lava, as at Kilauea and Nyiragongo, might permit complete oxidation of methane by steam while rapidly rising gas, characteristic of the orogenic belts, might not be completely oxidized

I regard the nitrogen content of volcanic gas from a lava lake as of limited value in assessing the extent of atmospheric oxidation because oxygen can be added to the magma without nitrogen through reaction on the lake surface and subsequent reduction of oxidized material carried downward by convection

A final answer to the question of origin cannot be given until more complete analyses (and especially isotopic analyses) of the Lake Kivu gas are available for comparison with the excellent results obtained for gas from Nyiragongo by Tazieff and his colleagues

KEVIN BURKE

Department of Geology,
University of the West Indies,
Mona, Kingston, Jamaica

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Primary and Secondary Arcs in the Scandinavian Caledonian Chain

PROF J TUZO WILSON *et al* [1] have given an outline of the European Caledonian and Hercynian mountains Regarding the Scandinavian Caledonian chain these authors mention that secondary arcs can readily be made out in Sweden, and that igneous rocks of primary arcs are exposed in the Lofoten Islands off Norway

I agree that secondary arcs can be made out in Sweden With regard to the Lofoten Islands, rhenium/osmium determinations of molybdenites from these Islands gave the pre-Caledonian ages 2080 and 2290 million years[2] According to my investigations other Norwegian coast districts form parts of primary arcs

The Scandinavian Caledonian chain became inactive tectonically in Devonian time The chain is now weathered down, and parts of it are beneath the sea We can, however, still trace a narrow mountain system[3] (Fig. 1) The primary arcs lie through the following Norwegian counties Møre and Romsdal, Nordland, and Troms The arcs are now exposed as Caledonian gneisses and schists

The junctions of these arcs at which secondary arcs formed are in central and in northern Scandinavia The secondary arcs consist of two parts, an uplifted massif or

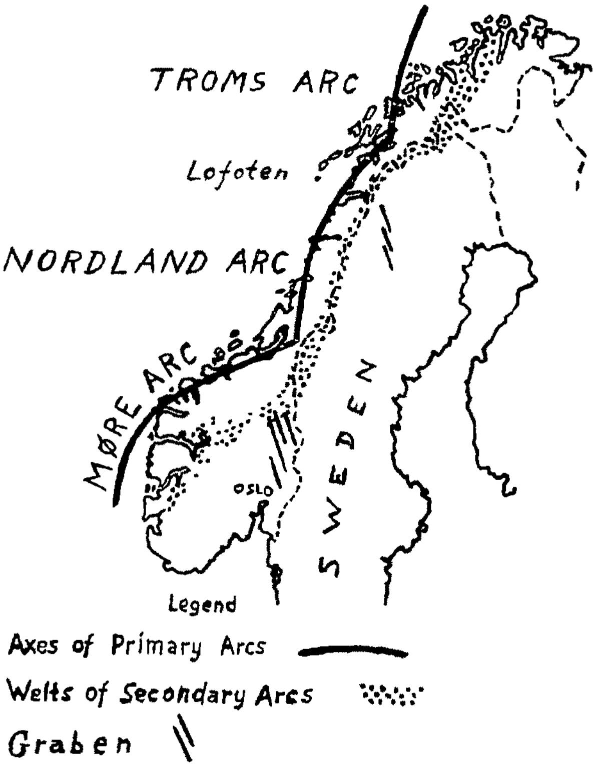

Fig. 1. The Scandinavian Caledonian chain, an inactive narrow mountain system

welt of the Precambrian basement, and, further from the primary arcs, a basin with thrust and folded Caledonian sediments. For example, the south Norwegian sparagmite region, with the Precambrian basement exposed in windows, forms a part of the uplifted massif, and the Oslo region is a preserved part of the corresponding basin of the southern secondary arc.

The graben corresponding to the southern secondary arc is exposed in the south Norwegian sparagmite region. Drilling has shown evidence of a buried graben[4], which corresponds to the northern secondary arc.

I. HERNES

Geological Institute,
University of Bergen,
Norway

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METALLURGY

Uhlig Model of Oxidation Kinetics

IT is well known that a space charge region is usually present in semiconducting oxides at metal-semiconductor junctions due to equalizing of Fermi levels at the junction[1]. Uhlig[2] has pointed out that this space charge layer may be a determining factor for growth rate and for growth law. Specifically, Uhlig proposes that the space charge layer limits the flow of electrons (and thus the flow of ions) from metal to oxide, so the variation of the space charge layer characteristics with film thickness determines the growth law. The purpose of this communication is to point out some basic errors in the treatment of the model so that further development of this approach, as well as comparison with new experimental results, can be fruitful.

The first objection to the treatment of Uhlig (see equation (5), ref. 2) is the misinterpretation of the position co-ordinate x for the total film thickness $y(t)$ in Poisson's equation. This leads to the application of physically meaningless boundary conditions, because the conditions are imposed for certain values of $y(t)$ [$y(t) = 0$ and $y(t) = l$] corresponding to certain fixed points in time, instead of for certain values of x [for example, $x = 0$ and $x = y(t)$] which hold for all time. In particular, the Uhlig treatment leads to a non-conservation of electrical charge during growth, provided the surface charge is constant at the air-oxide interface as claimed[2]. The predicted effects of space charge on rate are enhanced enormously for growth of thin films, since the parameter l denoting the extent of the space charge region in very thick films thus is introduced into the equations for growth of very thin films.

The second objection is the reduction of the metal-oxide work function by the electron affinity of oxygen adsorbed on the oxide even when the oxide film thickness is greater than the maximum distance which the electron can penetrate into the oxide from the metal crystal in a one-step process (for example, tunnelling or field emission into the oxide). We therefore offer the following modified derivation of the logarithmic law based on the same model.

An integration of Poisson's equation

$$\frac{d^2V}{dx^2} = -\frac{4\pi ne}{\varepsilon} \qquad (1)$$

for a constant density $n = n_0$ of trapped electrons of charge $e = -|e|$ gives

$$\frac{dV}{dx} = -E(0) + \frac{4\pi n_0|e|}{\varepsilon}x \qquad (2)$$

where $V(x)$ is the electrical potential, x is the position co-ordinate in the oxide normal to the metal surface with $x = 0$ being the metal-oxide interface, $E(0) = \frac{q\sigma}{\varepsilon}$ is the electrical field in the oxide at $x = 0$ due to the charged particle interfacial density, σ, at $x = 0$, q is the charge per particle at $x = 0$, and ε is the dielectric constant of the film. Further integration of equation (2) gives the electrical potential difference $[V(x) - V_0]$ from the interior of the metal to a point x in the film

$$V(x) - V_0 = -E(0)x + \frac{2\pi n_0|e|}{\varepsilon}x^2 \qquad (3)$$

The physically meaningful boundary conditions for the problem are constant electrical potential inside the metal, which can be designated as the zero of potential $[V_0 = 0]$ and over-all charge neutrality of the oxide-metal system. Any charged defects produced in the film induce an equal but opposite charge in the metal at $x = 0$. Thus for the case of zero surface charge density at $x = y(t)$, which is the outer surface of the oxide film

$$E(0) = \int_0^{y(t)} \frac{4\pi n_0|e|}{\varepsilon}\,dx = \frac{4\pi n_0|e|}{\varepsilon}y(t) \qquad (4)$$

and equation (3) reduces to

$$V(x) = -\frac{2\pi n_0|e|}{\varepsilon}[2y(t)x - x^2] \qquad (5)$$

Denoting by φ the Fermi-level E_0 of the neutral bulk oxide minus the Fermi-level E_m of the neutral metal, the total potential $\zeta(x)$ for an electron at position x in the oxide with respect to the interior of the metal is

$$\zeta(x) = \varphi - V(x) = \varphi + |V(x)| \qquad (6)$$

The injection of an electron from the metal into the oxide generally takes place against a potential barrier. The thickness of the barrier for thermionic emission is approximately one lattice constant a, but conceivably there could be thermal excitation to excited states with subsequent tunnelling through barriers of some greater thickness. We denote by δ the maximum tunnel thickness for the particular barrier $\zeta(x)$ given by equation (6) $[0 \leqslant x \leqslant \delta]$; this parameter δ must be computed or obtained experimentally for any given system, but is of the order of 50 Å If $y(t) < \delta$, then electrons can tunnel from the metal to adsorbed oxygen at the oxide–oxygen interface For this case, the activation energy ΔE for oxidation is given approximately by the difference in potentials at $x = 0$ and $x = y(t)$, minus K, where K is defined as the electron affinity of adsorbed oxygen on either the metal ($y(t) = 0$) or on the oxide ($y(t) > 0$) Using equations (5) and (6)

$$\Delta E = |e|\left[\varphi - K + \frac{2\pi n_0|e|}{\varepsilon} y(t)^2\right] \quad (y(t) < \delta) \qquad (7)$$

Since $y(t) < \delta$ for this case, the term in $y(t)^2$ is generally small compared with $\varphi - K$ (0 5–1 eV)^2 so long as $n_0 \ll 10^{18}/\text{cm}^3$, which we arbitrarily call the small space charge limit in this work For this limiting case

$$\Delta E \simeq |e|(\varphi - K) \qquad (8)$$

This is the same expression as the one suggested by Uhlig[2], although the present derivation is valid only in the small space charge limit and for film thicknesses $y(t)$ less than the maximum tunnel distance δ For $y(t) < \delta$ in the large space charge limit $[n_0 \geqslant 10^{18}/\text{cm}^3]$, equation (7) must be used to obtain ΔE

For $y(t) > \delta$, the parameter K cannot serve to aid the electron in its initial escape from the metal crystal, which is assumed to be the rate limiting step Equation (6) is then applicable, with x replaced by δ:

$$\Delta E = |e|\ \zeta(\delta) = |e|\left[\varphi + \frac{2\pi n_0|e|}{\varepsilon}\left\{2y(t)\delta - \delta^2\right\}\right] \quad (y(t) > \delta) \qquad (9)$$

For $y(t) \gg \delta$,

$$\Delta E \simeq |e|\left[\varphi + \frac{4\pi n_0|e|\delta}{\varepsilon} y(t)\right] \qquad (10)$$

Using an expression for the particle current J [note that $\mathrm{d}y(t)/\mathrm{d}t \propto J$] equivalent to that used by Uhlig[2]

$$J \propto e^{-\Delta E/kT} \qquad (11)$$

the following growth laws are obtained for the several limiting cases given above for ΔE

(*a*) for $y(t) < \delta$ and $n_0 \ll 10^{18}/\text{cm}^3$,

$$\frac{\mathrm{d}y(t)}{\mathrm{d}t} = A' \exp\left[-|e|(\varphi - K)/kT\right] \qquad (12)$$

(*b*) For $y(t) < \delta$ and $n_0 > 10^{18}/\text{cm}^3$,

$$\frac{\mathrm{d}y(t)}{\mathrm{d}t} = A' \exp\left[-|e|\left\{\varphi - K + \frac{2\pi n_0|e|}{\varepsilon} y(t)^2\right\}/kT\right] \qquad (13)$$

(*c*) For $y(t) \gg \delta$,

$$\frac{\mathrm{d}y(t)}{\mathrm{d}t} = A' \exp\left[-|e|\left\{\varphi + \frac{4\pi n_0|e|\delta}{\varepsilon} y(t)\right\}/kT\right] \qquad (14)$$

Thus the relation

$$y(t) = k_0 \log(1 + t/\tau) \qquad (15)$$

is obtained by integrating equation (14), where k_0 is given by $[\varepsilon kT/(4\pi n_0|e|^2\delta)]$ and τ is given by $(k_0/A')\exp(|e|\varphi/kT)$ Note from these results that for a given magnitude of space charge the value for k_0 is increased by ratio l/δ over that given in ref 2, where l represents some maximum width of the constant space charge region Since δ lies between 5 and about 50 Å, and $l \simeq 6 \times 10^{-4}$ cm, $l/\delta \simeq 10^3$ to 10^4 (Alternatively, this work requires an increase in the density of charged traps by this factor to give a certain value of k_0) Furthermore, in equation (14) the space charge term is competing with the large value φ compared with the much smaller value of $[\varphi - K]$ as given in ref 2, so that a change in rate is relatively slower to manifest itself in equation (14) In other words, the τ for equation (15) is found to be of the order of $(l/\delta)\exp\{|e|K/kT\}$ larger using equation (14) than is obtained in the work of ref 2 Since $K \simeq$ 3–4 eV, this factor is approximately exp (+100) (Alternatively, for a given τ these equations require a much smaller metal-oxide work function φ The φ must have a value of about 0 5–1 eV corresponding to the $(\varphi - K)$ in ref 2)

A linear growth law is obtained from equation (12), and a growth law with a sharper cut-off at the limiting thickness than the logarithmic law is obtained from equation (13) These two laws are not expected to be very important, however, because of the restriction to film thicknesses less than about 50 Å It should be mentioned that the Uhlig model does not provide for an electronic transport mechanism for $x > \delta$ In addition, ionic transport is totally ignored Thus some mechanism for easy ionic transport must be assumed in order to have any growth at all, and some easy electronic transport mechanism for $x > \delta$ must be assumed in order to obtain film thicknesses greater than δ

This work is not extended to the diffuse space charge layer[2] The case for any distribution of non-diffusing space charge can be treated in the above manner provided the expressions can be integrated An equilibrium distribution of charged defects, however, is the wrong basis for examining space charge effects in growing oxide films[3]

In summary, then, the correct use of Poisson's equation and the application of physically meaningful boundary conditions to the Uhlig space charge model for the logarithmic growth law has revealed that the predicted concentration of charged defects must be increased by a factor of 10^3–10^4 and the metal-oxide work function decreased by almost an order of magnitude to yield an appreciable effect on growth rate Thus a charged defect concentration greater than approximately $10^{18}/\text{cm}^3$, and a work function lower than about 0 5 eV are prerequisites for this model to be applicable to the growth of thin oxide films

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A T FROMHOLD, JUN

Sandia Laboratory,
Albuquerque,
New Mexico.

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Diffusion of Oxygen in Tungsten and some other Transition Metals

Stringer and Rosenfield[1] have recently calculated some activation energies for diffusion of oxygen in transition metals, which were not otherwise available One of these metals was tungsten In a plot of activation energy versus melting point of the solvent, the authors attempted to show that tungsten and other metals dissolving less than about 1 per cent oxygen lay on a straight line through the origin; while those metals dissolving more than about 1 per cent oxygen fell on a straight line, which did not pass through the origin and which had a small negative slope The line through the origin was supposed to be indicative of a normal lattice distortion mechanism and the other line an ionization mechanism

Diffusivity data for oxygen in tungsten are now available following an internal friction study[2] The experimental activation energy is observed in Fig 1 to fit the 'high-solubility' line of Stringer and Rosenfield Assuming that a real difference in diffusion mechanism does underlie the 'low-' and 'high-solubility' lines, the possibility of correlating the solubility with the mechanism now seems more remote

The 'high-solubility' line can be explained in the same terms of elastic strain as the line through the origin Zener's well-known theory of D_0 predicts a linear relation-

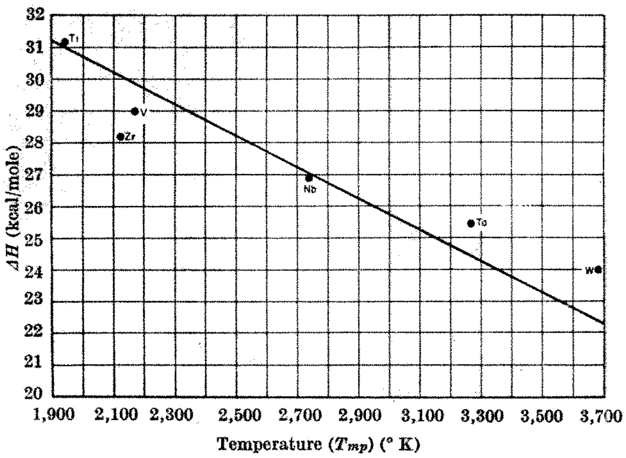

Fig. 1. Heat of activation for diffusion of oxygen in various transition metals having the melting points shown

ship between the energy of activation (ΔH) and the melting point of the solvent (T_{mp}) when:

$$T_{mp}\frac{\delta\left(\frac{\Delta S}{\beta}\right)}{\delta T_{mp}}+\frac{\Delta S}{\beta}=K$$

where ΔS is the entropy change, and β and K are constants. Since ΔS and β are always positive, a negative slope (that is, negative K) can only result when $\frac{\delta\left(\frac{\Delta S}{\beta}\right)}{\delta T_{mp}}$ is negative and $\left|T_{mp}\frac{\delta\left(\frac{\Delta S}{\beta}\right)}{\delta T_{mp}}\right|>\frac{\Delta S}{\beta}$. This condition is indeed met, as shown by the plot of theoretical $\Delta S/\beta$ versus T_{mp} in Fig. 2, and the good agreement with experimental points. The point for tungsten is the only one deviating to an appreciable extent from theory, and this is probably due to the instability of the internal friction peaks and a resultant high D_0. This instability would be expected to have a negligible effect on ΔH. Theoretical entropy changes were calculated from Zener's equation, $\Delta S = \beta\Delta H/T_{mp}$, using $\beta = 0{\cdot}4$ and the ΔH's listed in Table 1. The experimental values of ΔS were obtained from the formula, $D_0 = \gamma a_0^2 \nu \exp\frac{\Delta S}{R}$, using $\gamma = 1/6$, $\nu = 10^{13}$/sec and the lattice parameters (a_0) and D_0's appearing in Table 1.

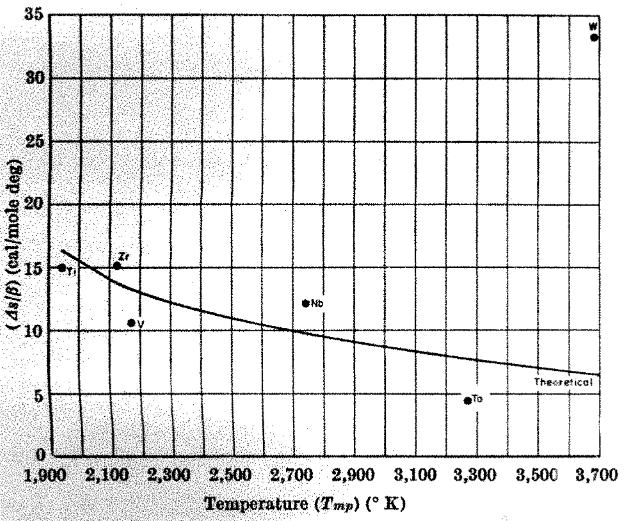

Fig. 2. Theoretical (curve) and experimental (points) changes in entropy for the diffusion of oxygen in various transition metals

Table 1. LATTICE PARAMETERS OF SOLVENT METALS AND DIFFUSION CONSTANTS FOR OXYGEN IN THESE METALS

Solvent	a_0 (Å)	D_0 (cm²/sec)	$\Delta H\left(\frac{\text{cal.}}{\text{mole}}\right)$
Zr	3·609 (862° C) (ref. 3)	0·0453 (ref. 4)	28,200 (ref. 4)
Ti	3·32 (900° C) (ref. 3)	0·083 (ref. 5)	31,200 (ref. 5)
V	3·039 (ref. 3)	0·013 (ref. 6)	29,000 (ref. 6)
Nb	3·301 (ref. 3)	0·021 (ref. 6)	26,900 (ref. 6)
Ta	3·303 (ref. 3)	0·0044 (ref. 6)	25,450 (ref. 6)
W	3·165 (ref. 3)	1·3 (ref. 2)	24,000 (ref. 2)

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A. J. JACOBS

Battelle Memorial Institute,
505 King Avenue,
Columbus 1, Ohio.

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Boron Constituents in Low-boron Steel

IT has been demonstrated by autoradiography that, in steel and related alloys containing boron, this element sometimes segregates to grain boundaries of the matrix. This has to be taken into account when considering the damaging effect of neutron irradiation, boron-10 capturing neutrons and emitting lithium and helium.

To determine the degree of boron enrichment by identifying possibly existent boron-containing phases, thin foils of a steel containing 0·0026 per cent boron have been examined by selected area electron diffraction. The material was austenitized at 1,110° C and isothermally transformed at 650° C for 1 h. This treatment is known to reveal the presence of particles of a particular boron constituent in the rim of ferrite which is formed along the austenite grain boundaries[1]. Such particles are shown in Fig. 1, which is a transmission electron micrograph of a thin foil steel specimen. When comparing the electron diffraction patterns obtained from these particles (Fig. 2) with known crystallographic data for conceivable boron-containing phases, correlation was found for Fe_2B and FeB.

This result is interesting both from the point of view of the application of such material in nuclear reactors, mentioned here, and as an advancement of the methods of detecting boron enrichment. Previously the necessary boron content in alloys in which segregation could be found, by autoradiography, was 0·005 per cent[2]. As to the exis-

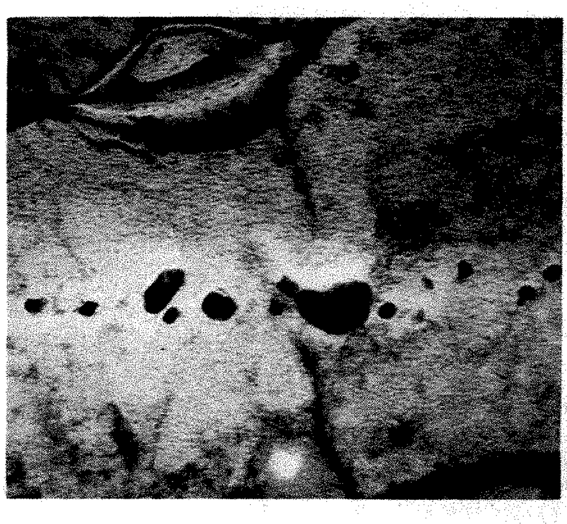

Fig. 1. Boron constituent particles (thin foil, × c. 23,700)

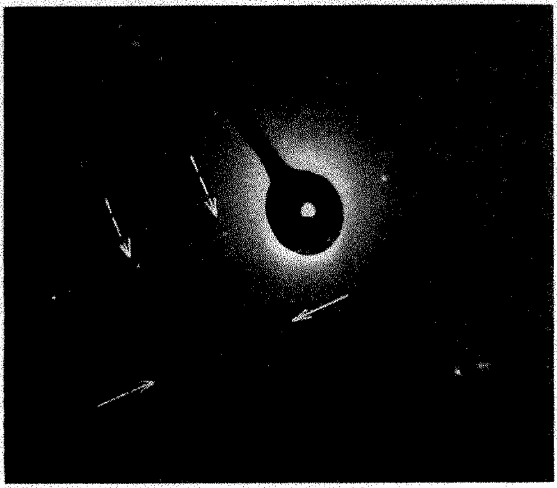

Fig. 2. Diffraction pattern of area containing Fe_2B (full arrows) and FeB (dashed arrows)

tence of FeB in addition to Fe_2B, this is not unexpected, since FeB has been found by electron microprobe analysis in a steel containing 0·1 per cent boron[3].

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GUSTAF ÖSTBERG

Aktiebolaget Atomenergi,
Lövholmsvägen 7,
Stockholm, 9.

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CRYSTALLOGRAPHY

Similarity of Octacalcium Phosphate and Hydroxyapatite Structures

THE formation of lamellar mixed crystals of hydroxyapatite and octacalcium phosphate have been postulated by Brown *et al.*[1] to account for 'defect apatites' and other properties of precipitated calcium phosphate salts. One of the mechanisms they suggested for the layering is that of epitaxial overgrowths, since some similarity of structure was recognized. The similarity which Brown[2] demonstrated, however, is not nearly so striking as the similarity that can be shown.

Brown recognized that both the hydroxyapatite and the octacalcium phosphate structures have alternating chains of calcium and phosphate along the *c* axis. Also, that octacalcium phosphate has part of a hexagon that is present in hydroxyapatite. Hydroxyapatite has flattened hexagons made of alternating calciums and phosphates around the hydroxyl positions. In advancing along the *c* axis of the crystal these hexagons are shifted 60° so that the projection of these on a single plane places the calciums and the phosphates in regular hexagons. The phosphate hexagons are slightly larger than the calcium hexagons. Brown showed that octacalcium phosphate has four of the six positions of each hexagon.

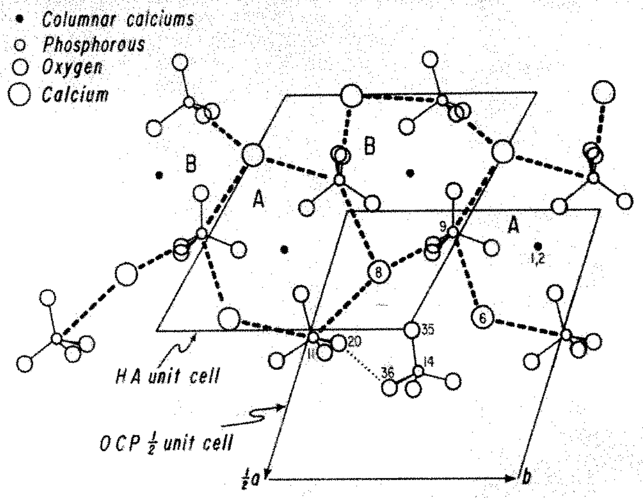

Fig. 1. Atomic positions in octacalcium phosphate for the plane *c* equal to approximately 0·25. The numbers given correspond to the numbering system used by Brown (ref. 2)

The more striking similarity is shown in Fig. 1. In this partial picture of octacalcium phosphate the complete hydroxyapatite structure can be seen except for the hydroxyls. The hydroxyl ions would be at the corners of the hydroxyapatite unit cell. In the case of octacalcium phosphate these positions are filled instead by water molecules (not shown) in the upper corners and by hydrogen phosphate in the lower corners (one shown, *P* number 14). Hexagons are formed around the axis of the columnar calciums such as around position 1, 2. There are two distinct types of hexagons: one quite regular (*A*) and the other distorted slightly (*B*). The distortion is due to shorter phosphorus to columnar calcium distances than the hexagonal calcium to columnar calcium distances. The two types alternate with each other along the '*c*' axis for both hydroxyapatite and octacalcium phosphate, that is, in the second plane of the unit cell in each crystal the positions of the two are reversed. More precisely, the second plane is the same as the first except for a 180° rotation of the hydroxyapatite unit cell content, including the water and hydrogen phosphate.

Table 1. COMPARISON OF DISTANCES IN HYDROXYAPATITE (HA) AND OCTACALCIUM PHOSPHATE (OCP)

Positions	Hydroxy-apatite	Octacalcium phosphate *
Columnar calcium to hexagonal calcium		
Regular hexagon	3·95	3·91 ± 0·06
Flattened hexagon	4·03	4·02 ± 0·08
Columnar calcium to phosphorus		
Regular hexagon	3·60	3·64 ± 0·06
Flattened hexagon	3·20	3·24 ± 0·06
Columnar calciums to oxygens in plane of		
Regular hexagon	2·42	2·43 ± 0·04
Flattened hexagon	2·44	2·44 ± 0·06
Columnar calciums to oxygens out of plane of		
Regular hexagon	3 +	3 +
Flattened hexagon	2·77 †	2·86 ± 0·16
Hexagonal calciums to oxygens in plane and inside		
Regular hexagon	2·71	2·79 ± 0·03
Flattened hexagon	2·36	2·37 ± 0·05
Hexagonal calciums to oxygens in plane and outside		
Regular hexagon	2·36	2·40 ± 0·03
Flattened hexagon	3 +	3 +
Hexagonal calciums to oxygens out of plane of		
Regular hexagon	3 +	3 +
Flattened hexagon	2·51	2·52 ± 0·06
Hexagonal calciums to oxygens		
Adjacent planes (usual chains)	2·37	2·36 ± 0·02
(exceptional chain)	—	2·24

* In each unit cell there are more than one of the described distance relationships. In hydroxyapatite these distances are essentially identical but in octacalcium phosphate they are quite variable, hence an average ± the standard deviation is given.

† The distances for octacalcium phosphate were calculated from the unit cell parameters given by Brown (ref. 2) and those for hydroxyapatite from those given by Posner *et al.* (ref. 3). These hydroxyapatite distances agree within 0·01 Å with those published by Posner except in the case marked with a dagger, where the difference is 0·03 Å.

Table 1 compares the dimensions of the two hexagons in the two structures. The two crystals are seen to contain almost identical hexagons. The distances in octacalcium phosphate are generally a few hundredths of an Å larger than in hydroxyapatite. The distances between calciums and phosphate are shorter in one of the chains along the *c* axis than the other 3 in a 1/2 unit cell of octacalcium phosphate or the 6 in hydroxyapatite.

This view of the two crystals suggests an explanation for the observation[1] that octacalcium phosphate grows more rapidly than hydroxyapatite, even though the latter is more stable. To explain the difference in growth rate, let us consider the lower edge of the figure as the surface of either crystal. Whether it grows like octacalcium phosphate or hydroxyapatite would appear to depend on whether a phosphate (atom 14) or a hydroxyl ion fills the corner position of the hydroxyapatite unit cell. The hydrogen phosphate ion pictured here has oxygen to calcium distances of 2.37, 2.39 and 2.63 Å and a hydrogen bond of 2.46 Å (20–36). A hydroxyl in the position of oxygen number 35 would have only two comparable calcium to oxygen distances, and only a weak hydrogen bond if any. This difference plus the fact that the hydroxyl ion in the surrounding solution would be present in much lower concentrations than the phosphate ions under physiological conditions would make it appear that generally there will be many more phosphates than hydroxyl in the surface positions. The size of the phosphate ion would prevent the completion of the hexagon around the hydroxyl position necessary for the hydroxyapatite structure since two of the hexagon positions are occupied by oxygens of the central phosphate (atom 14). Thus, phosphate appears to act like a crystal poison for lateral growth. Consistent with this hypothesis hydroxyapatite tends to form long needles[1]. The greater stability of hydroxyapatite appears logical since it achieves much better packing than does octacalcium phosphate. There are two calciums in each half of the 'water layer' of octacalcium phosphate (not shown here) close to those that they would have as columnar calcium in hydroxyapatite, but they are surrounded by only seven oxygens rather than the nine they would have in hydroxyapatite. In addition, the octacalcium arrangement creates a channel down the middle of the octacalcium unit cell along the c-axis, in which two water molecules are loosely held as shown by the ease with which they are lost[1,2].

I thank Mr. Robert Reedy for assistance with the computer and Dr. Isaac Feldman for advice.

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DONALD R. TAVES

Department of Radiation Biology,
University of Rochester,
New York.

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CHEMISTRY

Production of Acetylene from Coal, using a Plasma Jet

THE plasma jet is a convenient tool for the production of high temperatures (8,000°–15,000° K). It has been shown, in these laboratories, that the thermal decomposition of coal in a pure argon plasma results in about 20 per cent conversion of the carbon in the coal to carbon in acetylene, and that the addition of 10 per cent of hydrogen to the arc gas increases this conversion to about 40 per cent. The high-temperature stability of acetylene[1] and the rapid quenching provided by the jet are the main factors favouring this conversion.

The experiments were carried out with a plasma jet of conventional electrode arrangement, similar to that used by Leutner and Stokes[2] for the conversion of methane to acetylene but with easily removable electrodes. An electric arc was struck between a tungsten rod which acted as the cathode and a copper tube as the anode. The electric power was supplied from a welding generator, with an open circuit voltage of 80 V. The power consumption was about 6 kW with the pure argon plasma, increasing to about 9 kW when operating on the argon/hydrogen mixture.

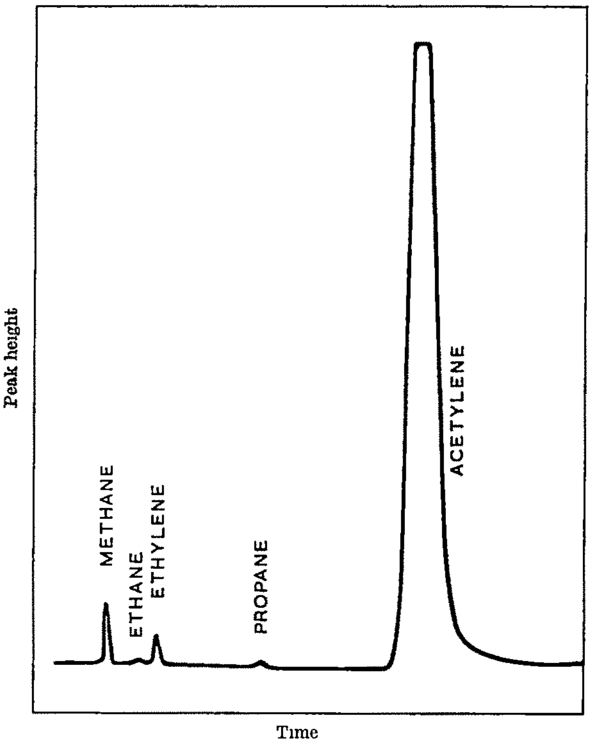

Fig. 1. Gas-chromatographic analysis of hydrocarbon gases. Methane, 0.014 per cent, ethane, 0.001 per cent, ethylene, 0.005 per cent, propane, 0.004 per cent, acetylene, 0.54 per cent

A high-volatile coal (83.6 per cent carbon, 5.3 per cent hydrogen, 7.7 per cent oxygen dry-mineral-matter-free basis, National Coal Board Rank Code No. 802) was ground to pass a 72 B.S. mesh sieve. The material was fed at rates of up to 1 g/min into the plasma jet from a fluidized bed by a subsidiary stream of argon. The coal was at first introduced into the primary gas feeding the jet, but this resulted in severe erosion of the electrodes. The coal/argon mixture was, therefore, passed into the region of intense heat through a single small hole drilled radially in the anode. The gases leaving the jet passed into a steel cooling chamber and were afterwards collected in a large polythene container. Chemical analyses were carried out either on the total gas sample in the polythene container (for wet chemical analysis) or on samples taken from the container (for gas-chromatographic or infra-red analysis).

The reaction products from the thermal decomposition of the coal in the plasma jet were almost entirely either solid (soot) or gaseous, there being negligible amounts of material of tarry nature that normally results from the thermal treatment of coal at relatively low temperatures and at slow rates of heating.

Gas-chromatographic analysis of the arc gas (Fig. 1) showed that acetylene represented more than 95 per cent of the hydrocarbons present. Calculation of the percentage conversion of the carbon in the coal to carbon in acetylene indicated that the yield of acetylene is considerable at the temperatures and under the thermal gradients prevailing in the jet, although the concentration of acetylene in the arc gas was low owing to the high throughput of argon (about 10 l./min). Maximum conversions of 25 per cent with pure argon and 45 per cent with an argon/10 per cent hydrogen mixture have so far been obtained with the present apparatus. Hydrogen, carbon monoxide and hydrogen cyanide (from the nitrogen in coal) are the other main gaseous components formed.

The elementary analyses of the soot are somewhat similar to those found for carbonized coal that has been heated in the temperature range 600°–800° C This suggests that at least some of the coal may not have reached the high temperatures associated with the arc Optical and electron microscope investigations indicate that the majority of the soot particles are in the submicron range

We thank Mr L A King, of the Electrical Research Association, Leatherhead, for his advice on the construction of the plasma jet, and the British Coal Utilisation Research Association for permission to publish this communication

R L Bond
I F Galbraith
W R Ladner
G. I T. McConnell

British Coal Utilisation Research Association,
Randalls Road,
Leatherhead

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Detection of Nitric Oxide by Gas-chromatography

In an investigation on the feasibility of using gas-chromatographic methods to determine the oxides of nitrogen in gaseous mixtures such as Diesel exhaust gases, we have successfully used a standard argon ionization cell to detect small volumes of nitric oxide The limit of detection was of the order of 0 01 µl A glass column was used, packed with molecular sieve No *5A*, and fitted to a Pye panchromatograph

The nitric oxide–argon mixtures used were prepared by serial dilution In order to limit the extent to which oxidation of the nitric oxide could take place during the preparation of nitric oxide–air mixtures, the following procedure was adopted A nitric oxide–argon mixture containing 0 1 per cent nitric oxide was first prepared, and aliquots containing the required amounts of nitric oxide were transferred to evacuated gas tubes These tubes were fitted with side arms closed with serum caps through which air was then admitted by the insertion of a hypodermic needle A comparison of the peak heights obtained from nitric oxide–argon mixtures with those obtained from nitric oxide–air mixtures is given in Table 1

Table 1 A Comparison of Nitric Oxide–Argon and Nitric Oxide–Air Mixtures

Nitric oxide (µl)	Peak height response (mm) Nitric oxide–air mixture	Nitric oxide–argon mixture
0 1	38 0	56 0
0 08	31 0	39 5
0 06	27 5	31 0
0 04	17 0	19 5
0 02	9 0	9 0
0 01	6 0	6 0

Column, 5 ft length molecular sieve No *5A* at 100° C, detector, argon ionization cell, 1,750 V, argon flow-rate, 36 ml /min

In the presence of oxygen (air) some oxidation of the nitric oxide occurred, but this diminished as the volume of added nitric oxide was reduced

We have confirmed the results of previous workers[1,2], who indicated the necessity of pretreating the column ('conditioning') in order to obtain reproducible results We have also been able to observe the non-linearity of the adsorption isotherm as an appreciable increase in retention time with decreasing nitric oxide concentration This is shown for the range 0 1–1 µl in Fig 1 In Fig 2, these chromatograms have been superimposed to show that the peak maximum appears to follow the trailing edge of the nitric oxide response As the retention time is a function of the amount of nitric oxide added, it should be possible to use this measurement for analytical purposes in place of the more usual peak height or peak area response This would give increased accuracy at the lower concentration-levels where the retention time can be recorded more accurately

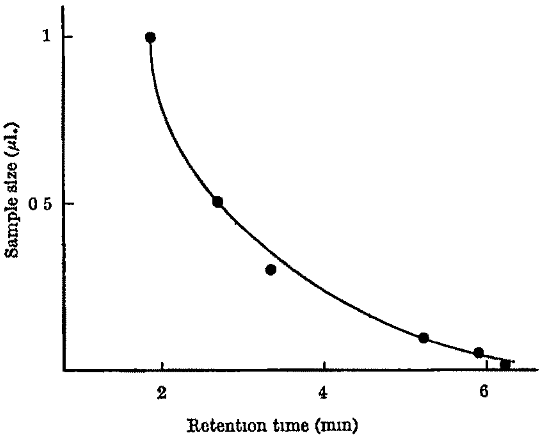

Fig. 1 Relationship between retention time and amount of nitric oxide added

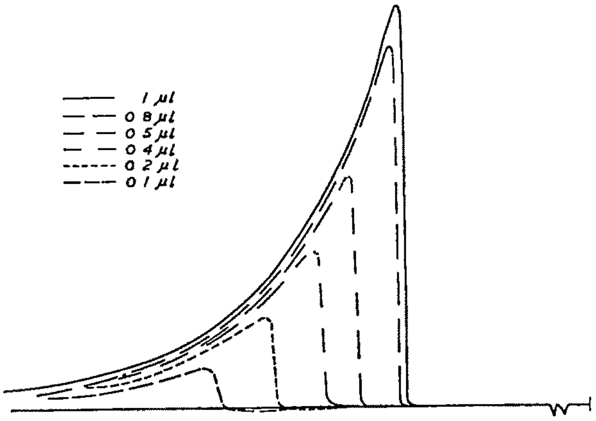

Fig 2 Superimposed chromatograms of various amounts of nitric oxide

P. J Kipping
P G Jeffery

Warren Spring Laboratory,
Department of Scientific and Industrial Research,
Stevenage, Herts

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Temperature as a Variable in Ion-exchange Paper Chromatography

Temperature is a variable which has received relatively little attention in chromatographic studies This is particularly true of paper chromatography in which more attention is given to controlling temperature as an aid to reproducibility than to using temperature to effect improved resolution.

In ion-exchange column chromatography effective use is made of changes in temperature[1]. The advantages claimed, however, are mainly associated with improved kinetics of the chromatographic process Specific effects, such as varying positions of chromatographed peaks, which cannot be assigned simply to the temperature dependence of *p*H have been observed but no systematic examination has been made of the effect of temperature on selectivities

This communication is an account of part of a general investigation of the effect of temperature on ion-exchange paper chromatography and was stimulated by the results of an earlier investigation which demonstrated the marked

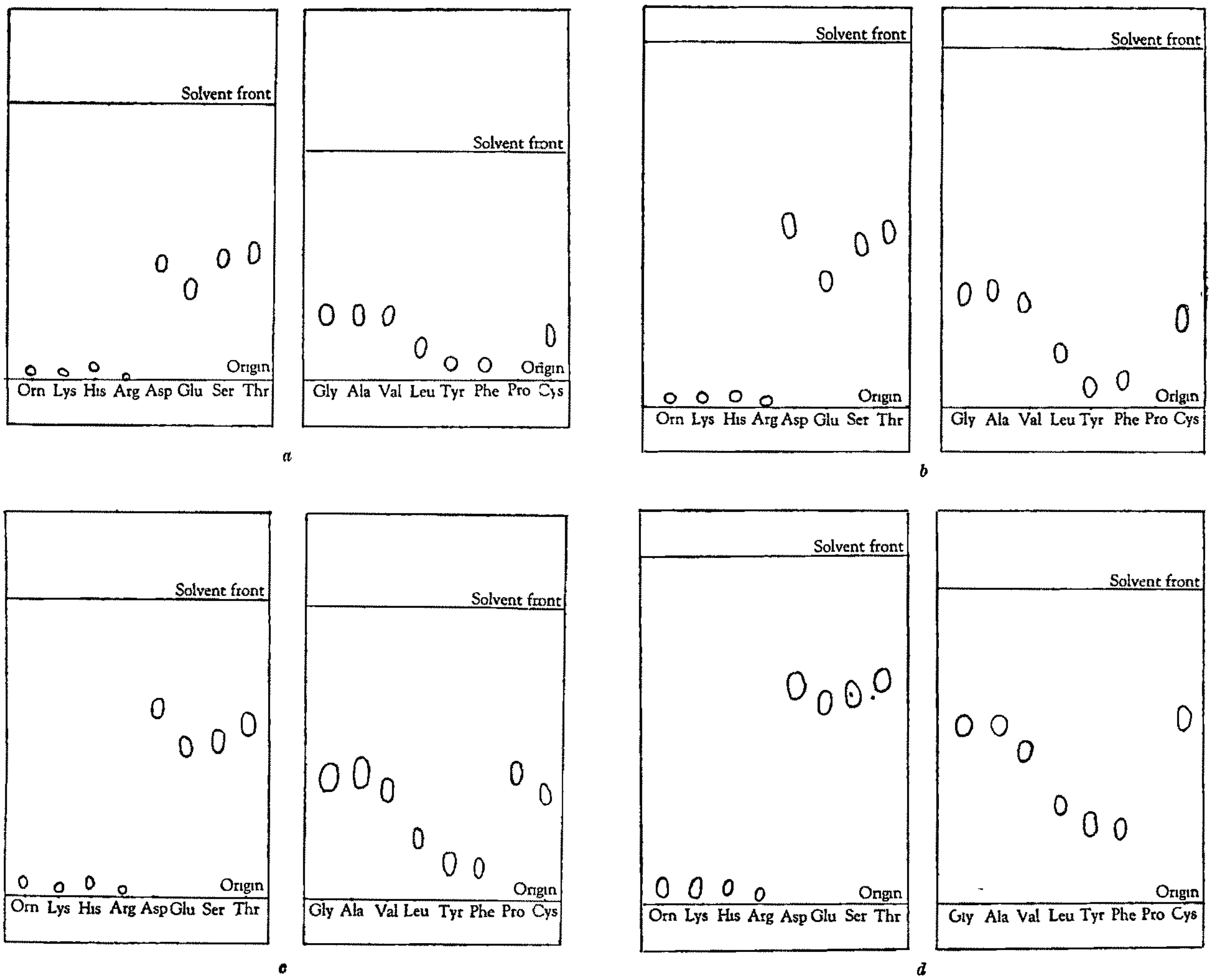

Fig. 1. Chromatograms showing the effect of temperature on one-dimensional separations of amino-acids on Whatman 'Zeo Karb 225' resin-loaded paper. Developing solvent, 0.2 M, *p*H 3.1 (20° C) sodium buffer. Temperature of development, (*a*) 3° C, (*b*) 20° C, (*c*) 45° C, (*d*) 75° C.

dependence of amino-acid sequence on non-ionic interactions between amino-acid side-chain and exchanger matrix[3].

Whatman 'Zeo Karb 225' resin-loaded paper (experimental product) was used throughout this investigation, the paper being used in its dry sodium form. Standard solutions of individual amino-acids, containing 0.2 g of amino-acid in 100 ml. of 0.01 N hydrochloric acid, were applied as individual spots along a line 1 in. from one edge of a 5½ in. × 4 in. sheet of paper according to previously described methods[2]. Ascending development with 0.2 M, *p*H 3.1 sodium buffer was carried out in a glass tank 7 in. high and 5 in. × 3 in. in cross-section. The tank containing the solvent was placed in a thermostatically controlled oven and the solvent allowed to attain the temperature of the oven before development of the chromatogram.

Chromatograms obtained at different temperatures are illustrated in Fig. 1. R_F values of the individual amino-acids and *p*H of the developing solvent at different temperatures are recorded in Table 1.

The amino-acids were applied to the paper in a sequence which allowed easy comparison of different side-chain structures. This is illustrated in Fig. 2, which is a reproduction of the separation at room temperature.

Fig. 1 and Table 1 show that there is a general increase in R_F values of the amino-acids with increasing temperature and that the changes in temperature are accompanied by changes in the relative positions of the individual amino-acids.

The degree of separation of the amino-acids is determined by the resultant of selective interactions between charged sites on the exchanger and on the amino-acid and between exchanger matrix and amino-acid side-chain. At room temperature, under the conditions of development used in this investigation, non-ionic interactions exert a significant influence on resolution. At elevated temperatures the non-ionic binding forces weaken relative to the ionic binding forces and as a result the relative rates of movement of the amino-acids change. The largest changes are associated with those amino-acids which interact most strongly with the exchanger matrix.

Table 1. EFFECT OF TEMPERATURE ON R_F VALUES AND BUFFER *p*H

Buffer temp.	Buffer *p*H	R_F values															
		Orn	Lys	His	Arg	Asp	Glu	Ser	Thr	Gly	Ala	Val	Leu	Tyr	Phe	Pro	Cys
3	3.22	0.02	0.02	0.02	0.01	0.40	0.29	0.42	0.46	0.30	0.30	0.28	0.13	0.05	0.05	—	0.19
20	3.15	0.02	0.02	0.02	0.01	0.48	0.34	0.43	0.47	0.32	0.32	0.28	0.13	0.06	0.06	—	0.25
45	3.00	0.04	0.03	0.04	0.02	0.61	0.49	0.52	0.58	0.40	0.40	0.36	0.15	0.12	0.12	0.45	0.39
75	2.85	0.07	0.04	0.07	0.02	0.72	0.58	0.60	0.64	0.58	0.57	0.48	0.32	0.24	0.23	—	0.59

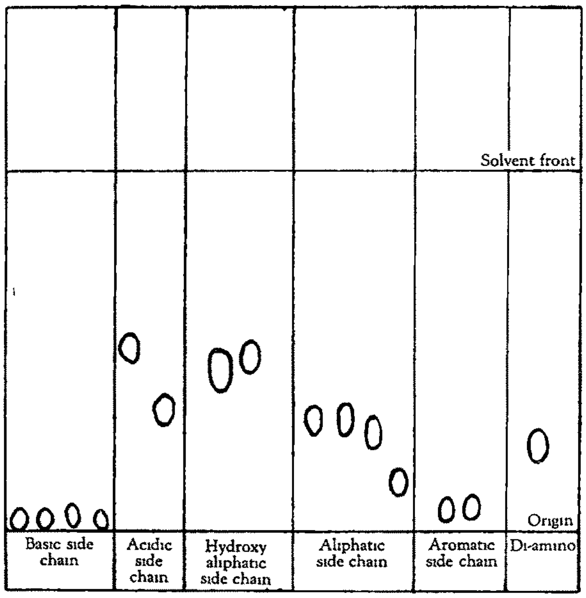

Fig. 2

Temperature dependence of *p*H (Table 1) and ionization of the amino-acids will also contribute to variation of R_F values with temperature. Each of these effects will tend to suppress the other, however, such that their resultant influence on R_F values will be relatively small[2].

The significant effect of changes in temperature on the R_F values of the acidic amino-acids cannot be explained in the foregoing terms, however, since the low degree of ionization of these, relative to the other, amino-acids does not support sufficiently strong interaction between their side-chain structures and the exchanger matrix.

It has previously been suggested that, by virtue of their low degree of ionization, the acidic amino-acids are retarded relative to the other groups by the operation of an ion exclusion effect[2]. In the case of these amino-acids, therefore, the dependence on temperature of R_F values probably results from a change in diffusion rates within the exchanger leading to a reduced selective penetration of these amino-acids at higher temperatures.

The influence of changes in temperature on zone definition is masked in ion-exchange paper chromatography by the simultaneous effect of temperature on rate of flow of the developing solvent. Some increased sharpness of zone formation with increasing temperature was observed, however.

The foregoing results have shown that temperature can be used, particularly, to vary the relative influence of ionic and non-ionic binding forces between amino-acids and exchanger and in consequence the relative rates of movement of amino-acids on a chromatogram.

I thank the directors of W. and R. Balston, Ltd., for permission to publish this work.

C. S. Knight

Whatman Research Laboratories,
W. and R. Balston, Ltd.,
Springfield Mill,
Maidstone, Kent.

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Kinetics of Isotope Redistribution Reactions

When a hydrocarbon with N equivalent, exchangeable hydrogen atoms is subjected to protium–deuterium exchange, molecules with 0, 1, . . . j . . . N deuterium atoms are formed, which we shall denote by . . . D_j . . . and their fractions by . . . d_j . . . . Neglecting isotope effects, the average degree of deuteration σ, expressed by

$$\sigma = \frac{1}{N}\sum_{j=1}^{N} j d_j \qquad (1)$$

which changes according to the exponential rate law for exchange[1,2].

Let us define a set of variables u_i

$$u_i = (-1)^i \sum_{j=i}^{N} \frac{j!}{i!\,(j-i)!}\, d_j \qquad (2)$$

Then the variable u_1 is directly related to σ

$$u_1 = -\sum_{j=1}^{N} j d_j = -N\sigma \qquad (3)$$

and the exchange rate law in terms of u_1 (at time $= t$) becomes

$$u_1 - u_1(\infty) = [u_1(0) - u_1(\infty)]\exp(-Akt) \qquad (4)$$

where A contains the dimensions of the system under investigation.

Redistribution (or equilibration) experiments constitute another case of isotope reactions. Here, the initial distribution of the isotope over the various isotopic species is not binomial (equilibrium 'statistical'). Equilibrium is approached by redistribution of the isotope within the same chemical compound; the average degree of deuteration σ (or u_1) is constant and hence equation (4) loses its meaning.

We have found from a fairly involved argument, the details of which will be published shortly[3], that the rate law governing redistribution reactions is analogous to equation (4). The variable u_2 is thus defined, according to equation (2), by

$$u_2 = \sum_{j=2}^{N} \frac{j!}{2!\,(j-2)!}\, d_j \qquad (5)$$

and it follows from exponential kinetics

$$u_2 - u_2(\infty) = [u_2(0) - u_2(\infty)]\exp(-A'k't) \qquad (6)$$

Two comments, elaborated elsewhere[3], concern the analogy of the kinetics for exchange (4) and redistribution (6). First, the ratio of k (4) and k' (6), as evaluated from corresponding systems, provides information about the reaction mechanism. Secondly, the redistribution rate law (6) lacks the complete generality of that for

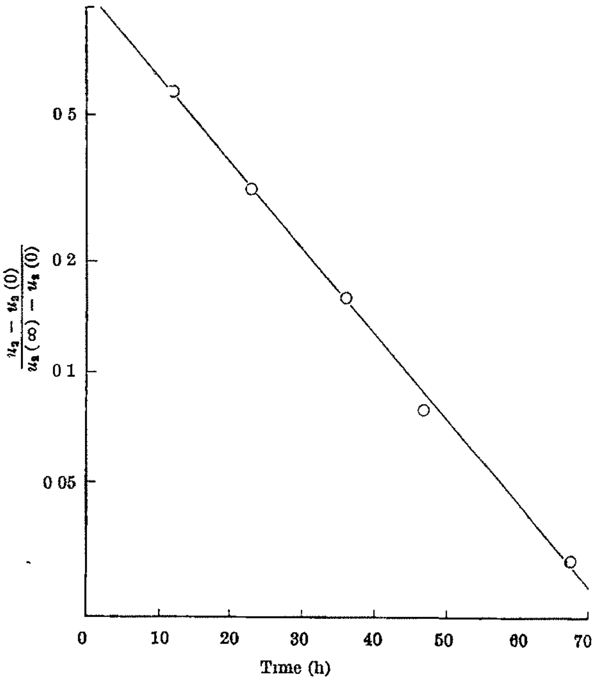

Fig. 1. Data of Wright and Taylor (ref. 4) (experiment denoted as *B*9) on the redistribution of hydrogen isotopes in methane over a nickel catalyst plotted according to an expression equivalent to equation (6)

exchange (4). It might fail for some, conceivable but presumably rare, reaction mechanisms. It certainly does hold for a stepwise process, that is, one proceeding by consecutive reactions, or for any process in which only one isomer D_2 (and, consequently, D_{N-2}) exists, as is the case for methane.

Fig. 1 illustrates the application of the redistribution rate law (equation (6)) to literature data[4] for methane. In this case ($N = 4$) u_2 equals: $1[CH_2D_2] + 3[CHD_3] + 6[CD_4]$. The complementary function $1[CH_2D_2] + 3[CH_3D] + 6[CH_4]$ may, of course, be used equally well. The previous[4] analysis of these kinetic results, based on exponential kinetics for each of the isotopic species, is without foundation. Such kinetics will only hold for either special initial conditions or for special mechanisms.

The simplest redistribution reaction concerns $N = 2$. The example most frequently investigated, particularly in heterogeneous catalysis, is:

$$H_2 + D_2 \rightleftharpoons 2\,HD$$

Exponential kinetics for [HD] (and the other isotopic species) are commonly observed. This result is another illustration of the redistribution rate law (equation (6)), since $u_2 = [D_2]$, and [HD] is linearly related to $[D_2]$ by the material balance.

H. Bolder
G. Dallinga
H. Kloosterziel

Koninklijke/Shell-Laboratorium,
Amsterdam.

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Partition Coefficients and Biological Activities

It was found[1] some time ago that the toxicities C_t of many physically toxic compounds could be estimated by the equation:

$$\text{Log}_{10}\, C_t/C_B = kP \qquad (1)$$

where k is a constant equal to 0·012, C_B is a measure of the intensity of the toxicity, and P is the parachor, which is used as a measure of molecular volume. Equations similar to (1) have been used successfully for the estimation of partition coefficients[2], x, for organic compounds between organic solvents and water and of solubilities[2,3] of organic compounds in water. When substances interact with a solvent, for example, by hydrogen bonding with the water, a further term E_A is required in the equation so that, for example:

$$\text{Log}_{10} x = kP + E_A \qquad (2)$$

This equation (2) was tested[2] on figures given by Collander[4] for partition coefficients x between isobutanol and water and between ether and water with k equal to 0·012 as before. For simple esters and alcohols E_A values were found between − 1 and − 2.

Recently[5,6], the effects of substituents on the biological activity of phenoxyacetic acids as plant hormones and on the toxicities to bacteria for analogues of chloromycetin have been related to two variables. The first is the well-known Hammett sigma factor σ and the second π is the logarithm of the ratio of the partition coefficients of the substituted, x^S, and unsubstituted, x^H, compounds respectively between 1-octanol and water at about 27° C. From equation (2):

$$\pi = \text{Log}_{10} x^S - \text{Log}_{10} x^H = 0{\cdot}012\,(P^S - P^H) + E_A{}^S - E_A{}^H \qquad (3)$$

Table 1 shows that the values of π for unionized phenoxyacetic acid molecules do agree well with 0·012 $(P^S - P^H)$ when the substituents contain only carbon and hydrogen. For such cases $E_A{}^S - E_A{}^H$ would be expected to be zero. The parachors were calculated by a method previously described[7]. Except for fluorine attached directly to the aromatic nucleus $E_A{}^S - E_A{}^H$ for halogen compounds is about + 0·35 (Table 2). The positive sign means that the interactions have increased the solubility in 1-octanol relative to water. All other interactions have so far been found to be negative. In the estimation of physical toxicities and solubilities no interaction terms seemed to be required for alkyl and aryl halides[2,3]. Figures for ortho-substituents are not given in Table 2 or in the following list because the oxyacetic acid group will itself interact with water and groups close to each other do not appear to interact independently[2]. The $E_A{}^S - E_A{}^H$ values calculated for the other groups are: 3–OCF_3, + 0·28; 3–SCH_3, − 0·49; 3–NO_2, − 0·57; 4–NO_2, − 0·61; 3–OCH_3, − 0·64; 3–SO_2CF_3, − 0·72; 3–OH, − 0·78; 4–OCH_3, − 0·79; 4–OH, − 0·89; 3–CN, − 0·92; 4–CN, − 0·91; 3–COOH, − 0·97; 4–$N{=}NC_6H_5$, − 1·16; 3–$COCH_3$, − 1·38; 4–$COCH_3$, − 1·44; 3–$NHCOCH_3$, − 2·21; 3–SO_2CH_3, − 2·77. With the exception of 3–OCF_3, all these values are negative.

Table 1

Substitution	π	$0{\cdot}012\,(P^S - P^H)$
None	0	0
2—CH_3	0·68	0·48
3—CH_3	0·51	0·48
4—CH_3	0·52	0·48
2—C_2H_5	1·22	0·96
3—C_2H_5	0·97	0·96
3,4—$(CH_2)_3$	1·06	1·07
3,4—$(CH)_4$	1·27	1·25
3,4—$(CH_2)_4$	1·40	1·55
3—n C_4H_9	1·91	1·91
3—C_6H_5	1·91	2·07

Table 2

Substitution	π	$0{\cdot}012\,(P^S - P^H)$	$E^S_A - E^H_A$
3—F	0·13	0·06	+ 0·07
4—F	0·14	0·06	+ 0·08
3—Cl	0·76	0·42	+ 0·34
4—Cl	0·70	0·42	+ 0·28
3—Br	0·95	0·59	+ 0·36
4—Br	1·02	0·59	+ 0·43
3—CF_3	1·09	0·67	+ 0·42
3—I	1·17	0·88	+ 0·29
4—I	1·26	0·88	+ 0·38
3—SF_5	1·51	1·15	+ 0·36
3—SCF_3	1·59	1·22	+ 0·37

The equation (3) should prove useful for the estimation of values of π. The term $0{\cdot}012(P^S - P^H)$ can be calculated immediately. It will be the same whenever a given substitution is made and equals π when the substitution involves only addition of carbon and hydrogen. The E_A values for an isolated interacting group and a given type of organic solvent appear to be nearly constant[2,3] so that it will often be possible to suggest a value for $E^S{}_A - E^H{}_A$. However, interaction energies do depend on the solvent and too much confidence should not be placed in values obtained from the partition coefficients because 1-octanol might give interaction energies which differed considerably from those for the biophase. Hansch *et al.*[6] realized this and when the value of π of − 1·26 for 3–SO_2CH_3 from 1-octanol proved unsatisfactory, they suggested that a value of π equal to − 0·47, which gave good agreement with all the biological results, should be used.

J. C. McGowan

Imperial Chemical Industries, Ltd.,
Research Department,
Welwyn Garden City,
Herts.

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Molecular Configuration of Benzil and α-Pyridil

THE molecular configuration of benzil has been the object of considerable interest for some time because of unusual physical characteristics, such as its large optical rotatory power. Caldwell and Le Fevre[1] measured its dipole moment in several solvents and compared it with that of phenanthroquinone, which is planar and contains two *cis* carbonyl groups. From the lower (3·5 *D*) electric dipole moment of benzil these authors concluded that the molecule must be skew and that the two C_6H_5—C=O halves must lie in two different planes making an angle of 90°–100° with each other.

Knaggs and Lonsdale[2] compared this suggestion with their X-ray diffraction results and found that it was quite reasonable. Furthermore, they concluded that neither a planar *cis* nor a planar *trans* configuration was acceptable for the interpretation of their X-ray data. Finally, Le Fevre *et al.*[3] have estimated from dielectric and Kerr constants that the molecule is twisted about the O=C—C=O bond.

In connexion with some work on 2,2′-dipyridyl glyoxal (α-pyridil), we have recorded the infra-red spectrum of benzil and of the foregoing substance. Comparison of our results with that in the literature revealed that previous measurements[4,5] of the infra-red spectrum of benzil showed a single band in the carbonyl region (1,700 cm^{-1}) and, furthermore, this was used as evidence[5] for a *trans* structure for benzil, in contrast with the results quoted above. We find two bands in the carbonyl region for both benzil and α-pyridil, as shown in Fig. 1, *A* and *B*. The splitting in benzil is about 14 cm^{-1} while in α-pyridil it is 23 cm^{-1}. Our results, together with that of other workers, are presented in Table 1.

The structure of α-pyridil was worked out in detail by Hirokawa and Ashida[6], who found that the molecule is rotated about the O=C—C=O bond by an angle of approximately 83 degrees. Since such a conformation makes the relevant point group of the molecule C_2, one should observe two carbonyl bands for this molecule. Fig. 1, *B* shows that this is the case. By analogy with α-pyridil, two carbonyl bands should be seen for a benzil molecule in the skew configuration discussed here, and this is shown in Fig. 1, *A*, to be true.

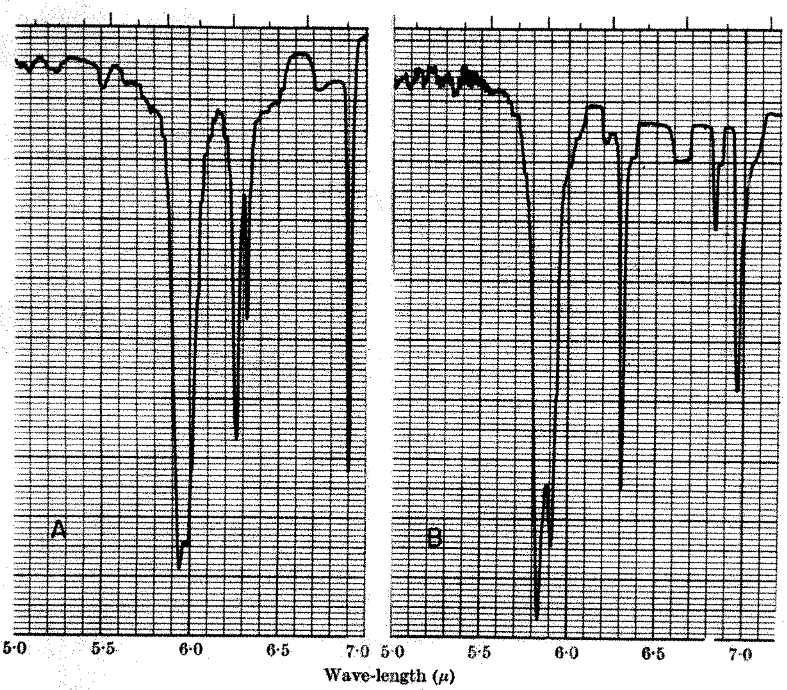

Fig. 1. *A*, Spectrum of benzil in chloroform; *B*, spectrum of α-pyridil in chloroform. Both solutions were approximately 0·03 M

Table 1

Ref.	Benzil	α-Pyridil
This work	1,686 cm^{-1} (*st, sp*)	1,715 cm^{-1} (*st, sp*)
	1,672 cm^{-1} (*sh*)	1,692 cm^{-1} (*st, sp*)
4	1,652 cm^{-1}	—
5	1,681 cm^{-1}	—

st, strong; *sp*, sharp; *sh*, shoulder.

The spectra were recorded using a Perkin–Elmer 237 grating spectrophotometer with IRTAN cells. Using an older spectrophotometer and the same solution used to record Fig. 1, *A*, we were unable to observe the splitting of the carbonyl band in benzil. As a result, the apparent conflict of the infra-red results with the other measurements is due to lack of resolution in the older spectrophotometers.

I. BERNAL

Department of Chemistry,
Harvard University,
Cambridge 38,
Massachusetts.

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BIOPHYSICS

Viscosity of Deoxyribonucleic Acid Solutions in the 'Sub-melting' Temperature Range

DEOXYRIBONUCLEIC acid (DNA) 'melting' has been mainly investigated using spectroscopic and optical rotation techniques[1]. To our knowledge, the viscosity behaviour associated with the 'melting' phenomenon has been examined only in the special cases of synthetic deoxyribopolynucleotides[2] and phage DNA[3]. In an investigation of this subject, using DNA preparations from three different sources, it was observed that the change in intrinsic viscosity parallels the change in optical density at 260 mμ, except for the 'sub-melting' temperature range. The peculiar behaviour found in this region is reported here. Briefly, this is characterized by a fall in viscosity which is completely reversed on cooling and seems to be caused by the local melting of deoxyadenylic-thymidylic (dA-T) clusters.

Viscosity was measured using a four-bulb viscometer built according to Eigner[4]. The average velocity gradients associated with each bulb were calculated using Kroepelin's formula[5]: values ranging from 100 to 20 sec^{-1} were found. Three DNA preparations (from calf thymus, chicken erythrocytes and *E. coli*, respectively), obtained essentially according to Kay *et al.*[6] and displaying intrinsic viscosities close to 70 dl./g, were used at concentrations of 20 μg/ml. in acetate buffer $\mu = 0·15$, *p*H = 5·6 (the *p*H of this buffer did not show any significant change within the explored temperature range). The viscometer was equilibrated at several different temperatures and fresh aliquots from stock DNA solutions were measured at each temperature. Ultra-violet 'melting profiles' were obtained at essentially the same DNA concentration used in the viscosity experiments.

Fig. 1 shows the behaviour of both optical density at 260 mμ and intrinsic viscosity at zero velocity gradient of calf thymus DNA as a function of temperature. Substantially similar results were obtained

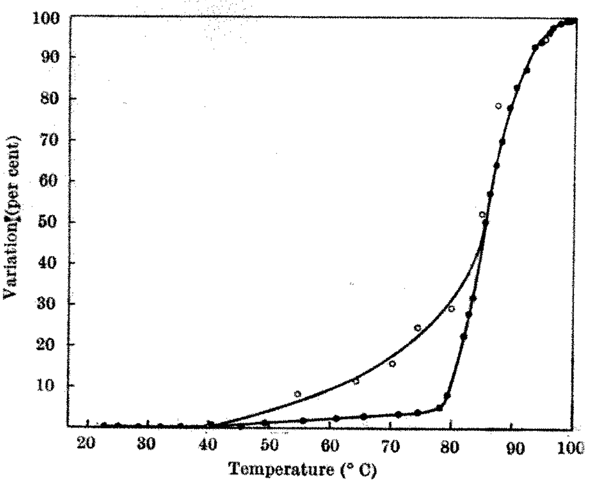

Fig. 1. Viscosity and optical density of calf thymus DNA (sample *B* 18) as a function of temperature. Viscosity decrease (○) and optical density increase (●) are given as percentage of total variation. DNA concentration was about 20 μg/ml. in acetate buffer *p*H = 5·6, $\mu = 0{\cdot}15$

with the other two samples. It is evident that viscosity undergoes a quite distinct drop in a temperature range where optical density is very slowly increasing in a linear fashion. Results to be published in detail elsewhere indicate that in the temperature range 50°–80° C, DNA hydrolysis occurs according to a 'double hit' mechanism, at a very slow rate and shows a lag-time of more than 40 min. Therefore, the results shown in Fig. 1 (in the range 50°–80° C), which were obtained within 20 min after introducing the solutions in the viscometer, do not need any correction for hydrolysis phenomena. Viscosity results in the temperature range 80°–95° C were corrected for hydrolysis.

In phosphate buffer *p*H 7·6, $\mu = 0{\cdot}15$ (where the melting point is the same as at *p*H 5·6), the difference between the viscosity and optical density curves was less pronounced.

When the temperature was lowered from the 'submelting' range down to 25° C (no matter how fast was the drop) a complete recovery of the initial viscosity value was obtained.

The foregoing results suggest that the 'sub-melting' behaviour of viscosity is due to a change in the configuration of DNA molecules. That this change is not related to some trivial aggregation phenomenon mediated by residual protein seems to be suggested by its reversibility. A possible explanation is that it is due to the local melting of dA-T clusters, the existence of which, in runs of up to 9 units, has been shown by Spencer and Chargaff[7]. In connexion with this interpretation it may be recalled that the melting temperature of a synthetic dA-T polymer is about 65° C (ref. 8), well within the range where the 'submelting' phenomenon occurs in DNA, and that Greer and Zamenhof[9] found that although guanine is more labile than adenine at high temperatures, depurination almost exclusively affects adenine at temperatures below the 'melting point'.

We thank Prof. C. Sadron for his advice.

Anne-Marie Freund
Giorgio Bernardi
Centre de Recherches sur les Macromolécules,
6 rue Boussingault, Strasbourg,
France.

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Photoconduction of Adenosine in Various Morphological Forms

Optically clear films can be cast on a substrate when a neutral adenosine aqueous solution is slowly evaporated at about 60° C. Along the perimeter of the film, white needle-shaped crystallites are observed. If the film is exposed afterwards to high humidity, its clear portion will become cloudy. When viewed under the microscope between crossed nicols, this cloudy portion is seen to consist entirely of spherulites as shown in Fig. 1. The infra-red spectra of: (1) potassium bromide pellet of powdered needle-shaped crystallites; (2) non-spherulitic clear film; (3) spherulitic film of adenosine are shown in Fig. 2. From these spectra it is evident that the molecular arrangements of adenosine in these three morphological forms are different. Further work has to be done in order to elucidate the structures of the material in these various forms. In the present work it is intended to report some results on semi- and photo-conduction of the three different morphological forms of adenosine surface cells.

The experimental details for measuring the conduction of organic materials are similar to those reported previously[1]. The temperature dependence of the resistance of adenosine is shown in Fig. 3. Except for the deviation towards the lower temperature end, the resistance follows the well-known equation $R = R_0 \exp (E_d/2kT)$, where R_0 is a constant and E_d is the activation energy for dark conduction. The activation energy of the pellet is 4·52 eV, in agreement with the value reported by Eley and Leslie[2]. The activation energies of the spherulitic and non-spherulitic films are 4·03 and 3·68 eV respectively. The temperature dependence of photocurrent of adenosine shown in Fig. 4 follows the equation $i = i_0 \exp (-E_p/kT)$, where i_0 is a constant and E_p is the activation energy for photoconduction. The values of E_p are about 1·90 eV for the pellet, 1·87 eV for the spherulitic film, and 1·73 eV for the non-spherulitic film.

The activation energies for dark- and photo-conduction of adenosine are much higher than the corresponding values of polyadenylic acid[3] and NaDNA in the dry state[4].

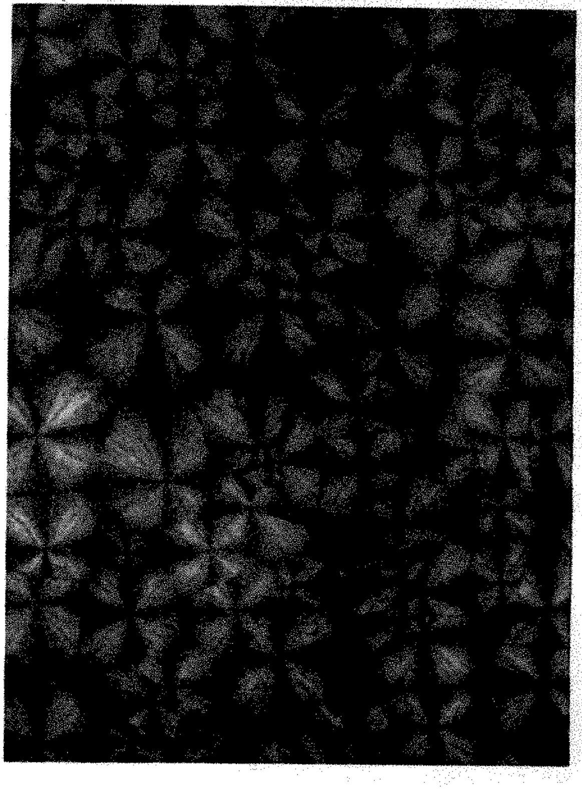

Fig. 1. Adenosine spherulites (crossed nicols × 385)

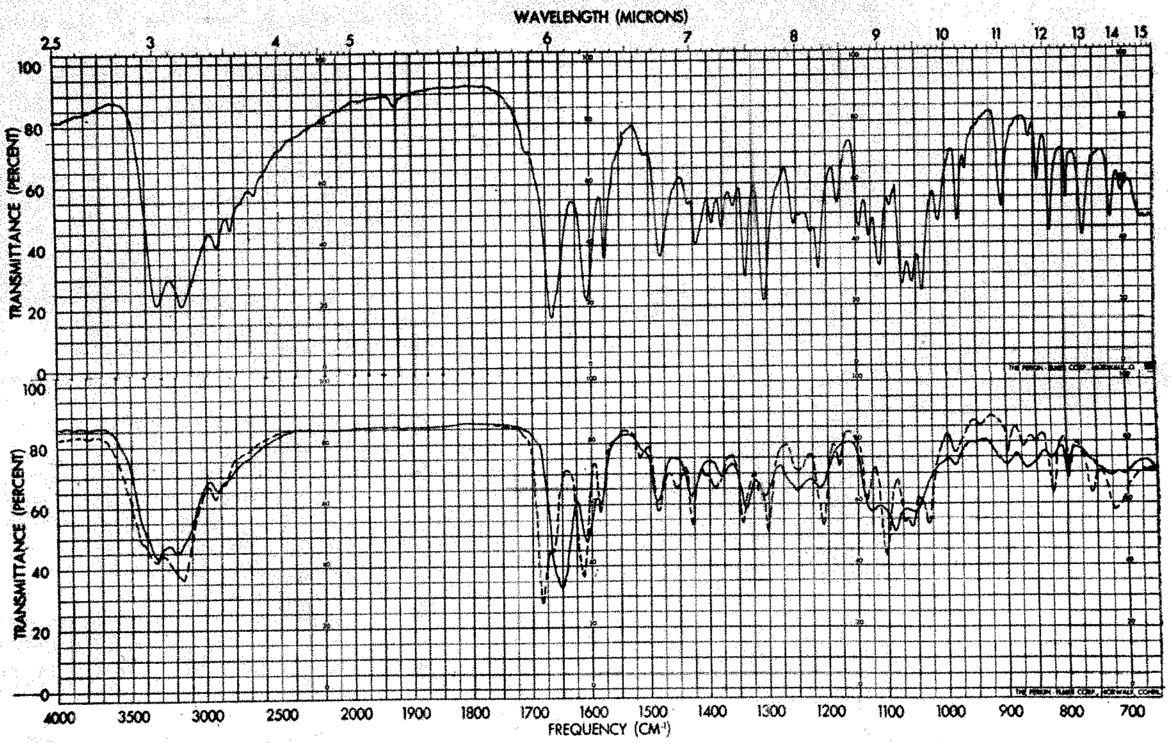

Fig. 2. Infra-red spectra of adenosine samples. Top, potassium bromide disk of powdered needle-shaped crystallites; bottom (solid curve), clear film on silver chloride plate; (dotted curve), same film exposed to high humidity and dried (spherulitic film)

These higher values may be largely due to the higher barrier between neighbouring molecules of this nucleoside. Let us assume that the energy required for electronic conduction in adenosine is due partly to the energy E_N required for charge carrier generation and partly to the energy E_μ required to overcome the barriers for charge carrier transport. Then we have[4] $E_d = E_N + E_\mu$, $E_\mu = 2E_p$, and $E_N = E_d - 2E_p$. From the experimental results E_N can be calculated to be about 0·72 eV for the pellet, 0·29 eV for the spherulitic film, and 0·22 eV for the non-spherulitic film. It may be noted that white light with peak intensity near 1,200 mμ was used for photocurrent measurement. The carrier generated by such a light radiation may not be due to the excitation of the π-electrons in the bases, since such an excitation would require a light source of wave-lengths near 259 mμ, the

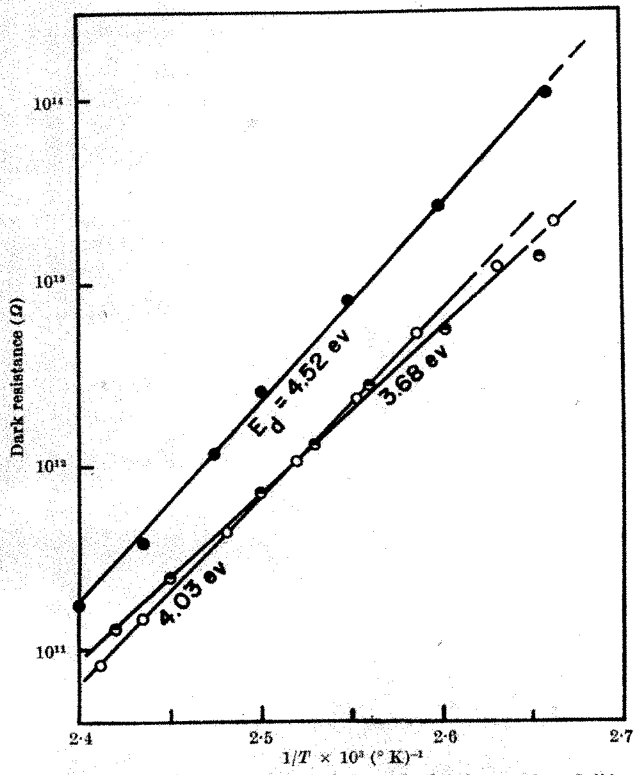

Fig. 3. Resistance of adenosine versus inverse absolute temperature. Solid circles, pressed pellet from powdered needle-shaped crystallites; open circles, spherulitic film; half-open circle, non-spherulitic film

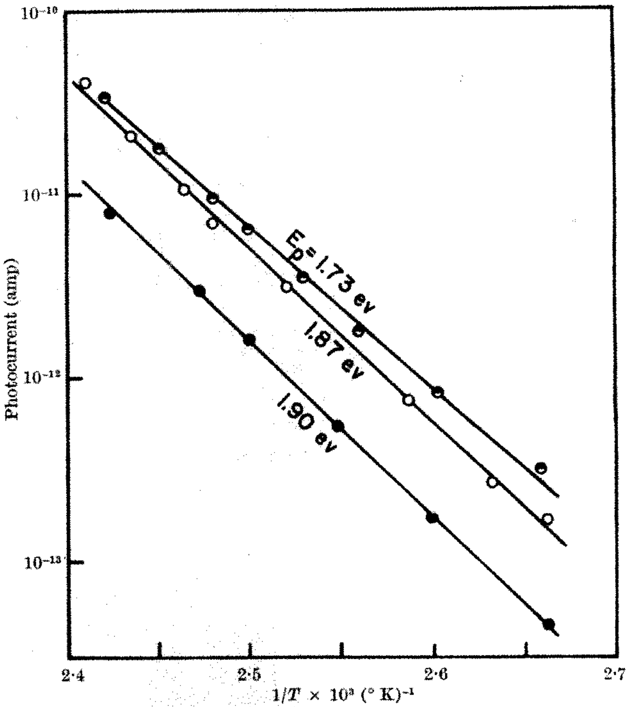

Fig. 4. Photocurrent of adenosine versus inverse absolute temperature. Solid circles, pressed pellet from powdered needle-shaped crystallites; open circles, spherulitic film; half-open circles, non-spherulitic film

absorption maximum of adenosine. It is assumed that the carriers are excited from impurity- or imperfection-levels, and the small values of E_N calculated are in agreement with this point of view.

It has been noted that the measured value of E_p for a given sample does vary somewhat from measurement to measurement. The small difference of E_p for adenosine obtained for the various morphological forms may thus not be considered as significant. It is concluded that the value of E_p is primarily a measure of the average barrier between neighbouring adenosine molecules and the effect due to morphological differences is rather small.

C. Y. Liang *
E. G. Scalco

Exploratory Research Laboratory,
American Viscose Corporation,
Marcus Hook, Pennsylvania.

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* Present address: Research Laboratories, Lear-Siegler, Inc., Santa Monica, California.

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Periodogram Analysis of Precipitates

Thiele *et al.*[1] have provided many examples of partially periodic precipitation phenomena, some of which resemble in a surprising manner the patterns found in tissues. The analysis of these patterns has a special interest because the pattern of the periodic precipitates is not quite regular, but more or less influenced by chance or by more than a single factor.

The material to be analysed is in the form of electron microscope negatives on pieces of film 2 cm × 2 cm, kindly sent to me by Prof. H. Thiele. These pieces fit into the graduated disk of a scanning apparatus already described[2], in which they can be oriented and scanned at any angle from 90° to −90°. The apparatus consists of a microscope with a modified motor-driven stage, a photocell, an amplifier, and a recorder. It is calibrated with standard neutral filters to measure optical density. Sometimes the precipitates appear to be arranged in one direction rather than another; it may then be convenient to place the film in the graduated disk so that this direction lies along the line joining 90° and −90°, but there is often much uncertainty in making this adjustment.

The negative is scanned at 20° angles from 90° to −70°, that is, in 9 different directions. This results in 9 periodograms, on which two kinds of calculation can be carried out. The first is to take each periodogram separately and to find the frequency with which maxima and minima occur. If there are M maxima and minima in a scanning distance of 10,000 Å, the half-period P_1 will be $10{,}000/M$, and there will be one such value for each periodogram. This tells us nothing about the size of the maxima and minima. The second kind of calculation is to find the mean and the standard deviation (root-mean-square deviation) for each of the 9 periodograms, and then to plot the standard deviation against the scanning angle θ. The result is a curve which shows maxima and minima, usually occurring regularly. After completion of this second kind of calculation, the average value of P_1 which corresponds to the largest half-periods P_2 of the σ versus θ graph can be selected as being specially interesting, and can be called $P_{1\max}$; alternatively the values of P_1 for all 9 periodograms can be tabulated.

If the periodic precipitates were entirely regular, the minimum value of the standard deviation provided by the second kind of calculation would be zero, but for the material under consideration it is h, which can be interpreted as a measure of the extent to which a regular arrangement is disturbed by secondary factors or by chance irregularities. These disturbances lead to an increase in the value for the minima and to a decrease in the value for the maxima, and if the value for the maximum of the σ versus θ graph were M in the case of a regular periodic precipitate, it would be $(M - h)$ when the entirely regular arrangement was disturbed by secondary factors or by chance.

Table 1

	Half-period P_2 (radians)	Half-period $P_{1\max}$ (Å)	h	Amplitude of *S.D.*
Copper alginate	1·57	1450	0·23	0·24–0·42
Cadmium alginate	1·57	1100	0·24	0·25–0·45
Sodium alginate	n.s.	n.s.	0·17	n.s.*
Copper alginate †	0·70	760	0·25	0·20–0·40
Cadmium alginate †	0·61	830	0·23	0·23–0·50
Lead alginate †	0·53	910	0·24	0·25–0·61
Lead pectinate	1·57	1000	0·12	0·12–0·30

* Not significant.
† Probably represent mixtures.

The periodic precipitates of micelles which Prof. Thiele has sent me give the values shown in Table 1.

There are two important special cases. The first is when the material is precipitated at random, as in the case of sodium alginate or in the case of the human red cell ghost[2]; then the period and the amplitude of the oscillations are not significantly different at any angle. The second is when there is a mixture of micelles. In this case, the period and the amplitude of the root-mean-square deviation changes significantly in the course of the scanning operation, or the half-period is less than 1·57 radians and the curve of σ versus θ can usually be reduced to two or more curves, each with a half-period of 1·57 radians but with maxima and minima at different scanning angles (for example, the curve for copper alginate seems to be the result of a mixture of two species each with a half-period of 1·57 radians, but the one with a maximum at 20° and the other with a maximum at 90°).

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Eric Ponder

Service de Biophysique,
Institut Pasteur, Paris.

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BIOCHEMISTRY

Variant Forms of Arachin

In the past few years, analysis of samples from individual animals has revealed variants of many of the blood proteins. In man, for example, hæmoglobin, haptoglobins, transferrins, albumin and several glycoproteins are known to exist in two or more closely related forms, the distribution of which follows a simple hereditary pattern. In milk, β-lactoglobulin and α-lactalbumin show similar polymorphisms.

So far, I believe that no such polymorphism has been demonstrated in plant proteins though there is no obvious reason why one should not exist. In an attempt to find variants, the proteins of single seeds from Virginia and Spanish strains of peanut (*Arachis hypogaea*) have been examined by using acrylamide-gel electrophoresis[1]. Seeds were homogenized in electrophoresis buffer, centrifuged to remove oil and debris and the protein concentration adjusted to about 2 per cent before electrophoresis. Arachin exists under these conditions in a monomer–dimer form[2]; these are shown in Fig. 1, which also shows the variant, more rapidly migrating form found in some of the seeds of the Virginia strain. The form with highest mobility is called arachin *A* and the slower arachin *B*. Although the differences are most clearly seen in the monomers, the dimers also showed small differences in mobility. In addition, it was just possible to resolve the two forms by electrophoresis in agar gels at *p*H 8·6.

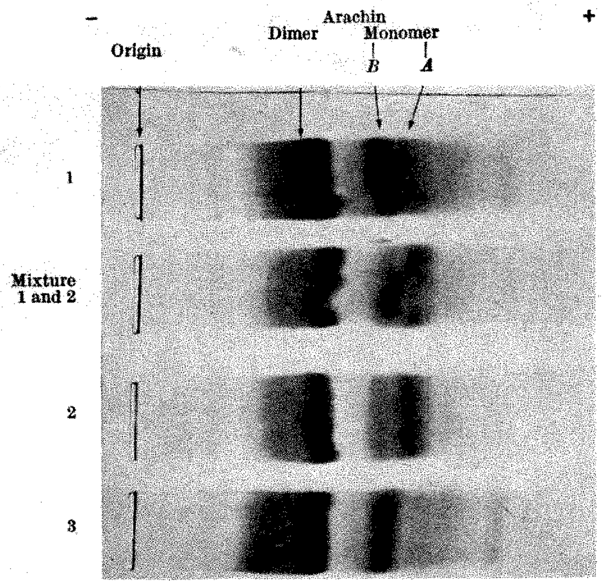

Fig. 1. Polyacrylamide-gel electrophoresis of peanut cotyledon proteins in a *tris*–citrate–sodium hydroxide–borate discontinuous buffer system, *p*H 8·6. Gel concentration was 7·5 per cent. Samples 1 and 2 show two types of mixed seeds; sample 3 contains arachin *B* only

Of 53 seeds examined, 32 were of the *AB* type, while 21 contained arachin *B* only. In some cases it was possible to distinguish between two types of mixed seeds, with either a preponderance of *A* or *B*. In five cases, pairs of seeds were examined, each pair being taken from a single pod. In every case the seeds comprising the pair were identical. This is consistent with a hereditary mechanism and, although no breeding experiments have been done, genetic control of the two forms of arachin is almost certain. All ten seeds of the Spanish variety examined contained both *A* and *B* arachins, but other minor proteins in this strain differed from those in the Virginia. No example contained arachin *A* alone.

Although such a form might be expected by a superficial analogy with known polymorphism the peanut is tetraploid and self-fertile and the genetic situation is quite different from that in animals. It is unlikely that the two forms are controlled by alleles at a single locus and this is not therefore a true polymorphism.

It is clear from Fig. 1 that both arachins may form dimers and the existence of the two forms should be taken into account in interpreting results obtained previously.

M. P. TOMBS

Unilever Research Laboratory,
Colworth House,
Sharnbrook, Bedford.

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Electrophoretic Separation of the Phenolases from Potato Tubers

EARLY investigations of the properties of polyphenol oxidase from plants showed that most preparations catalysed the oxidation of several polyphenolic substrates. In the past few years it has been demonstrated that crude polyphenol oxidase preparations actually are mixtures of several phenol oxidase proteins, each exhibiting unique catalytic and physical properties. Smith and Krueger[2] used a column of hydroxyl apatite and chromatographically separated crude extracts of mushrooms into a series of purified fractions. These contained several enzyme types, including the classical catecholase and cresolase enzymes. Sisler and Evans[3] showed that chlorogenic acid was a better substrate for the crude polyphenol oxidase from tobacco than for a comparable preparation from mushrooms. The ratios of rates of oxidation of chlorogenic acid to rates of oxidation of catechol as catalysed by each of a series of purified extracts from tobacco varied widely, indicating the presence of more than one type of polyphenol oxidase enzyme. Uritani[4] used starch-gel electrophoresis and separated crude extracts of sweet potato roots into three distinct bands each showing a capacity to catalyse the oxidation of chlorogenic acid. From these experiments it seems clear that organisms contain several types of polyphenol oxidase enzymes.

This investigation was undertaken to determine whether the classical phenolase of potato tubers could be separated into more than one active fraction by electrophoretic techniques.

Crude extracts of frozen peelings from commercially available Russet Burbank potatoes were prepared by a modified method of Smith and Krueger[2]. For the preparation 500 g of frozen peelings in 2 l. cold 15 per cent ethanol containing 0·05 M acetate buffer at *p*H 5·3 were homogenized in a Waring blender for 1 min. The mash was strained through cheesecloth and the filtrate centrifuged in a Servall continuous centrifuge at 18,000*g*. The precipitate recovered after centrifugation was mixed with the mash and the entire mixture was resuspended in 0·1 M sodium chloride solution (400 ml. 0·1 M sodium chloride per 500 g of original starting material). Centrifugation yielded the first salt extract, which was discarded. The pulp was resuspended overnight in 0·1 M sodium chloride, using the proportions already described, and the salt extract was collected after centrifugation. The sodium chloride extraction procedure was repeated three times. The three sodium chloride extracts were combined and brought to 70 per cent saturation with solid ammonium sulphate. The precipitate was collected at 18,000*g* and dissolved in 0·01 M phosphate buffer at *p*H 7. The extract was dialysed overnight against 0·001 M phosphate buffer at *p*H 7. In a large-scale fractionation, 40 lb. of potato peelings yielded 275 ml. of dialysed enzyme solution containing 30 mg protein/ml. An assay of the activity of the extract by the methods of Sisler and Evans[3] showed a specific activity of 117 u./mg protein. A sample (92 ml.) of the dialysed preparation was further purified by use of a preparative electrophoresis apparatus in the laboratory of Dr. H. S. Mason. The extract was introduced into an Elphor continuous electrophoretic separator, model *FF*, at a rate of 10 ml./h. *Tris* buffer (0·01 M) at *p*H 7·0 was supplied at a rate of 80 ml./h. The material was subjected to electrophoresis at 2,400 V for a period of approximately 10 h. Forty-nine fractions were obtained, each with a volume of 15 ml.

The fractions were assayed for polyphenol oxidase activity by the indirect assay procedure of Sisler and Evans[3], using chlorogenic acid, catechol and *p*-cresol as substrates. The concentrations of different reactants in a final volume of 3 ml. were: polyphenolic substrate $5{\cdot}7 \times 10^{-5}$ M; ascorbic acid $2{\cdot}8 \times 10^{-5}$ M; ethylenediamine tetraacetic acid 10^{-3} M. One unit of enzyme activity was expressed as an optical density decrease of 0·01 per min at 265 mμ. The protein content of the extract was determined by the method of Lowry *et al.*[1].

The curves in Fig. 1 show that the phenolase enzyme complex from potatoes was separated into two distinct components by electrophoresis. Both components catalysed the oxidation of chlorogenic acid more rapidly than the other substrates tested. The peak fractions (tubes 21 and 37, Fig. 1) showed similar ratios of activity when catechol was compared with chlorogenic acid as substrate. The cresolase activity in relation to the activity with other substrates was greater in the first peak than in the other peaks, showing that cresolase activity was concentrated in the first component.

The pronounced shoulders which preceded the peak fractions in Fig. 1*A* suggest that both components are electrophoretically heterogeneous in relation to phenolase

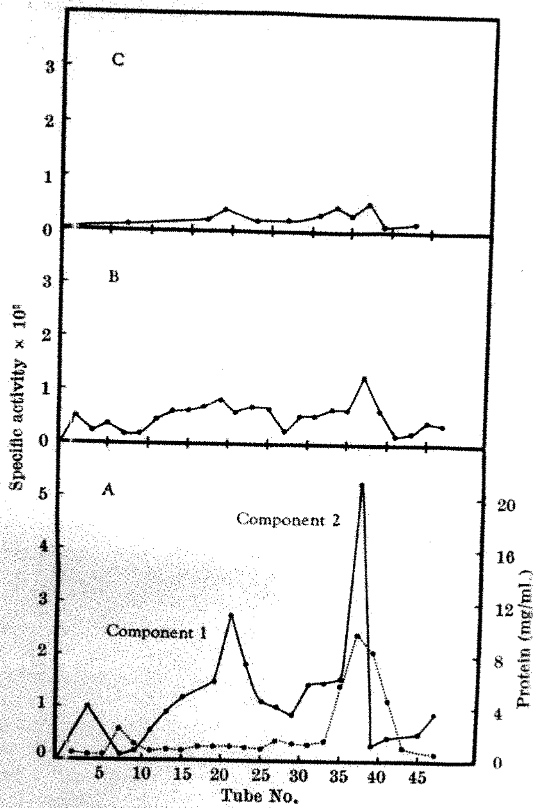

Fig. 1. Purification of an extract from potato tubers by electrophoresis. *A*, Phenolase activity using chlorogenic acid as the substrate; *B*, phenolase activity using catechol as the substrate; *C*, phenolase activity using *p*-cresol as the substrate. ●——●, in *A*, *B* and *C* refers to units per mg protein; ● . . . ●, in *A* refers to mg protein per ml. (this curve also represents the protein content of the enzyme used in *B* and *C*). In this experiment 350,000 units of enzyme (using chlorogenic acid as the substrate) were subjected to electrophoresis and 60 per cent of the total activity was recovered in the fractions

enzymes. The comparison of the chlorogenic acid to catechol activity ratios from the results in Figs. 1*A*, *B* show that the specificity for substrate of certain shoulder fractions is different from the substrate specificity of peak fractions. For example, the ratios of activity with chlorogenic acid (Fig. 1*A*) to activity with catechol (Fig. 1*B*) for shoulder fractions 17 and 33 are 1·6 and 2·6 respectively; whereas, comparable ratios for the peak fractions 21 and 37 are 4·6 and 4·3 respectively. Similarly, the ratios of activity with chlorogenic acid to activity with *p*-cresol for shoulder fractions 17 and 33 are 6·1 and 3·5; whereas the comparable ratios for peak fractions 21 and 37 are 6·9 and 17·5 respectively.

It is clear from the curves in Fig. 1 that crude extracts of potato tubers contain electrophoretically different polyphenol oxidases. Further purification of the proteins that constitute these components will be necessary in order to characterize more completely the different phenolase enzymes from this source.

SURESH PATIL
HAROLD J. EVANS

Department of Botany and
Plant Pathology,
Oregon State University,
Corvallis, Oregon.

P. McMAHILL

Department of Biochemistry,
University of Oregon Medical School.
Portland, Oregon.

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Effects of X-rays on Nuclear RNA of Lymphatic Cells

THE metabolism of ribonucleic acid in various animal tissues is known to be resistant to ionizing radiation. Recently, nuclear RNA was found by several investigators[1–4] to be inhibited in incorporating its precursors, and further investigations on nuclear RNA seemed necessary to analyse the effects of ionizing radiation on animal cells. In the work recorded here, nuclei of rabbit appendix were used as material, as rapid incorporation of phosphorus-32 into nucleic acids *in vitro*[5] has been found in these isolated nuclei.

Nuclei were isolated from rabbit appendix essentially according to the method of Allfrey and Mirsky[6], using 0·25 M sucrose solution as the suspending medium. The nuclei suspended in the medium, to which were added 5 mM magnesium chloride and 5 μg/ml. streptomycin, were irradiated ice-cold with X-rays, using a filter of 0·25 mm copper and 1·0 mm aluminium, a dose rate of 217 r. or 512 r. per min, 200 kV and 19 m.amp. Immediately after irradiation, the nuclear suspension and the unirradiated control were incubated with 2 μc. ^{32}P/ml. for 30 min at 37° C. The suspensions were then chilled and washed with 0·01 M phosphate buffer, *p*H 7·0. RNA was extracted with 90 per cent phenol by Kirby's method[7]. Nuclear RNA obtained was fractionated with the column of DEAE-cellulose (0·89 m.equiv./mg) by the gradient elution with linearly increasing concentration of sodium chloride up to 1·0 M in 0·01 M phosphate buffer, and finally by 0·5 N sodium hydroxide, collecting the elute each by 4 ml. The amounts of RNA was measured with a spectrophotometer at a wave-length of 260 mμ. Aliquots were taken from eluted samples to count the radioactivity of phosphorus-32 with a Geiger–Müller counter.

Fig. 1 illustrates the chromatogram of normal nuclear RNA. Seven groups of the fractions were separated: *F*–I (fraction I) eluted by the buffer; *F*–II by sodium chloride at 0·1 M; *F*–III at 0·15–0·2 M; *F*–IV at 0·5 M; *F*–V at 0·6 M; *F*–VI at 1·0 M; *F*–CII eluted by 0·5 N sodium hydroxide.

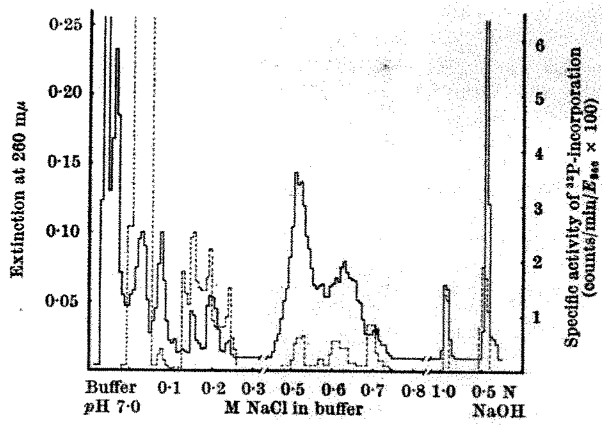

Fig. 1. Chromatographic pattern of normal nuclear RNA. 2–5 mg of phenol-extracted RNA was charged on 1·5 g of DEAE-cellulose. Elution was carried out with sodium chloride of linearly increasing concentration buffered with phosphate buffer at *p*H 7·0, and finally with 0·5 N sodium hydroxide, at the flow-rate of 0·6–0·7 ml./min at room temperature. ——, Amounts of RNA (extinction at 260 mμ); —, specific activity of ^{32}P-incorporation (counts/min/*E*260 × 100)

Irradiation with 1,000 r. caused no significant changes in the chromatographic pattern of RNA. By irradiating with 2,000 r., *F*–IV and V decreased slightly, while *F*–I and II increased as compared with those of normal (Fig. 2). After 10,000 r.-irradiation, *F*–IV and V decreased remarkably, and *F*–I was much more increased than that at the 2,000 r. of irradiation (Fig. 3).

Incorporation of phosphorus-32 into the fractions of RNA was also influenced by X-ray. In the normal nuclei,

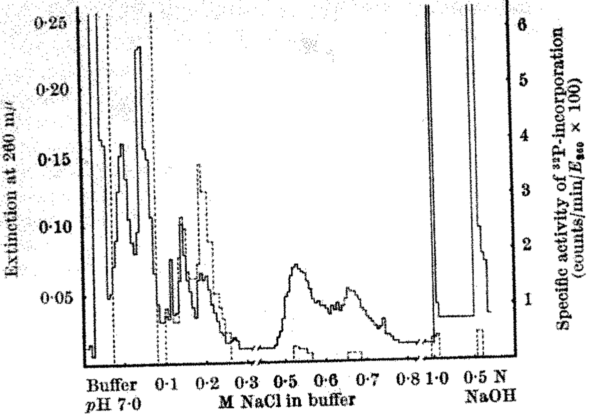

Fig. 2. Chromatographic pattern of 2,000 r.-irradiated nuclear RNA. Nuclear suspension was irradiated with X-ray, at the dose-rate of 217 r./min, followed by the incubation in the 0·25 M sucrose solution with 2 μc. ³²P/ml. for 30 min at 37° C. Other procedures were the same as in the legend of Fig. 1

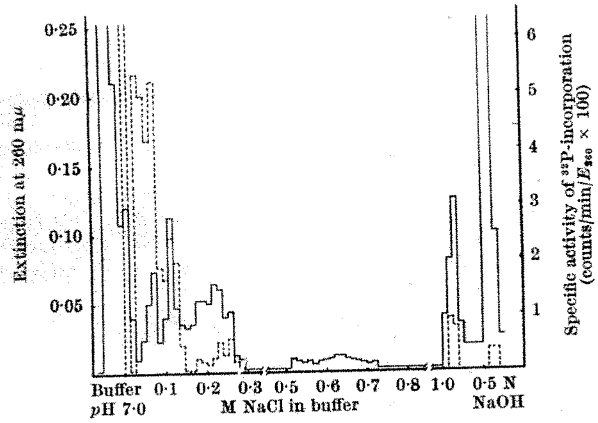

Fig. 3. Chromatographic pattern of 10,000 r.-irradiated nuclear RNA. All procedures were the same as in the legend of Fig. 2; but the dose-rate was 512 r./min

the degree of incorporation was highest in *F*–II, and *F*–III also showed considerable incorporation. Other fractions did not present the high incorporation as such (Fig. 1). These incorporations into the fractions were depressed by the irradiation of 2,000 r. except into *F*–II in which it was accelerated from 1·1 to the extreme case of 400 times normal. 10,000 r. of irradiation caused great inhibition of the incorporation into all fractions. In *F*–IV and V, the incorporation was not detectable and in *F*–II it was only 1/20–1/80 of normal.

This experiment demonstrates that RNA is reduced both in amounts and in the degree of incorporation of phosphorus-32 by X-ray irradiation, and that the RNA incorporating phosphorus-32 at a low rate is more sensitive to X-rays than that incorporating at a high rate.

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KAZUHIRO J. MORI
TOSHITERU MORITA

Department of Nuclear Science,
Faculty of Science,
Kyoto University,
Japan.

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Bacteriophage Plaque-count Assay and Confluent Lysis on Plates without Bottom Agar Layer

WITH so much research being carried out on bacteriophage, improvements to simplify and save time in the routine techniques of assay and preparation would be of great value. Having spent considerable time on preparation of 'bottom' agar plates for the agar layer assay by the plaque count method[1,2] and for high-titre bacteriophage stock preparation on a large number of plates by the confluent lysis method[3], we investigated the possibility of saving time by using plates without 'bottom' agar for assay and stock preparation.

The following medium was found satisfactory on glass plates without 'bottom' agar. The components per litre were: Difco bacto-casamino-acids (acid hydrolysed casein) 10 g, Difco bacto-nutrient broth 15 g, sucrose 10 g, dextrose 1 g, crystalline magnesium sulphate 5 g, sodium chloride 5 g, and agar 8 g.

The results for efficiency of plating and for yields are presented in Table 1.

Table 1. PLAQUE FORMATION AND YIELD OF DIFFERENT BACTERIOPHAGES ON PLATES WITHOUT 'BOTTOM' AGAR

Type of bacteriophage	Efficiency of plating by our method* (% plaques compared with standard assay†)	Yields observed (No. of phage per plate) On plates with 'bottom' agar‡	On plates without 'bottom' agar§
*T*1	101·1 ± 5	5·0 × 10¹¹	6·2 ± 10¹¹
*T*2	52·7 ± 5	1·1 × 10¹²	1·1 × 10¹²
*T*3	98·7 ± 5	4·5 × 10¹¹	7·1 × 10¹¹
*T*4	51·2 ± 5	5·8 × 10¹¹	7·9 × 10¹¹
*T*5	53·7 ± 5	9·0 × 10¹¹	1·1 × 10¹²
*T*6	53·2 ± 5	6·0 × 10¹¹	6·6 × 10¹¹
*T*7	104·5 ± 5	3·7 × 10¹¹	4·6 × 10¹¹

* 1·5 ml. of *E. coli* suspension in the log phase of growth and 0·1 ml. of appropriate dilutions of phage in tryptone broth were mixed with 4·5 ml. of our melted medium and poured on sterile Petri dishes immediately; it solidified in about 15 min. Errors represent maximum deviation in six experiments.

† Agar layer assay method of Adams (ref. 2).

‡ Prepared as described by Swanstrom and Adams (ref. 3).

§ 3·0 ml. of bacterium suspensions, infected with enough phage to give confluent lysis (3·5 × 10⁴ of *T*1; 2·1 × 10⁶ of *T*2, *T*4 and *T*6; 2·1 × 10⁴ of *T*3 and *T*7; 5·3 × 10⁶ of *T*5) were mixed with 4·5 ml. of our melted agar, and poured on sterile Petri dishes. After overnight growth, 10·0 ml. of tryptone broth was added to each plate, and the agar was broken up and filtered fast through Whatman No. 2 paper in a size 3 Buchner funnel (losses were noted if it took too long to filter, therefore larger funnels should be used for larger volumes).

Let us first consider the formation of plaques on plates without 'bottom' agar. It is seen that the efficiency of plating for *T*1, *T*3 and *T*7 is the same as by the conventional assay over 'bottom' agar. However, the number of plaques for *T*2, *T*4, *T*5 and *T*6 formed on plates without 'bottom' agar is only approximately 50 per cent of that formed on plates with 'bottom' agar; therefore, in order to arrive at the correct value, plaque counts obtained by our method should be multiplied by two. The results are consistent if *E. coli* is in the same phase of growth.

Second, let us consider the yields of various phages by confluent lysis. The yields obtained on plates without 'bottom' agar were slightly better than the yields obtained on plates with 'bottom' agar. (However, our yields of phages prepared according to the method of Swanstrom and Adams[3] were lower than those reported by the original investigators.)

As the area for confluent lysis in a glass plate (Petri dish) is limited and as Petri dishes with large areas are not available, stainless steel trays (Volrath, size 17½ in. × 11⅝ in. × ¾ in.) with aluminium covers were used. Confluent lysis on such trays was conveniently achieved as follows: to 100 ml. of *E. coli* suspension, grown overnight in an incubator by shaking but without aeration, was added 100 ml. of tryptone broth, 40·0 ml. of special solidifying medium (the solidifying medium was similar to our assay medium as described before, except that it contained twice as much casamino-acids, nutrient broth and agar) and a proportionally larger number of phage (30 times the number used for confluent lysis per plates). The mixture was then poured on the trays and stored overnight at room temperature (25°). Then the soft agar was broken up and filtered on Whatman No. 2 paper through a large (size 6) Buchner funnel. The average yields (× 10¹³) obtained per tray were: 3·2 for *T*1, 3·8 for *T*2, 1·2 for *T*3, 2·0 for *T*4, 6·0 for *T*5, 4·3 for *T*6 and 1·1 for *T*7. These values are 25–50 times the yield obtainable in a normal size glass Petri dish without 'bottom' agar.

Summing up one can say that: (1) Routine assay of bacteriophage samples can be made on glass plates without 'bottom' agar, but a correction factor of 2 has to be taken into account for bacteriophages *T*2, *T*4, *T*5 and *T*6. (2) High yields of bacteriophages can be obtained when confluent lysis is carried out on glass plates without 'bottom' agar. (3) Confluent lysis can be adapted for large-scale bacteriophage production by carrying it out on large stainless steel trays.

SAIYID RIZVI
PETER T. MORA

National Institutes of Health,
Bethesda, Maryland.

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PHYSIOLOGY

A New Concept of Temperature Regulation by Amines in the Hypothalamus

NEARLY ten years ago it was found[1,2] that adrenaline, noradrenaline and 5-hydroxytryptamine (5-HT) are present in relatively high concentrations in that part of the wall of the third ventricle known as the hypothalamus. Yet no definite function could be attributed to their presence in this diencephalic structure.

The hypothalamus is involved in the central control of a variety of important functions, one of which is concerned with regulation of body temperature. But what goes on in the hypothalamus to bring about this regulation? The experiments recorded here suggest that it is the release of adrenaline, noradrenaline and 5-HT.

The experiments are the outcome of two independent observations. First, that pyrogens injected into the cerebral ventricles[3,4] or directly into the anterior hypothalamus[5] of a cat cause fever and shivering, and second that, injected into the cerebral ventricles, adrenaline and noradrenaline abolish drug-induced shivering, whereas 5-HT has the opposite effect and evokes shivering. These effects, too, have been attributed to an action on the hypothalamus[6–8]. We have now shown that the injection of these amines through an implanted Collision cannula into the cerebral ventricles of an unanæsthetized cat also modify body temperature: adrenaline and noradrenaline lower, and 5-HT raises, the temperature.

To demonstrate the effect of adrenaline or noradrenaline, the body temperature has first to be elevated. This can be done by pyrogens. When a minute amount of pyrogen—about 1/1,000 of the dose effective on intravenous injection—is injected intraventricularly into an unanæsthetized cat the rectal temperature rises after a latency of about 1 h to a level of 2°–2·5° C above normal and remains elevated for several hours. The high temperature can then be brought down to normal, or to nearly normal, by an intraventricular injection of 50–100 μg of either adrenaline or noradrenaline. Within a few minutes the temperature begins to fall and the maximal fall is reached within about 45 min; then the temperature slowly climbs during the next 2–3 h until fever-level is again attained. When smaller doses of adrenaline or noradrenaline are injected intraventricularly at half-hourly intervals a reduction in temperature is maintained for longer periods. The injections of the catecholamines also cause cessation of the shivering produced by pyrogens.

To produce with 5-HT a rise in body temperature as well as shivering, 200 μg are injected intraventricularly into the unanæsthetized cat. Usually the rise occurs in two peaks. There is first a transient, relatively quick rise, which is followed within 1 h after the temperature has returned to normal by a second more gradual but prolonged elevation of up to 2° C. It is sometimes more than 20 h before the rectal temperature has returned to normal. When 5-HT is given during the pyrogen fever it appears to be even more effective. The intraventricular injection of 200 μg then causes an additional steep elevation of up to 1° C and it requires more than 30 h before the pyrogen–5-HT fever subsides.

The fever and shivering produced by 5-HT are affected in the same way by the intraventricular injection of adrenaline or noradrenaline as the fever and shivering produced by pyrogens. The temperature is brought down and shivering ceases.

The findings reported here lead to a new concept about the mechanisms involved in the hypothalamic regulation of body temperature. They suggest that normal temperature is maintained by a delicate balance in the release of adrenaline, noradrenaline and 5-HT in the hypothalamus, thus attributing an important physiological function to the occurrence of these amines in this part of the brain; this, however, may not be their sole function. The effect of adrenaline and noradrenaline in lowering body temperature is seen only when the temperature has first been elevated. The reason may be because these amines are continuously released in sufficient amounts in the hypothalamus to keep the temperature down, in the same way as their release is thought to prevent us from shivering continuously[9]. Additional adrenaline or noradrenaline applied artificially to this region, therefore, would have no action either on the temperature which is already down, or on shivering which is not present. A rise in temperature as well as shivering could then be visualized as the result of two mechanisms: an inhibition of the release of adrenaline and noradrenaline, and an initiation or augmentation of the release of 5-HT. Fever produced by pyrogens, that is, the fever of infectious diseases, may be brought about in just this way. In addition, pyrogens may render the hypothalamus more sensitive to the action of 5-HT, and there is the further possibility that pyrogens do not act solely through the hypothalamic amines and their release, but also mimic the effect of 5-HT.

W. FELDBERG
R. D. MYERS*

National Institute for Medical Research,
Mill Hill, London, N.W.7.

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Integration at an Inhibitory Interneurone: Inhibition of Renshaw Cells

THE spinal interneurones known as Renshaw cells have as their function the regulation of motoneurone activity: they appear to terminate on motoneurones directly inhibiting their firing[1], and to inhibit other inhibitory interneurones, thereby disinhibiting motoneurones and enhancing their discharge[2]. Because of this widespread involvement of Renshaw cells in the control of motor activity, the regulation of the firing of Renshaw cells themselves is of considerable interest. Since the discovery of these interneurones by Renshaw[3] and their subsequent detailed examination by Eccles, Fatt and Koketsu[1] and others, attention has centred on their excitation. However, it has been observed that Renshaw cells can be inhibited as well as excited by supraspinal stimuli[4], and an observation of Renshaw's[3] suggests that they can also be inhibited by antidromic stimulation of motor nerves. In view of the importance of Renshaw cells as the only clearly identified inhibitory interneurones in the spinal cord, we have investigated the influence of various types of stimuli on their discharge, and find that widespread inhibitory action contributes to the regulation of their activity.

All experiments were performed on acute decapitate cats; the carotid arteries were tied and vertebral arteries clamped. Artificial respiration was applied. In order to minimize movement all preparations were paralysed by intravenous injections of 'Flaxedil' (American Cyanamid Co.). Laminectomy was performed in the usual manner. In some preparations the dorsal roots on the side on which recording was to take place (ipsilateral side) were cut from *S2* to *L5* to prevent the entry of orthodromic impulses into the cord, and various leg nerves were dissected to make possible antidromic stimulation of motor nerves. With such preparations it was possible to test the effect on Renshaw cell discharge of natural stimulation of all limbs other than the ipsilateral hind limb and of antidromic stimulation of the ipsilateral motor nerves. For other experiments the ipsilateral dorsal roots were left intact and the ventral roots cut; on the contralateral side the ventral roots were cut, and leg nerves prepared for stimulation. This type of preparation was used for natural stimulation of the ipsilateral hind limb and electrical stimulation of contralateral muscle and cutaneous nerves.

Recording from Renshaw cells was extracellular, and was performed by means of glass micropipettes filled with 4 M sodium chloride and having a resistance of 1–2 Meg. Cells were found by tracking in the *L7*–*S1* region while stimulating either motor nerves or ventral roots, and were identified by their characteristic high-frequency response to a single antidromic shock[1,3]. Most cells were found within a few hundred microns of the dorsal root entry line and at depths between 2 and 3·5 mm. Once a cell was located and its pattern of discharge was determined, attempts were made to condition the discharge by electrical stimulation of various nerves and by natural stimuli applied to various parts of the body surface.

In agreement with Eccles, Eccles, Iggo and Lundberg[5], it can be stated that Renshaw cells can be activated by more than one muscle nerve, although often one nerve is dominant. In our experiments the discharge has consisted of up to 30 spikes, with a total duration of up to 60 msec. So far, we have been unable to inhibit this discharge by antidromic stimulation of any of the available muscle nerves (deep peroneal, flexor longus digitorum, plantaris, gastrocnemius-soleus, posterior biceps-semitendinosus, biceps anterior and semimembranosus). However, it has been possible to obtain powerful inhibition of the response by stimuli, such as squeezing and pinching applied to the various limbs. Such an inhibition is shown in col. 1 of Fig. 1. It can be seen that the inhibition is quite profound. The group of frames in *B* illustrates a typical occurrence, namely, that the inhibition becomes less powerful during a maintained stimulus. Strong inhibition has usually been obtained from the contralateral hind limb; in addition, inhibitory effects have often been seen as a result of stimulating the ipsilateral hindlimb, ipsilateral forelimb and tail. From the nature of the stimuli applied it is evident that much of the inhibition must be due to activity in cutaneous fibres. Experiments to determine the nature of the nerves involved are under way at present.

Column 2 of Fig. 1 shows that this inhibition is depressed by strychnine. Intravenous injection of 0·1 mg/kg strychnine sulphate resulted in some irregularity of the test discharge and occasional burst responses of the cell. It can also be seen in *B* of col. 2 that after strychnine the conditioning stimulus frequently resulted in slow waves, which were not, however, associated with discharge of the cell. Comparison of cols. 1 and 2 shows that in this instance the inhibition is almost abolished. Evidently this inhibition resembles, in its susceptibility to strychnine, the various post-synaptic spinal inhibitions that have been investigated (cf. ref. 6), and differs in this respect from various post-synaptic inhibitions at higher levels of the nervous system[7].

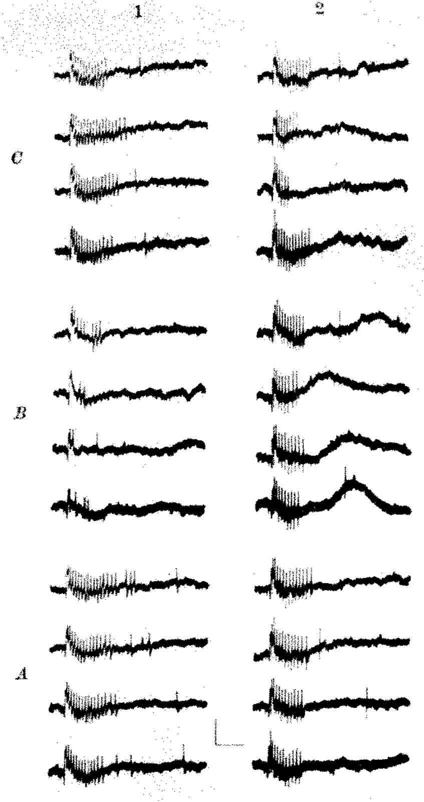

Fig. 1. Inhibition of Renshaw cell discharge. This cell was activated only by antidromic stimulation of posterior biceps-semitendinosus. The inhibition illustrated was obtained by squeezing of toes of the contralateral hind foot. In col. 1, reading from bottom to top, *A* shows control responses, *B* responses during squeeze of the toes, and *C* subsequent controls. Squeeze of the toes alone evoked no discharge. Col. 2 shows an identical series obtained 5 min after intravenous injection of 0·1 mg/kg strychnine sulphate. It can be seen that the inhibition is strongly depressed. The spikes in col. 2 are somewhat larger than those in col. 1, because the electrode moved closer to the cell. Upward deflexion negative. Time marker, 10 msec.; voltage calibration, 500 μV

It is clear from the results recorded here that Renshaw cells can be inhibited by means of segmental reflexes, both ipsilateral and contralateral, and by means of long spinal reflexes. Inhibition of inhibitory interneurones has been proposed in other regions of the nervous system[8,9], but our experiments provide positive evidence for inhibition of an interneurone the only known function of which is to inhibit motoneurones and other interneurones. Since depression of Renshaw cell discharge would result in release of motoneurones, these findings illustrate another pathway which can lead to disinhibition of spinal motoneurones. It is now apparent that the control of the recurrent collateral system which leads to inhibition and disinhibition of motoneurones[2,10] is even more complex than previously supposed.

It would seem reasonable to expect that inhibition of inhibitory interneurones, together with changes in excitatory drive, would play a part in the integration of motor activity. It is more economical to suppress an inhibition at its origin than to override it at its destination.

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VICTOR J. WILSON
WILLIAM H. TALBOT

The Rockefeller Institute,
New York.

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Prevention by Ascorbic Acid of Liver Glycogen Depletion in Endotoxin Intoxication

IT is well established that there is a striking depletion of liver glycogen in endotoxin intoxication, and insulin, glucose and lactate are relatively ineffective in increasing liver glycogen storage[1]. This depletion of liver glycogen is not necessarily due to epinephrine discharge or to circulatory disturbances attendant on intoxication, but it seems to be related to the accumulation of endotoxin in liver[2]. The characteristic prostration common to all animal species in the later stage of endotoxin intoxication seems to be related to this depletion of glycogen[2]. It is especially pronounced in adrenalectomized animals, and death results from hypoglycæmic deterioration with or without cramp. It is well known that pretreatment with cortisone prevents death[1]. Although the mechanism of glycogen depletion has been discussed in relation to inhibition of hepatic enzymes, especially succinic dehydrogenase[3], or to the effect on mitochondria leading to oxidative uncoupling[4], no conclusive evidence indicating special relations of endotoxin to these disturbances could be obtained. In this respect it has been shown that the effect of endotoxin in depleting liver glycogen in rabbits is much lessened in the hot summer season even in adrenalectomized animals[5]. It has therefore been suggested that the depletion of liver glycogen might be determined by the metabolic response of liver induced by the uptake of endotoxin in the hepatic reticuloendothelial cells. Here it was shown that saturation with ascorbic acid could ameliorate the depletion of liver glycogen in cold weather in which the susceptibility of the animal is markedly enhanced.

Albino rabbits weighing about 2·5 kg were used. They were maintained by 'Okara' (soy-beans curds waste 300 g with salt 0·5 g a day). Ascorbic acid (500 mg/day, mostly 'Hi-C' tablet of Takeda Chemical Industries, Ltd., Osaka) was mixed with 'Okara'. For attaining blood total ascorbic acid level at the saturation-level (about 1·2 mg/dl., DNP method), at least 4 days' administration was necessary. Experiments were performed both on intact and adrenalectomized animals in order to determine the effect of ascorbic acid saturation independent of glucocorticoids with which ascorbic acid has been claimed to be synergistic[6]. It has been repeatedly shown that adrenalectomized rabbits do not necessitate any replacement treatment and eventual existence of accessories can easily be checked by a water diuresis test[7]. Purified endotoxin (300 µg/kg of *Salmonella typhosa*, Difco) was administered intravenously after fasting overnight. Liver specimens for glycogen analysis were taken after 3 h under light 'Nembutal' anæsthesia (20 mg/kg, intravenously).

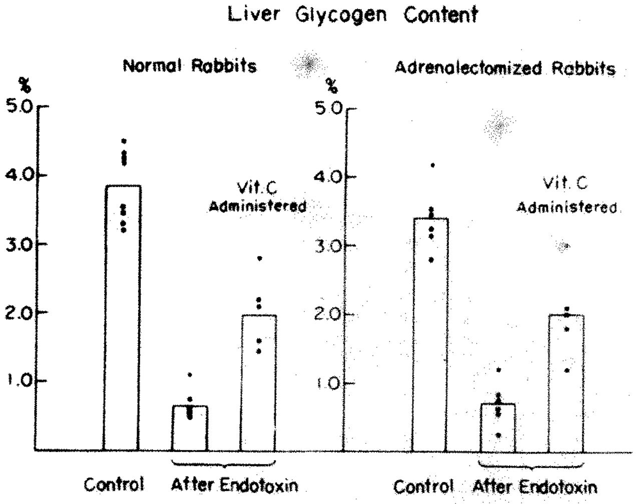

Fig. 1. Prevention of liver glycogen depletion due to endotoxin by ascorbic acid saturation (3 h after endotoxin)

As shown in Fig. 1, ascorbic acid saturation could markedly ameliorate the depletion of liver glycogen after endotoxin both in intact and adrenalectomized animals. With this the attendant hypoglycæmia was completely prevented and in intact animals the preceding initial hyperglycæmia due to epinephrine discharge[2] became marked and prolonged. Even adrenalectomized animals could tolerate the toxic dose used here (100 per cent lethal for adrenalectomized rabbits[2]). Thus the effectiveness of ascorbic acid in preserving liver glycogen independent of glucocorticoids could be substantiated. There was no indication that ascorbic acid consumption might be increased in endotoxin intoxication, since no appreciable changes in ascorbic acid content (total and reduced) in blood and liver occurred after endotoxin. Therefore, the effectiveness of ascorbic acid did not seem to be concerned with the supplement of ascorbic acid consumed. No gluconeogenetic effect[8] could be expected. Stabilization of hepatic metabolism by its antioxidative effect seemed to be the underlying mechanism. In cases of severe endotoxin intoxication the blood glutathione (GSH, after Brückmann and Wertheimer) became reduced (from about 30 to 20 mg/dl. 3 h after endotoxin inoculation). This was prevented with lessening of the intoxication by ascorbic acid saturation, just as the case with tolerant animals.

Saturation with ascorbic acid did not materially increase the ascorbic acid content of liver (13–18 mg per cent, total ascorbic acid) and yet could show the foregoing remarkable effect. In this respect, the observation by Fukuda and Horiuchi[9] in man that, with increasing saturation with ascorbic acid, the consumption of ascorbic acid estimated by the deficit in the daily urinary output compared with the amount ingested increased markedly might throw some light on this problem. In any event, significance of ascorbic acid saturation could be evaluated in endotoxin intoxication leading to hepatic glycogen depletion.

TOKURO FUKUDA
TAKEICHI KOYAMA

Department of Physiology,
Chiba University School of Medicine,
Chiba, Japan.

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Alcohol and Evoked Potentials in the Cat

THE effect of alcohol on the electrical activity of the cortex and deep structures of the brain in various animals has been the subject of investigation of many workers, including Horsey and Akert[1] and Caspers[2]. There is a general agreement that this drug has a synchronizing effect on the spontaneous electrical cortical activity and, depending on the dose of alcohol, shows various degrees of reduction in frequency and decrease in amplitude. The underlying mechanism of this action is still in doubt. Kalant[3], in a comprehensive review, quotes Caspers[2] and furthers the idea that alcohol exerts its earliest effect at the level of the reticular formation and that the changes in electrical cortical activity are subsequent to the primary reticular depression. In this preliminary communication are presented results on the effects of alcohol on the evoked responses recorded from the reticular formation and cortex following stimulation of the sciatic nerve.

Acute experiments were carried out in 14 intact adult cats. Under ether anæsthesia the animals were tracheotomized and both femoral veins and one femoral artery cannulated. The animals were placed in a stereotaxic apparatus and electrodes (concentric needles) were stereotaxically placed through a burr hole into the mesencephalic reticular formation at intercollicular level (*F*; 2·5; *L*; 1·5; *H*; −2·5) according to the co-ordinates of Jasper and Ajmone Marsan[4]. Recording electrodes (chloride silver balls) were also located on the somatosensory cortex. The wound margins and pressure points were injected with local anæsthetic and the ether then discontinued. The animals were immobilized with 'Intocostrin' (Squibb), maintained on artificial respiration and kept warm under a heating pad. The sciatic nerve contralateral to the recording electrodes was cut before the ether was discontinued and the central stump stimulated electrically, 2–3 h post-operative, with single rectangular pulses of 7–9 V and 0·1–0·2 msec duration, delivered every 2 sec. The evoked potentials in the midbrain reticular formation and in the somatosensory cortex were simultaneously amplified and recorded on the face tube of a 502 dual beam 'Tektronix' oscilloscope. Photographs were taken with a 'Grass' kymograph camera. Arterial blood pressure was continuously monitored via the cannula inserted in the femoral artery which was connected to a mercury manometer. A 20 per cent solution of ethyl alcohol at a dose-level of 1 g/kg was slowly injected through one of the femoral cannulas which was used for this purpose only. The duration of injection was 2–3 min. Blood samples for the determination of alcohol-levels were drawn from the other femoral vein. Ethyl alcohol in the blood was determined enzymatically according to the method described in the *Sigma Technical Bulletin*, No. 330, April, 1963. Electrode placements were checked by histological examination of unstained frozen sections of the brain stem prepared as described previously[5].

Fig. 1 illustrates the effect of alcohol on the evoked potentials in the midbrain reticular formation and somatosensory cortex on stimulation of the sciatic nerve. Records of five superimposed frames were taken 2, 5, 30 and 60 min after the injection to compare with three sets of records taken as controls before the injection of alcohol. Blood samples were collected before and 5, 30 and 60 min after alcohol injection for determination of alcohol-levels. In the fourteen animals examined the following results were obtained: in nine experiments (Fig. 1, series *A*), the response from the somatosensory cortex was markedly depressed while the reticular response was only partially depressed 2 min after the administration of alcohol. The recovery process was evident 15 min after alcohol injection. Both responses recovered to some extent 1 h after alcohol treatment though the recovery of cortical response was retarded, in comparison with that of the reticular formation. In five other experiments (Fig. 1, series *B*), both the cortical and reticular responses were markedly suppressed two minutes after alcohol injection. However, in these animals the partial recovery of the reticular response was more marked than that of the somatosensory cortex 1 h after the injection of alcohol. In general, the cortex was more profoundly affected by alcohol than the reticular formation.

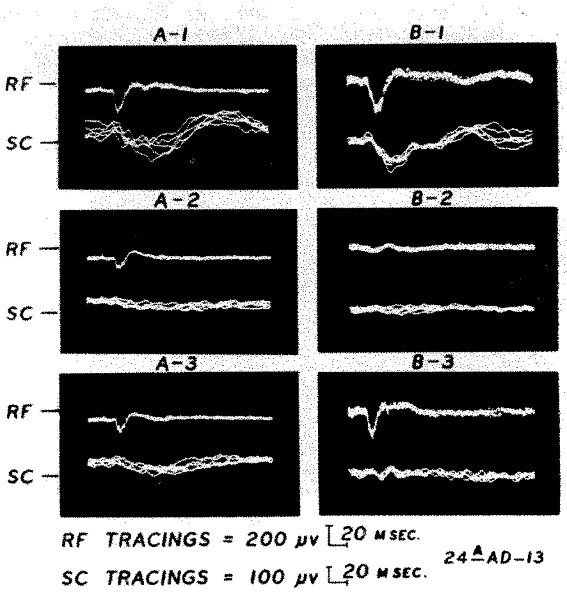

Fig. 1. Effect of alcohol on the evoked potentials in the reticular formation (*RF*) and somatosensory cortex (*SC*) on the stimulation of sciatic nerve. *A*-1, Control; *A*-2, 2 min after alcohol injection, a depression which was marked in *SC* and only partial in *RF*; *A*-3, 15 min after alcohol injection, onset of the recovery process which, however, was retarded in *SC*; *B*-1, control; *B*-2, 2 min after alcohol injection, marked depression of both responses; *B*-3, 1 h after alcohol injection, marked recovery in *RF* but very little in *SC*. Each response displays five superimposed traces. Upward deflexion indicates negativity

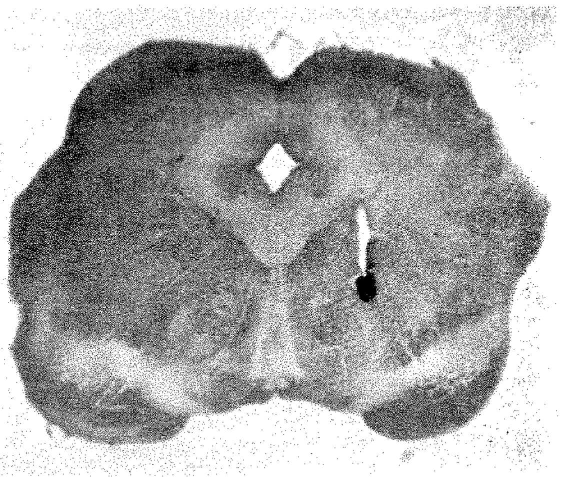

Fig. 2. Photomicrograph of an unstained coronal section of the midbrain. Track of electrode tip and a number of brain structures are clearly identifiable (× *c*. 4·75)

Control experiments were undertaken to rule out the effect of the volume of injection and of systemic changes introduced by blood pressure effects. Similar volumes of normal saline injected intravenously had no effect on blood pressure and the evoked potentials. The systemic effects of alcohol solution administered were checked by monitoring the blood pressure. About 50 sec after the injection, a moderate drop in blood pressure of about 15–20 mm mercury was observed. The initial value recovered in less than 2 min after alcohol administration. As a rule, the blood pressure values recuperated by the time the first record was taken after injection. In about 5–15 min, in most instances, the blood pressure would increase 20 mm mercury above the control value and remain so for the rest of the experiment. However, in others, the blood pressure recovered its control value within 30 min after injection. These changes in the blood pressure did not seem to influence the amplitude of the evoked potentials.

In Table 1 are shown the average levels of blood alcohol. It can be seen that 5 min after the administration of alcohol, when the responses were markedly affected, both in the reticular formation and the somatosensory cortex, the blood alcohol-levels increased from the average control value of 8 mg per cent to 123 mg per cent. These levels decreased gradually with time but were still high, as compared with the control values, after 1 h.

Table 1. BLOOD ALCOHOL-LEVELS BEFORE AND AFTER INTRAVENOUS ADMINISTRATION OF ALCOHOL (1 GM/KG)

Time after injection (min)	Control	5	30	60
Mg alcohol per 100 ml. blood	8·8 ± 3·8*	123·3 ± 27·2	107·2 ± 22·8	95·4 ± 24·4

* *S.D.*

Our experimental evidence does not confirm Caspers's contention[2] of a causative relationship for his findings of increased cortical excitability and depression of the reticular formation by ethanol. In no instance did we find increased cortical potentials. However, it should be pointed out that excitability and amplitude of evoked potentials are not necessarily correlated.

All the same, the striking observations presented here point to the fact that under our experimental conditions the cortex is affected more by ethanol than is the reticular formation. The time-course of recovery brings added evidence that alcohol exerts its effect more profoundly on the cortex. The recovery phase was not followed beyond the period of 1 h. It seems that the reticular formation and the cortex may have different thresholds of response to ethanol-levels.

A. R. DRAVID
RAOUL DI PERRI
A. MORILLO *
H. E. HIMWICH

Thudichum Psychiatric Research Laboratory,
Galesburg State Research Hospital,
Galesburg, Illinois.

* Present address: Departamento de Fisiologia, Universidad Javeriana, Facultad de Medicina, Bogota, Colombia.

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PHARMACOLOGY

Anti-œstrogenic Effects of Bovine Pineal Glands

GONADOTROPIC activity of the pineal glands has been the subject of several extensive researches[1,2]. However, there is some discrepancy in the reported effects of pineal implants or pineal extracts on the œstrogenic manifestations. It is still uncertain whether the pineal gland contains both œstrogenic and anti-œstrogenic, antagonistically acting substances, or only one of them. Moreover, the reported results have been inconclusive as to the influence on pineal gonadotropic activity of such factors as species, age and sex of animals used as tissue donors[1-6].

In our experiments we decided to adopt the male frog spermatogenic test[7,8] for investigations of pineal anti-œstrogenic properties. Chorionic gonadotrophin ('Pregnyl', N.V. Organon-Oss, Holland) and the homogenized cow pineal glands (so adjusted with saline solution that 1 ml. contained 50 mg or 100 mg of fresh tissue) were used. In further experiments, extracts of cow pineal glands were also used. The extracts were prepared either according to the method described by Altschule[9] or by a method described by Bergmann[10] in such a way that, having made it up with saline solution, 1 ml. of extract was equivalent to 200 mg of fresh pineal gland. The preparations were injected into the dorsal lymph sac of male edible frogs (*Rana esculenta* L.). The homogenized pineal glands were applied 6 h, and the extracts 3 h, prior to the injection of chorionic gonadotrophin.

In the pilot experiments performed on 80 frogs no response, as regards spermatozoa excretion, was found in any one case after injection of bovine pineal gland. The results seem to indicate that the assayed pineal glands did not contain any œstrogenic agents.

The spermatogenic sensitivity of male frog is liable to change with the season of the year[11]. The experiments were carried in December 1962 and January 1963. Therefore, in the course of preliminary investigations it has been found that in conditions of the experiments the most suitable dose was 50 I.U. of chorionic gonadotrophin and the best response was received while examining the urine 6 h after the injection of the hormone.

In the first experiments we noticed that the bovine pineal glands taken from slaughtered adult cows inhibited very significantly the spermatozoa excretion in frogs. On the contrary, no effects were noticed after the application of the pineal glands collected from 2–4-week-old calves. At first, we were inclined to explain this observation as a matter of difference in the age. However, among the calves killed at that time at the local slaughterhouse there were male and female specimens in more or less equal proportions; among adult cattle there were only females. So male frog tests were also made separately with the pineal glands of male and female calves, and it was found that the pineal glands of female calves inhibited the frog spermatogenic reaction in a manner almost as intensive as those of the cows, while with the pineal glands of the male calves the reaction obtained was insignificant and it could not be confirmed statistically. In addition, when the pineal glands of males and females were combined together in a ratio 1 : 1 no inhibitory properties were found. Also the extracts by Bergmann and Altschule, when assayed for inhibition of the frog spermatogenic test, did not produce any clear, statistically provable effects, in spite of the fact that they were prepared using the pineal glands of cows.

Table 1. Effects of parenterally injected homogenized tissues and extracts from bovine pineal glands on spermatogenic response of the male frog, *Rana esculenta*, to chorionic gonadotrophin injection

No. of group	Treatment	No. of frogs	Spermatogenic response No. of frogs	 Relative (%)	P*
1	Control group; 'Pregnyl' 50 I.U.	40	26	100·00	—
2	Cow pineal, 0·05 g; 'Pregnyl' 50 I.U.	45	16	54·62	< 0·02
3	Cow pineal, 0·1 g; 'Pregnyl' 50 I.U.	49	7	22·00	< 0·001
4	Calf pineal (♀) 0·1 g; 'Pregnyl' 50 I.U.	42	9	32·90	< 0·001
5	Calf pineal (♂) 0·1 g; 'Pregnyl' 50 I.U.	66	33	70·70	> 0·1
6	Calf pineal (♀ ♂) 0·1 g; 'Pregnyl' 50 I.U.	49	31	97·23	> 0·5
7	Cow pineal extract by Bergmann (0·2 g of a gland); 'Pregnyl' 50 I.U.	23	11	73·53	> 0·2
8	Cow pineal extract by Altschule (0·2 g of a gland); 'Pregnyl' 50 I.U.	25	16	98·46	> 0·5

* Compared with the control group No. 1 by the χ^2 method.

The results of the experiments are shown in the Table 1. For closer comparison, the number of frogs which rendered a positive spermatogenic response in a control group was taken as 100 per cent (relative per cent). In relation to that figure, the percentage of frogs reacting in the experimental groups was calculated (Fig. 1).

It seems that controversies on the subject of œstrogenic and anti-œstrogenic properties of the pineal gland met with in the available literature might be the result of not taking into consideration those peculiarities which are

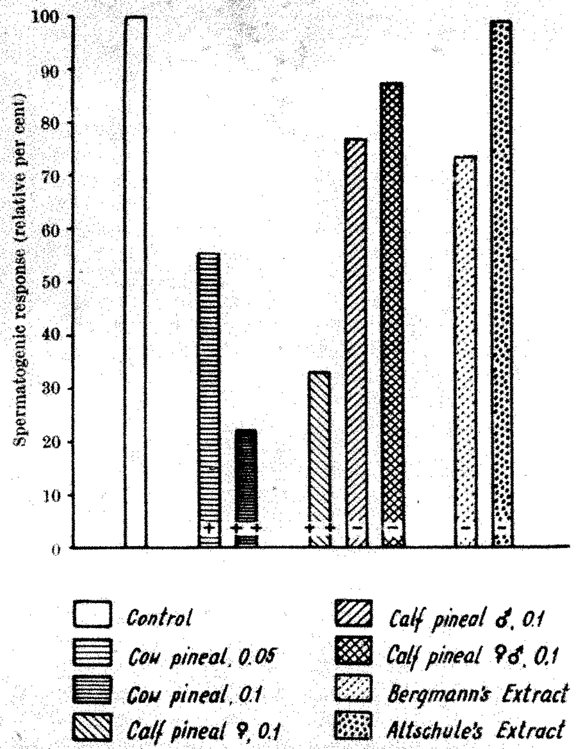

Fig. 1. Inhibition with pineal preparations of male frog spermatogenic reaction. ++, $P < 0·01$; +, $P < 0·05$; −, $P > 0·05$

connected with the sex of the animals used as pineal tissue donors. Moreover, it should be pointed out that after an application of 50 mg of cow pineal gland the inhibition of the frog reaction was nearly twice as weak as after a dose of 100 mg. It is to be expected that the described reaction of the inhibition of the frog spermatogenic test might be applied as a method of biological assay of the anti-œstrogenic properties of the pineal gland. Although several methods have already been suggested[1,2,10] for assaying the potency of pineal preparations, our method seems to have some advantages because of its simplicity, speed and cheapness.

TEODOR JUSZKIEWICZ
ZOFIA RAKALSKA

Department of Experimental
Pharmacology and Therapeutics,
Veterinary Research Institute,
Puławy, Poland.

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Excretion of Neostigmine labelled with Carbon-14 in Urine

ALTHOUGH neostigmine (prostigmin) has been used for about thirty years for the treatment of myasthenia gravis, and later for the post-operative reversal of tubocurarine, relatively little seems to be known about the mechanisms involved in its metabolism and excretion. The inhibition of plasma cholinesterase has been used as a convenient indicator of neostigmine concentration, and it has been shown that after administration to dogs and to patients with myasthenia gravis, elimination of neostigmine depends on the dose and method of administration[1]. Some of these conclusions have been confirmed in more recent work where a colorimetric method was used for the measurement of neostigmine in urine[2]. Thus when neostigmine methylsulphate is given by intramuscular injection to patients with myasthenia gravis, about 50 per cent of the dose is excreted unchanged, whereas after oral administration little or no unchanged drug can be detected in the urine[2,6].

Further work in this laboratory has shown that a high proportion of each dose by intramuscular injection is excreted by myasthenic patients within 2 h. Confirmation that neostigmine is rapidly excreted was obtained by administration of neostigmine labelled with carbon-14 to rats, when up to 50 per cent of the dose was detected in the urine within 60 min of intramuscular injection. This evidence suggested that the rapid excretion of neostigmine might be due to renal tubular secretion.

Using the technique for estimating renal tubular secretion in the hen described by Sperber[7,8], we have found that after intramuscular injection of labelled neostigmine considerably more of the dose was excreted by the ipsilateral kidney than by the contralateral kidney (Table 1). Similar results were obtained when the drug was infused at a constant rate into the saphenous vein.

Table 1. RENAL EXCRETION OF NEOSTIGMINE BY THE HEN AFTER INTRAMUSCULAR INJECTION OF NEOSTIGMINE LABELLED WITH CARBON-14

Hen No.	Dose neostigmine (mg)	Percentage of dose recovered in urine from each kidney		Duration of collection (min)
		Ipsilateral	Contralateral	
1	0·667	39·7	5·7	90
2	0·333	65·2	16·1	90
3	0·333	59·8	7·7	120
4	0·333	57·4	6·5	135

The renal tubular transport of *N'*-methylnicotinamide (NMN) and of a number of other quaternary nitrogen compounds has been shown to be inhibited by the basic cyanine dye, 1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride ('Cyanine 863')[3–5]. Experiments in which labelled neostigmine was injected in single doses and by continuous infusion into the saphenous vein of the hen have shown that the apparent tubular excretion factor is substantially reduced by prior or concurrent administration of 'Cyanine 863'.

We conclude from this evidence that when neostigmine is injected a substantial proportion of the dose is eliminated by renal tubular secretion.

The urine collected in the experiments on hens has been examined by paper chromatography and electrophoresis; most of the carbon-14 is present as neostigmine but there is also evidence of *m*-hydroxyphenyltrimethylammonium, a metabolite of neostigmine previously reported by Scott *et al.*[6].

We thank the Distillers Co., London, for a grant in support of this research from which one of us (B. H. T.) is in receipt of a maintenance grant. The 'Cyanine 863' was kindly supplied by Dr. L. G. S. Brooker of Eastman Kodak Co., New York.

J. B. ROBERTS
B. H. THOMAS
A. WILSON

Department of Pharmacology
and General Therapeutics,
University of Liverpool.

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Platelet Damage induced in Plasma by Certain Fatty Acids

THE implication of lipids in the production of hypercoagulability of the blood has been recognized for a number of years. For example, the presence of certain lipid fractions has been shown to facilitate blood clotting *in vitro*, and hypercoagulability has been demonstrated in blood taken from human subjects after a meal of high fat content[1-3]. It has been shown recently that the addition of certain fatty acids to plasma accelerates the clotting time[4], and of especial interest are observations by Poole and Connor that small quantities of long-chain saturated fatty acids, but not unsaturated congeners, evoke a marked hypercoagulability of blood in the thrombus-generator[5-7] This instrument, originally described by Chandler, utilizes a revolving loop of plastic tubing into which blood is placed. The clot produced by this procedure has been shown to closely simulate thrombi occurring in human thromboembolic disease; that is, with a white head of platelets and leucocytes and a fibrin tail[8,9].

We have recently reported that rabbit plasma contains a factor which causes platelet damage as manifested by release of platelet histamine and serotonin, and that the plasma factor, though calcium dependent, acts even in the presence of a high concentration of heparin[10]. We have, therefore, examined the effects of fatty acids on platelets in plasma rendered incoagulable by heparin.

Platelet-rich plasma was prepared in siliconed glass centrifuge tubes as described previously[11]. Fatty acids were dissolved in distilled water with an excess of sodium hydroxide, and the *p*H was adjusted to about 7·8 with hydrochloric acid. Heparin was added to a final concentration of 80 μg/ml., followed by fatty acid solution. The tubes were then recalcified and incubated for 30 min at 37° C. Platelets were removed by centrifugation at 1,200*g* for 30 min at room temperature. The histamine and, in some tubes, serotonin content of the supernatant plasma were then measured fluorometrically[12,13] and compared with the amine content of platelet-rich plasma.

Table 1. EFFECT OF FATTY ACIDS ON RELEASE OF HISTAMINE FROM RABBIT PLATELETS UNDER VARIOUS CONDITIONS*

Condition	Histamine release (control = 1·0)†
Caprylic acid (50–250 μg/ml.)	1·0 (0·9– 1·3)
Myristic acid (25 μg/ml.)	2·2 (1·3– 2·9)
Palmitic acid (25–250 μg/ml.)	1·3 (1·0– 1·9)
Stearic acid (10 μg/ml.)	3·0 (1·9– 5·1)
Stearic acid (25 μg/ml.)	5·9 (3·0– 9·8)
Stearic acid (50 μg/ml.)	6·5 (3·6–10·4)
Arachidic acid (1–25 μg/ml.)	5·2 (2·4– 8·0)
Behenic acid (1–10 μg/ml.)	3·2 (2·4– 4·4)
Oleic acid (25–250 μg/ml.)	1·0 (0·8– 1·2)
Linoleic acid (100–250 μg/ml.)	1·1 (1·0– 1·3)
Linolenic acid (100–250 μg/ml.)	1·2 (0·9– 1·4)
Stearic acid (50 μg/ml.), no calcium	0·8 (0·6– 1·2)
Stearic acid (50 μg/ml.) cold (5° C)	0·7 (0·5– 1·2)
Stearic acid (25 μg/ml.) albumin solution	0·8 (0·6– 1·1)

* Fatty acids were incubated with rabbit platelet-rich plasma in the presence of heparin (80 μg/ml.), as described in text.

† Figures for histamine release denote ratio of percentage available histamine released in experimental tubes divided by histamine released in control tube (plasma + heparin + calcium) at 37°. Figures in parentheses denote range. Serotonin release (not shown) was about the same as histamine in all tubes measured.

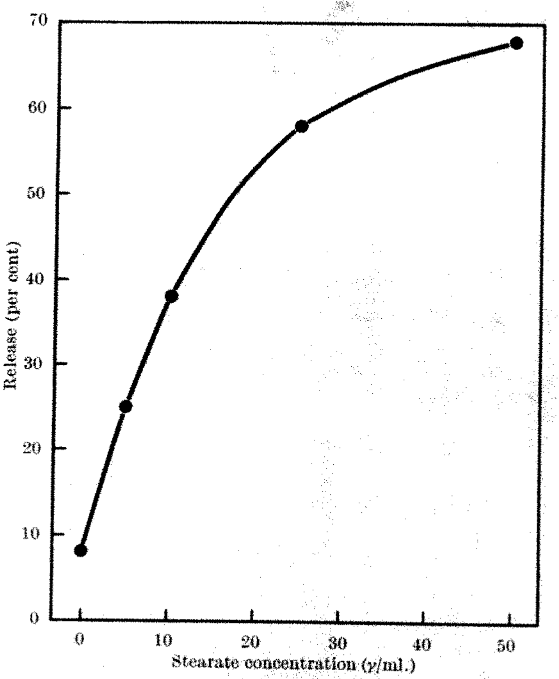

Fig. 1. Effect of various concentrations of stearic acid on release of platelet histamine

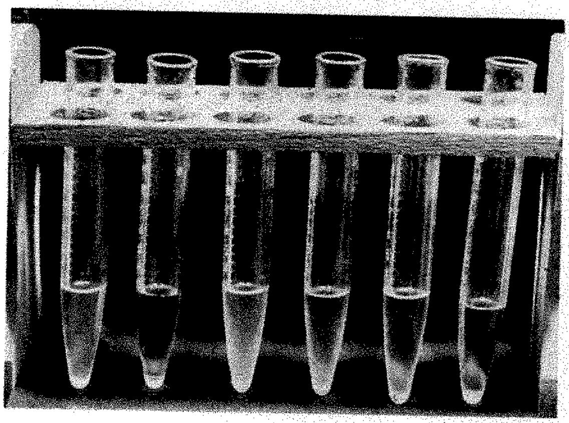

Fig. 2. Effect of stearic acid (25 μg/ml.) on rabbit platelets. Tubes were incubated and centrifuged as described in text. Left to right: (1) plasma—no stearate; (2) plasma + stearate—no calcium; (3) plasma + stearate; (4) plasma (50 per cent) + bovine serum albumin (50 per cent) + stearate; (5) plasma (25 per cent) + bovine serum albumin (75 per cent) + stearate; (6) bovine serum albumin + stearate. All tubes contained heparin (80 μg/ml.) and (except for No. 2) excess calcium ion. Figures for histamine release were: (1) 10, (2) 5, (3) 60, (4) 38, (5) 25, (6) 8 per cent

As shown in Table 1, a marked release of platelet histamine occurred in tubes to which small quantities of stearic, arachidic, or behenic acids were added, while relatively little or no release occurred with lower molecular weight saturated fatty acids or with unsaturated fatty acids, even at much higher concentrations, indicating a considerable degree of specificity in the mechanism. Fig. 1 illustrates the effect of various concentrations of stearic acid. As shown in Table 1, no histamine release occurred in the absence of calcium ion or in tubes kept in the cold (5° C). The involvement of a plasma factor is suggested by the finding that stearic acid did not act on washed platelets suspended in buffered bovine serum albumin solution (Table 1). Fig. 2 illustrates the effect of stearic acid on platelets suspended in various combinations of plasma and bovine serum albumin solution. In the presence of plasma, stearate, and calcium ion, most of the platelets have adhered to the vessel wall, but platelet damage, as estimated visually or by histamine release, is minimal in the absence of any one of these factors.

The platelet damage described here occurs even in the presence of sufficient heparin to block the action of thrombin on fibrinogen or on platelets. The fatty acid effect thus may act through an early stage of the coagulation mechanism, one not affected by heparin. The mechanism possibly involves the action of Hageman factor, thought to be responsible for the effects of fatty acids on whole blood coagulation[6,7,14]. The heparin-insensitive mechanism of this type of platelet damage may be of importance in the ætiology of white thrombus formation and hypercoagulability. The experimental system described in this communication could provide a technique for the study of certain early coagulation steps.

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P. A. Shore
H. S. Alpers

Department of Pharmacology,
University of Texas Southwestern Medical School,
Dallas.

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IMMUNOLOGY

Hæmolytic Complement and Serum Bactericidin

In a previous communication[1], serum bactericidin was related to a clotting factor extracted from fibrin. Several properties of this bactericidin are known to be shared by hæmolytic complement, such as inactivation by heating at 56° C for 30 min and removal by filtration through Seitz filter pads. Complement, like bactericidin, is absent from uncoagulated cell-free plasma[2]. Further investigations comparing the two serum activities were undertaken.

Both guinea pig and human sera served as sources of complement. Experiments were set up as follows. To fresh serum dilutions made up in 0·2 ml. of 0·9 per cent sodium chloride were added 0·2 ml. of 0·75 per cent sheep erythrocyte suspensions. Such cells had to be sensitized with immune hæmolysin when guinea pig serum was used, whereas with human serum this was not necessary. Tubes were incubated in a 37° C water bath for 1 h and read for complete hæmolysis. The smallest amount for this reaction or unit of complement was 0·005–0·01 ml. of guinea pig serum or 0·01–0·02 ml. of human serum. The difference between the action of guinea pig and human serum was explained by the finding that human serum normally contained both complement and sheep hæmolysin. Heating at 56° C for 30 min destroyed complement but left behind antibody which would bring about sheep cell lysis with guinea pig serum. The antibody could not be removed by repeated washings of the sensitized cells with saline. Its titre was determined by lysis with guinea pig serum (2 units of complement) as well as agglutination with anti-human globulin. Identical results were obtained. With most sera the minimal reactive dose was 0·005 ml. Normal human serum also contains sheep red blood cell agglutinins but of a much lower titre. A high titre of such agglutinins, as present in the sera of patients suffering from infectious mononucleosis, did not affect the hæmolysin titre.

No difference in the action of human and guinea pig complement was observed. Neither one was able to bring about the lysis of human Rh-positive cells sensitized with low-titred antibody, which strongly reacted with anti-human globulin. Like bactericidin, both were inactivated by lytic factor and heparin[1]. Only 0·01 ml. of potent lytic factor preparation was required to block the action of 2 units of complement. The reaction was immediate. It could be reversed by the addition of excess complement. Barium sulphate in 10 or 25 mg quantities absorbed in 10 min all complement from 0·1 ml. of human or guinea pig serum but did not affect serum bactericidin. Both complements also differed from bactericidin in thermostability at temperatures below 56° C, as reported previously[1].

Sera of a number of patients suffering from a variety of infectious and organic diseases were analysed for complement and sheep hæmolysin. Considerable reduction in either one factor or both was noted in many instances, but no consistent pattern emerged. High bactericidin-levels often coincided with reduced complement activity. Guinea pig serum, though rich in complement, had no bactericidin.

From the results of this investigation as well as previous studies[1,3] it would seem that clotting factor extracted from fibrin, *C* reactive protein, serum bactericidin, and hæmolytic complement share a chemical grouping that arises immediately prior to or during fibrin formation. Indirect evidence[4] points to a steroid glucuronide. It would also appear most likely that their differences in physico-chemical and physiological properties are due to association with different proteins.

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Paul H. Kopper

Biology Department, Washburn University,
and Stormont-Vail Hospital,
Topeka, Kansas.

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Specificity of Isoantisera against Leukæmic and Thymic Lymphocytes

This communication concerns the specificity of isoantisera prepared against leukæmic and against thymic lymphocytes of *AKR* mice, as determined by immune cytolysis[1-3]. Discovery of a strong antigen associated with the thymic lymphocytes of *AKR* mice is also reported. A preliminary report has been made[4].

For immune cytolysis of *L*4946 leukæmic lymphocytes of *AKR* mice, the small-scale assay system previously described in detail[2] was used. The only change was use of 100,000 cells per assay tube. Essentially the same cytolysis system was used for thymic lymphocytes, except that absorbed hamster serum at a final concentration of 10 per cent was used as source of complement[3].

Isoantisera were prepared by injection of leukæmic and of thymic lymphocytes of *AKR* mice into mice of strains *C57BL*/6 and *C3HeB*/*Fe*(ref. 3). The cytolytic potencies of each of the four isoantisera were determined against *L*4946 leukæmic cells and against thymic lymphocytes (Table 1). Cytolytic potency is expressed as 100/cytolytic titre, where the cytolytic titre is the final concentration of isoantiserum (per cent) giving 50 per cent cytolysis of cells present in an assay tube. Specificity is expressed as the ratio of cytolytic potencies[5]; it refers to specificity against cell types, and has no direct bearing on the presence or absence of specific tumour antigens.

The relatively high potency of *C57BL*/6 isoantisera for cytolysis of *L*4946 leukæmia (Table 1, *A*) correlates well with the complete resistance of *C57BL*/6 mice to intraperitoneal transplantation of *L*4946 cells. The low potency of *C3HeB*/*Fe* isoantisera for cytolysis of *L*4946

Table 1. Specificity of Isoantisera against Leukæmic and Thymic Lymphocytes

Isoantisera prepared against	Cytolytic potency for cytolysis of *L*4946 leukæmia (*AKR*)	Thymic lymphocytes (*AKR*)	Specificity against *L*4946 leukæmia
A. Isoantisera prepared in *C57BL*/6 mice.			
*L*4946 leukæmia	78	4	19·5
Thymic lymphocytes	24	450	0·053
Specificity against *L*4946 leukæmia	3·2	0·0089	366
B. Isoantisera prepared in *C3HeB*/*Fe* mice.			
*L*4946 leukæmia	4	340	0·012
Thymic lymphocytes	3	450	0·0067
Specificity against *L*4946 leukæmia	1·33	0·76	1·8

leukæmia (Table 1, *B*) also correlates well with the semi-sensitive state of *C3HeB/Fe* mice to implantation of the *L*4946 tumour, the LD_{50} being 200,000 cells. In contrast, the LD_{50} of *L*4946 cells injected intraperitoneally into *AKR* mice, in which this tumour originated, is approximately 11 cells[6]; so far we have been unable to demonstrate cytolytic antibody in this situation.

The isoantiserum prepared in *C57BL/6* mice against *L*4946 leukæmia (top line, Table 1, *A*) showed a 78/4 or 19·5-fold higher specificity in its reaction against *L*4946 cells relative to its reaction against thymic lymphocytes. In contrast, the *C57BL/6* isoantiserum to thymic lymphocytes (second line, Table 1, *A*) showed a specificity of 0·053. The ratio of the specificities of these paired isoantisera (*C57BL/6* anti-*L*4946 leukæmia and *C57BL/6* anti-thymic lymphocytes), as tested against the same paired cell types (*L*4946 leukæmia and thymic lymphocytes), was 19·5/0·053 or 366. The overall specificity of 366 for the two isoantisera prepared in the resistant *C57BL/6* mice (Table 1, *A*) contrasts strongly with the overall specificity of 1·8 for the two isoantisera prepared in the semi-sensitive *C3HeB/Fe* mice (Table 1, *B*). This difference is mainly due to the unexpected reverse in specificity of the *C3HeB/Fe* isoantiserum to *L*4946 cells (top line, Table 1, *B*), as compared to the specificity of the *C57BL/6* isoantiserum to *L*4946 cells (top line, Table 1, *A*).

The allele H-2^b present in *C57BL/6* mice differs from the H-2^k or H-$2^{k'}$ allele of *AKR* mice, while *C3HeB/Fe* mice possess the same allele as *AKR* mice[7-9]. Moreover, tumour cells isologous to a given mouse strain carry antigens corresponding to the H-2 allele of that strain[7,8]. For these reasons, isoantisera prepared in *C57BL/6* mice are relatively potent against *L*4946 cells which carry antigens corresponding to the H-2^k or H-$2^{k'}$ allele of *AKR* mice, while the isoantisera prepared in *C3HeB/Fe* mice and also tested against *L*4946 cells are barely active.

Presence of one or more strong antigens on the surface of *AKR* thymic lymphocytes is revealed by the strong cytolytic potencies shown against these cells by isoantisera prepared in *C3HeB/Fe* mice (Table 1, *B*), which were obtained despite compatibility in the H-2^k or H-$2^{k'}$ allele. Detailed information regarding these antigens can be deduced from the results given here. This will be published elsewhere when further experiments have been completed.

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ARNOLD E. REIF
JOAN M. V. ALLEN

Biochemistry Section, Department of Surgery,
Tufts University School of Medicine,
Boston City Hospital, Boston, Mass.

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PATHOLOGY

Sampling of Cell Material from Human Tumours by Aspiration Biopsy

CELL material which is aspirated from human carcinomas through a fine needle consists largely of plugs of epithelial cells with admixture of interstitial fluid and variable amounts of blood. In earlier investigations[1-3], the epithelial component was isolated by centrifugation for quantitative enzymatic analysis. The activity of dipeptidase and of acid and alkaline phosphatase enzymes was determined in cells aspirated from mammary and prostatic carcinomas. These values were compared with results from analyses of epithelial plugs aspirated from benign mammary and prostatic lesions.

Since patients experience relatively little discomfort from aspiration biopsy with a fine needle, repeated biopsies are readily tolerated. The method should therefore be useful for examining the biological behaviour of tumour cell populations at different stages of the disease. This communication reports use of aspiration biopsy for examining the viability of cancer cell populations during radiotherapy.

A Luer–Lock syringe with a special handle[4] which allows a one-hand grip was used together with a stainless steel needle approximately 8 cm long and 0·7 mm in outer diameter (22 gauge). Use of this fine needle minimizes admixture of blood. Biopsy is performed as follows. The skin is washed with antiseptic solution and the tumour is fixed with one hand in a favourable position for needle biopsy. The needle is then introduced into the tumour area. In order to obtain sufficient material, especially from fibrotic tumours, the needle must be moved back and forth in the tumour four or five times. When the specimen has been aspirated, the pressure in the syringe is allowed to equalize before the needle is withdrawn from the tumour area. The material in the needle is carefully expressed into 0·2 ml. of sterile Simms's balanced salt solution in a 2-ml. centrifuge tube. A cell suspension is prepared by gentle pipetting with a sterile Pasteur pipette. Aliquots are taken for cell counts in a Bürker hæmocytometer chamber and for vitality tests, using the membrane permeability for eosin *Y* or lissamin green dyes as indicators of death of the cells[5]. The remainder of the suspension is sedimented by centrifugation at 20*g* in a refrigerated centrifuge at 4° C and resuspended in tissue culture medium for short-term tissue culture investigations (results to be published later).

Fig. 1 shows the percentage of permeable cells in cell populations aspirated from two tumours on consecutive days during radiation therapy. A conventional X-ray apparatus (200 kV) was used and the skin doses were recorded. The filled circles indicate death of cells in aspirate from a lymph node metastasis (diam. about 1 cm) of a poorly differentiated squamous carcinoma. The open circles denote cell death in aspirate from a tonsillar lymphoblastic lymphoma (diam. about 3 cm).

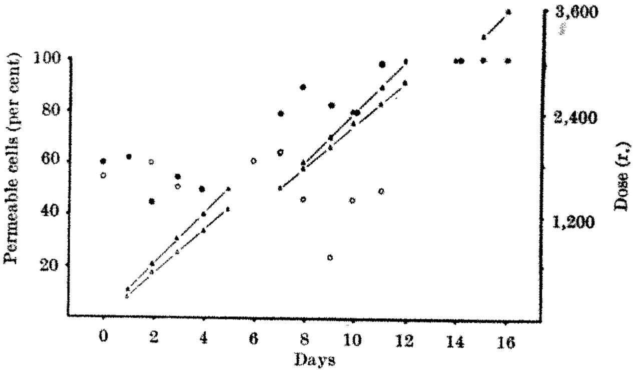

Fig. 1. Percentage of permeable cells in tumour populations aspirated on consecutive days of radiotherapy. ●, Lymph-node metastasis from squamous carcinoma; ○, lymphoblastic lymphoma; triangles, irradiation dosage

Clinically the metastatic squamous carcinoma proved to be fairly resistant to irradiation and persisted after treatment, whereas the lymphoma was radiosensitive and disappeared on the eleventh day.

In apparent contrast, Fig. 1 shows that death of cells in aspirate from squamous cell carcinoma increased during radiotherapy, the maximum being reached during the last days of treatment. No measurable increase of death of cells was found in the radiosensitive lymphoma, even on the eleventh day, when the tumour had almost completely disappeared.

The results hitherto obtained do not indicate if the findings in this latter tumour were attributable to selective removal of damaged cells from the tumour site during irradiation, before death of cells could be registered by means of dye permeability tests. However, it seems pertinent to mention that cells aspirated from tumours which clinically are regarded as radiosensitive (for example, lymphoblastic lymphomas, seminomas, or some low-differentiated carcinomas or sarcomas) display in smear preparations or suspensions marked decrease of mutual adhesiveness and behave as individual free cells. Contrary behaviour is shown by highly differentiated carcinomas and sarcomas. The possibility must therefore be taken into account that during radiotherapy the cell populations of clinically radiosensitive tumours may become mobilized as a result of low mutual adhesiveness[6] before death of the cells can be demonstrated by dye tests.

Bo Johansson
Josef Zajicek

Cytology Department of Radiumhemmet,
Institute of Radiopathology,
Stockholm 60.

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BIOLOGY

Silurian Echinoid Pedicellariæ from New South Wales

So far pedicellariæ are unknown in echinoids older than Carboniferous. The Silurian echinoid with which pedicellariæ occur has been recorded by Sherrard[1], and is now catalogued as *F*8554 in the University of New England Palæontological Collection. It was collected from Vallance's Hill, Portion 177, Par. Namina, some 12 miles south-east of Yass, New South Wales, from rocks regarded as Ludlovian in age.

The echinoid is the mould of an imbricate lepidocentroid in which the corona has disintegrated leaving an accumulation of isolated plates, radioles, maxillæ and (?) teeth. Although both secondary limonitic staining and the coarseness of the enclosing matrix to some extent obscure finer detail, the remains of eight pedicellariæ are visible among the debris.

The pedicellariæ (Fig. 1) are all small, 0·34–0·4 mm in height, with the base reaching a maximum diameter of 0·16 mm. They possess narrow elongated blades more than two-thirds their height. All are three-valved, typical tridentate pedicellariæ. No details of apophyses can be seen, nor can any stalks be identified.

Knowledge of Palæozoic echinoid pedicellariæ is confined almost solely to the studies of Geis[2], who described isolated pedicellariæ collected from Carboniferous strata of Texas, Illinois and Missouri. Jackson[3] also has recorded a single pedicellaria associated with a specimen of *Meekechinus elegans* Jackson from the Permian of Kansas. Geis found not only globiferous and tridentate types, but also ophicephalous pedicellariæ, which Mortensen[4] attributed to the Lepidocentroida by analogy with those of living echinothuriids. In passing it must be noted that Durham and Melville[5] were unaware of the occurrence of Palæozoic ophicephalous pedicellariæ when they proposed an echinoid classification in which the sub-classes were based virtually on the presence or absence of these[6].

The seeming absence of ophicephalous pedicellariæ in the Silurian lepidocentroid reported here, is, perhaps, to be expected. Their absence in living cidaroids suggests that they were as yet undeveloped in lepidocentroids when the cidaroids arose from this order, probably in the Devonian. Regnéll[7] has described some isolated radioles from the Upper Silurian of Gotland as *Silurocidaris clavata*. These, however, cannot be taken as indicating the Silurian occurrence of the Cidaroida, for they lack the microstructure characteristic of representatives of this order.

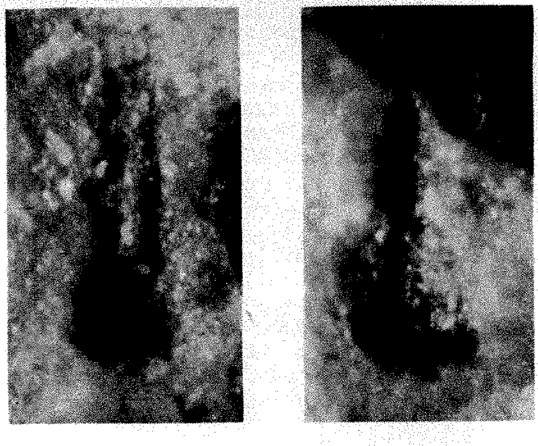
Fig. 1. Unretouched photographs of moulds of two tridentate pedicellariæ from the Silurian of New South Wales (× 100)

Döderlein[8] has deduced from the examination of pedicellariæ of living echinoids that the tridentate type is the primitive parental form from which other types have been differentiated. While an argument based on such premises lacks sure foundation, support for Döderlein's conclusion is now provided by the oldest echinoid pedicellariæ from the fossil record.

This communication establishes the early origin of simple tridentate pedicellariæ within the Echinoidea, and suggests that the possession of pedicellariæ may be a necessary attribute of this echinoderm class.

G. M. Philip

Department of Geology,
University of New England,
Armidale, New South Wales.

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Glucose-6-phosphate Dehydrogenase and Potassium Concentration in Sheep Erythrocytes

THE relationship of glucose metabolism to electrolyte-levels in erythrocytes has been the subject of numerous investigations.

Evans and King[1] established that the potassium concentration (K^+) in sheep erythrocytes was under genetic control and could be greatly altered by the presence of one or another of a pair of alleles at one locus, the gene for high potassium being recessive to that for low potassium. The Australian Merino belongs predominantly to the low-potassium group and its erythrocytes have potassium-levels even lower than those of the low potassium groups in British breeds[2]. New-born lambs of all breeds examined have high erythrocyte potassium-levels, which decrease gradually and reach adult values when the animals are about 2 months old[3].

Wright *et al.*[4], investigating glucose metabolism in young lambs, found that as potassium-levels decreased with the age of the animal, so the rate of uptake of oxygen of their erythrocytes in the presence of glucose also became lower, but anaerobic glycolysis did not show any similar trend.

Significantly higher uptake of oxygen, $^{14}CO_2$ and lactate production were observed with erythrocytes from high-potassium Southdown sheep when they were compared with low potassium Merinos[5].

In earlier experiments[6] on Peppin Merinos erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) activity has been found to be very low, about 5 per cent of normal human red cell values. Subsequent tests on Merino and British cross-bred animals, however, yielded somewhat higher values. It seemed of interest, therefore, to investigate the relationship between (K^+) and glucose-6-phosphate dehydrogenase activities in the erythrocytes of different breeds.

The present tests were done on animals of different sexes and ages chosen at random from: (1) a closed flock of Southdown sheep; (2) inbred families of Peppin Merinos. The experimental techniques used for the estimation of potassium[3] and glucose-6-phosphate dehydrogenase[6] in erythrocytes were described earlier. Experimental details and results are given in Table 1.

Table 1. CORRELATION BETWEEN ERYTHROCYTE POTASSIUM CONCENTRATION AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

Breed	Potassium type	No. of animals	(K^+) (m.equiv./l. red blood cell)		G-6-PD (Δ $O.D._{340}$/min/ml. red blood cell at 20° C)*		Correlation coefficient
			Mean	Standard deviation	Mean	Standard deviation	
Southdown	low	36	18·84	4·50	0·328	0·082	0·480†
Southdown	high	19	78·86	10·38	0·329	0·102	0·323 N.S.
Merino	low	56	9·68	2·41	0·248	0·068	0·437‡

* G-6-PD activity was estimated spectrophotometrically by the rate of NADP reduction in a system containing glycylglycine buffer *p*H 7·6, 40 mM; $MgCl_2$, 16·7 mM; NADP, 77 μM; G-6-PD, 8·3 mM; hæmolysate, 0·2 ml.; water to 3·0 ml. final volume.

† $P < 0·01$.

‡ $P < 0·001$.

The correlation coefficients calculated for (K^+) and G-6-PD activity are significant in the low-potassium groups of both the Southdown and the Merino sheep ($P < 0·01$, and $P < 0·001$, respectively). The correlation in the high-potassium Southdown group is not statistically significant.

The levels of G-6-PD activity were very similar in the two potassium groups of the Southdown animals. The erythrocytes of Merino sheep, however, seemed to have a lower level of this enzyme than those of the British breed. A Student's *t* test performed on the enzyme values obtained from all 55 Southdown and 56 Merino sheep showed the difference to be significant at the 0·1 per cent level.

These results differ from those of Budtz-Olsen *et al.*[7], who recently failed to demonstrate G-6-PD activity in both high- and low-potassium sheep erythrocytes and concluded that sheep were deficient in this enzyme. The methods used for the determination of enzymatic activities were, however, different from those used in our experiments.

I thank Mrs. J. Williams for the statistical analysis of the experimental data.

J. H. KOCH

C.S.I.R.O.,
Division of Animal Health,
McMaster Laboratory,
Glebe, New South Wales, Australia.

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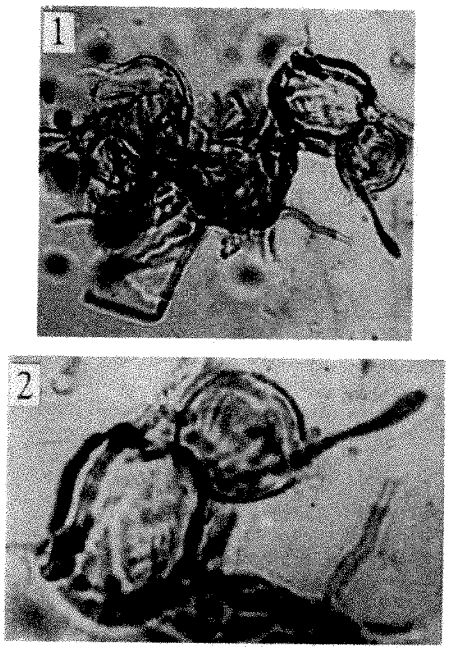
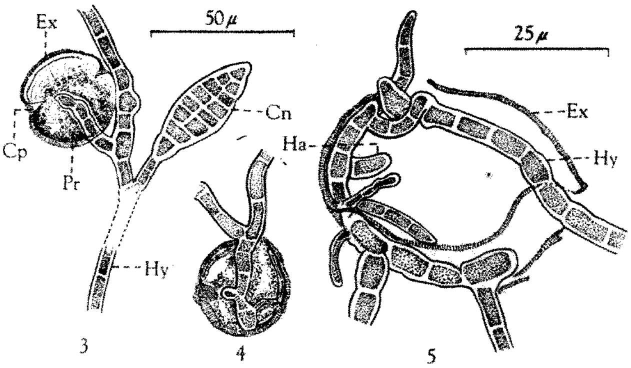
Fungal Infection on the Pollen Grains of *Schizanthus pinnatus* Ruiz and Pav.

ALTHOUGH fungal infections of the floral heads have often been observed in a number of plants, the nature of such infections on the pollen grains has usually escaped attention. The examination of the fungal relations of pollen grains is a new field of research, and may be deemed to be of importance in considerations of pollen viability and the dissemination of parasitic fungi.

Fungal infected floral heads of *Schizanthus pinnatus* (Solanaceae) were collected from the nursery of the National Botanic Gardens, Lucknow, in March, 1963. The infected flowers were crushed in lacto-phenol, warmed over a flame, and stained with cotton blue, for microscopic examination. Pollen grains of *Schizanthus* are provided with three germinal furrows, and with highly vacuolated protoplasmic contents. The infection of these pollen grains (Figs. 1–5) is caused by *Alternaria* sp., in various ways. The hyphæ enter the pollen at the furrows, ramify in the protoplasm, and sometimes pass out through the other furrows. The region of the hyphæ inside the pollen grains ('intrapollinous hyphæ' as opposed to 'extrapollinous hyphæ') produce,



Figs. 1 and 2. Photomicrographs showing the fungal (*Alternaria*) infection on the pollen of *Schizanthus pinnata* (Fig. 1, × 285; Fig. 2, × 570)



Figs. 3–5. Line diagrams of Figs. 1 and 2. *Cp*, Colpus; *Cn*, conidia; *Ex*, exine of pollen; *Ha*, haustoria; *Hy*, hyphæ; *Pr*, protoplasm of pollen

'haustoria' which possibly helps the fungus to absorb food materials from the pollen.

The 'intrapollinous' hyphæ remain either vegetative or form conidia and lie either along the pollen wall or at the centre of the grain. The fungal infection seems to cause the dissolution of the protoplasm either partially or completely. Similar infections have been observed in the pollen of *Cocos nucifera* and also other cultivated plants.

We thank Prof. K. N. Kaul, director of the National Botanic Gardens, Lucknow, for his advice.

P. K. K. NAIR
H. A. KHAN

Palynology Laboratory,
National Botanic Gardens,
Lucknow.

Embryos derived from Callus Tissue Cultures of the Wild Carrot

INDEPENDENT regeneration of root or shoot primordia is common in higher plants. However, the simultaneous, integrated development of both primordia from a single small mass of meristematic cells is found almost exclusively in ovular embryos. We have observed the development of structures bearing both these primordia, from callus tissue cultures of the wild carrot, *Daucus carota* L. These structures closely resemble *in situ* pro-embryos and embryos both morphologically and in their pattern of development.

The callus was obtained by aseptically culturing root tissue on a semi-solid medium designed by Caplin and Steward[2]. It was sub-cultured through several transfers prior to use. Embryos were obtained by transferring single 4–6 cm pieces of callus to a liquid version of the culture medium and rotating these in an apparatus similar to that described by Steward *et al.*[3]. Ten days after transfer, some cultures had produced 50–100 of the structures pictured in Fig. 1. All stages of development, from compact nearly spherical bodies 0·2 mm in diameter to those with projections resembling cotyledonary nodes and radicles, were observed at this time. Subsequent development of these pro-embryo-like structures is pictured in Fig. 2. Green cotyledon-like appendages and typical roots have formed. If these plantlets were removed from the liquid nutritive medium at this stage and transferred to a moist agar substrate containing only the mineral components of the culture medium, development continued along normal seedling patterns and young wild carrot plants were produced.

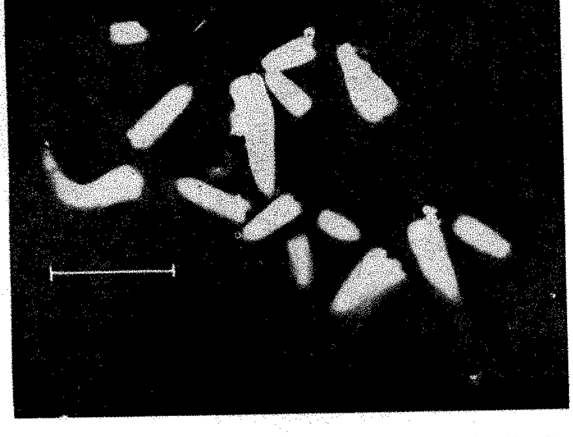


Fig. 1. Embryo-like structures taken from a ten-day-old liquid culture of wild carrot callus. Cotyledonary-node-like projections are present at one end of nearly all structures. The two largest structures each have a well-developed root tip. Scale, 1 mm

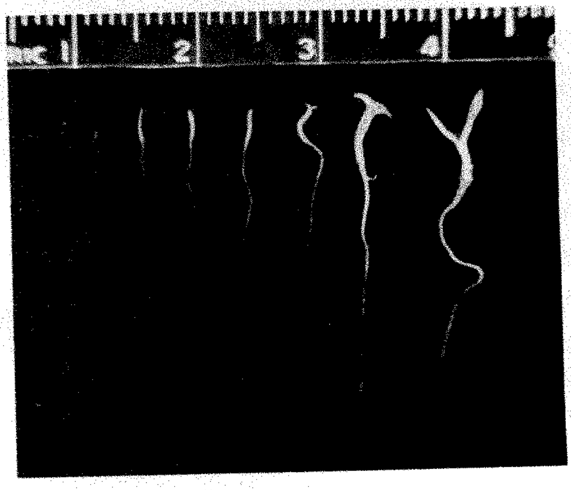


Fig. 2. Wild carrot plantlets which have developed from the structures shown in Fig. 1 after 2–3 weeks' further culture. Scale units, cm

The origin of these embryo-like structures is uncertain. They appeared freely suspended in the culture medium at the earliest stage at which they could be distinguished from the debris. They may have been formed at the surface of the callus mass or from clumps of free-cells which were commonly observed in these cultures. Their development is distinctly different from the plantlet formation described by Steward *et al.* in cultures of the commercial carrot[1]. These authors reported that first, minute tissue masses were formed in cell aggregates of freely-suspended cells. When these 'nodules' reached a certain size, inner cells became lignified and 'tracheid-like' and became enclosed by a sheath of 'cambium-like elements'. Root primordia formed in the cambial region. Shoot primordia did not appear until rooted 'nodules' were transferred to stationary agar media.

In our cultures, the smallest recognizable organized structure (about 0·1 mm diameter) consisted of densely packed small meristematic cells. Even these small cell masses appeared to be polarized, with the long axis of the cells oriented in one direction. No lignified vascular elements and no cambial sheath were formed. As the size of these structures increased they became cylindrical (Fig. 1) with long axis parallel to the long axis of the cells. Root primordia appeared at one end of the elongated pro-embryo-like structure while green cotyledon-like appendages developed simultaneously at the opposite end. Normal appearing roots, with a root cap, root hairs and occasional lateral rootlets were then formed By the time the roots were several cm long, the region corresponding to the hypocotyl of a seedling was approximately 1 cm long and strongly red-pigmented as is often true of the hypocotyl of seedlings of this species. Extensive development of the shoot apex and true leaves was not observed in rotating liquid cultures but appeared in all plantlets soon after transfer to stationary cultures.

The basic difference between the phenomenon reported here and that reported by Steward *et al.*[1] seems to be in the time of development of the polarizing forces which are thought to direct differentiation and axial development of the root and shoot meristems. In our cultures this polarity seemed to exist in the earliest stages. This leads to a very orderly development in which root and shoot poles developed simultaneously and in a manner similar to that of normal embryogenesis. In the case studied by Steward *et al.*[1], the organization of root primordia was delayed until a sizable mass of cells was formed by relatively disorganized growth. Steward[4], in fact, stresses the role of isolation of the inner cells of the 'nodules' from the nutrient medium as an important step in the initiation of orderly growth. The initiation and subsequent development of the root primordia were more typical of branch roots which are pericyclic in origin than it was of the radicle-primary root sequence of embryos. Shoot development apparently did not occur until rooted 'nodules' were subjected to the unilateral influence of gravity.

From the results of this work it would seem that the essential conditions for embryogenesis can be duplicated *in vitro*. The opportunity, provided by these tissue cultures, to examine the early stages of the organization of co-ordinated root-shoot systems may eventually permit the identification and elucidation of the controlling forces of embryogenesis.

D. F. Wetherell
W. Halperin

Department of Botany,
University of Connecticut, Storrs.

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Production of Pectic Enzymes by *Rhizoctonia solani* and Orchid Endophytes

Rhizoctonia solani has frequently been isolated from roots of the purple marsh orchid, *Orchis purpurella*, with which it may form a normal mycorrhizal symbiosis. Such endophytic strains and others isolated as pathogens from diseased crop plants often show symbiosis to *O. purpurella* protocorms in culture tests[1,2]. In the absence of any knowledge of the pectolytic potentiality of orchid endophytes a development of this investigation has been a comparative examination of the production of pectic enzymes by various *Rhizoctonia* strains *in vitro*.

The fungi used included pathogenic *R. solani* strains *Rs* 6 from potato and *Rs* 10 from rice (respectively parasitic and symbiotic to *O. purpurella* protocorms) and two typical orchid symbionts *R. repens* (*Rr*) from *O. purpurella* and *R. goodyerae-repentis* (*Rgr*) from *Goodyera repens*. Cultures were grown at *p*H 5·6 in static liquid media containing 1 per cent glucose or apple pectin as carbon source, 0·35 per cent casein hydrolysate, 0·05 per cent yeast extract, 0·1 per cent KH_2PO_4, 0·05 per cent $MgSo_4 7H_2O$ and trace elements. With all the strains tested, growth and sclerotial formation followed a similar pattern on the two media, pectin being only slightly less good a carbon source than glucose.

Culture filtrates were collected daily and assayed at predetermined *p*H optima for the enzymes endopolygalacturonase (PG)[3], endopolymethylgalacturonase (PMG)[3] and protopectinase. PG and PMG activity was determined viscometrically and expressed in arbitrary enzyme units per volume of culture filtrate using a modified form of the method of Roboz, Barratt and Tatum[4]. Protopectinase activity was expressed as a maceration index using Brown's method[5].

Pectic enzyme production was negligible on glucose, but comparatively large amounts were secreted throughout the growth period on pectin media. Typical results for actively growing cultures on pectin are given in Table 1.

Table 1. PECTIC ENZYME ACTIVITY OF CULTURE FILTRATES OF ACTIVELY GROWING *Rhizoctonia* STRAINS ON A PECTIN MEDIUM

Strain symbol	*Rs* 6	*Rs* 10	*Rr*	*Rgr*
Age of culture (days)	4	4	7*	4
Mycelium dry wt. (mg)	119	90	67	109
PG activity (units)	15·8	41·5	427·6	5·0
PMG activity (units)	1·7	5·4	42·7	1·2
Maceration index	6·3	3·9	6·1	3·7

*Growth-rate of *R. repens* was lower than that of other strains.

It can be seen that all the strains have a potentiality for the secretion of each of the pectic enzymes tested, in quantities unrelated to their symbiotic or parasitic character. In particular, the pectic enzyme potential of *R. repens* is surprisingly high. It has not yet been possible to investigate enzyme production by orchid endophytes *in vivo*, but it seems unlikely that parasitic and symbiotic activity can be discriminated on the basis of pectic enzyme production alone. The symbiotic host endophyte complex must incorporate factors controlling the production of pectic enzymes which otherwise might give rise to a pathogenic condition. It is suggested that these factors arise only as the result of the interaction of the metabolic systems of the two partners.

So far as is known, this is the first demonstration of pectic enzyme production by symbiotic mycorrhizal fungi.

G. Hadley
M. Pérombelon

Department of Botany,
University of Aberdeen.

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MICROBIOLOGY

β-D-1,6-Glucanase in *Streptomyces*

According to Reese *et al.*[1], β-D-1,6-glucan rarely occurs in Nature, yet many fungi are able to produce an enzyme capable of hydrolysing it. Production of β-D-1,6-glucanase by *Streptomyces* has not been reported.

Previously[2,3] we reported that a newly isolated micro-organism produced lutease (β-1,6-glucan-6 glucanohydrolase) when grown in a Czapek medium containing peptone and higher-molecular-weight luteose instead of sucrose. This latter substance is a β-D-1,6-glucan prepared from mucilage which was produced from glucose by *Penicillium aculeatum* Raper and Fennell var. *apiculatum* Abe strain No. 2282 isolated by us from soil[4].

Since the details of taxonomic position of lutease-producing micro-organism were as yet uncertain, although the strain seemed to belong to *Streptomyces* species, morphological and cultural characteristics of the strain were examined. From the characteristics, that is, straight aerial hyphæ (with smooth surface spore), water-green aerial mycelium, medium to strong proteolytic action, *Streptomyces* strain No. *OP*–4–5 was identified as belonging to *Streptomyces griseus*.

Tests were made to search for lutease production in *Streptomyces* species. Among the 89 species, 92 strains, of *Streptomyces* tested, *Streptomyces griseus* N.I.H.J.13, *Streptomyces griseus* N.I.H.J.107, *Streptomyces parvus* N.I.H.J.115, and *Streptomyces niveoruber* N.I.H.J.413, produced lutease more or less by previous culture for 48 h at 30° C in a Czapek medium containing peptone and higher-molecular-weight luteose instead of sucrose on a shaker. The lutease production of these 4 strains was defined by lutease activity assayed at each culture time. Results are shown in Figs. 1 and 2. Two strains of

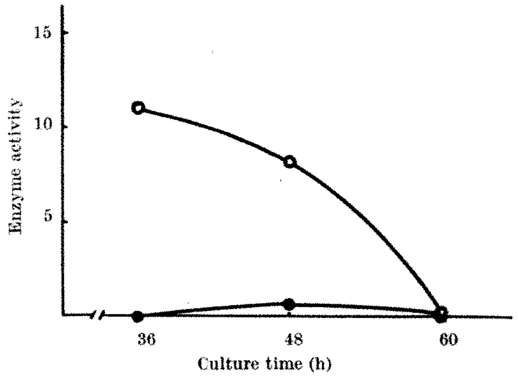

Fig. 1. Production of lutease by *Streptomyces griseus* N.I.H.J.13, and *Streptomyces griseus* N.I.H.J.107. ○, *S. griseus* N.I.H.J.13; ●, *S. griseus* N.I.H.J.107. Each strain was cultured in Czapek medium containing 0·2 per cent peptone and 0·5 per cent higher-molecular weight luteose instead of sucrose at 30° C on a shaker. The assay method of lutease activity (ref. 2) was used in the same way

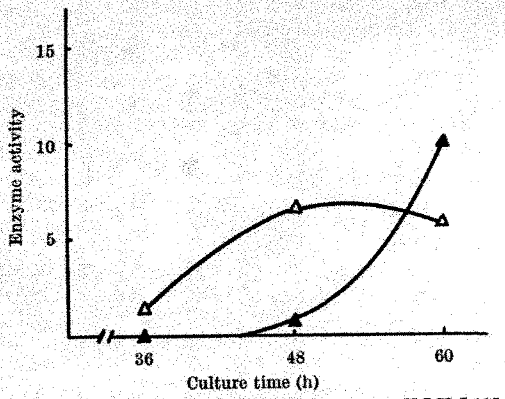

Fig. 2. Production of lutease by *Streptomyces parvus* N.I.H.J.115, and *Streptomyces niveoruber* N.I.H.J.413. △, *S. parvus* N.I.H.J.115; ▲, *S. niveoruber* N.I.H.J.413

Streptomyces griseus tested produced lutease, but the production by *Streptomyces griseus* N.I.H.J.107, was very low. Lutease activity produced by *Streptomyces griseus* strain No. *OP*–4–5 was 25·7 after culture for 36 h culture under the same conditions. It may be that *Streptomyces griseus* generally produces lutease when grown in a medium containing higher-molecular-weight luteose. According to Ettlinger *et al.*[5], *Streptomyces parvus* also belongs to *Streptomyces griseus* series. Although the 4 strains produced lutease, the amount was smaller than that of the enzyme produced by *Streptomyces griseus* strain No. *OP*–4–5 which was isolated by us.

We thank Dr. H. Umezawa and Dr. Y. Okami, of the National Institute of Health, Tokyo, for supplying *Streptomyces* species and for their advice.

NORIO NAKAMURA
OSAMU TANABE

Fermentation Research Institute,
Ministry of International Trade and Industry,
Inage, Chiba City, Japan.

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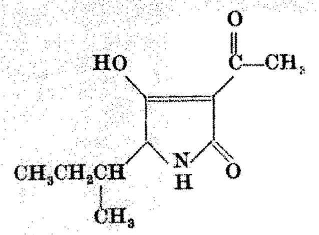
VIROLOGY

Antiviral Activity of Tenuazonic Acid

TENUAZONIC acid (I) was first isolated by Rosett *et al.*[1] from culture filtrates of various strains of *Alternaria tenuis*. The recent report[2] of antitumour activity of tenuazonic acid prompts us to report our results with this mould metabolite. During an antiviral screening programme culture filtrates of an *Aspergillus* were found to inhibit the cytopathic effect of measles virus in *HEp*2 (Moore) cell cultures (and to exhibit antibacterial activity referable to penicillins). An antiviral compound which later was shown to be tenuazonic acid was isolated from these filtrates. Isolation was assisted by measles and poliovirus plaque tests.

The *Aspergillus*, strain *F*1404, had been isolated from a Venezuelan soil sample and fermented aerobically at 26° C for 4–5 days in a medium containing glucose, soy-bean meal, brewers' yeast, and sodium chloride. Crude tenuazonic acid was first obtained from such culture filtrates by 'Darco' adsorption, followed by elution with aqueous acetone and *n*-butanol extraction of the concentrated eluate. Later the crystalline copper salt and the pure liquid acid were isolated following the procedure of Rosett *et al.*[1] The chemical and physical properties of our acid and its derivatives were shown to be virtually identical with those reported by the English group[1,3] and with those of an authentic sample synthesized from ethyl *L*-isoleucinate and diketene using the method developed by Lacey[4].



I

Tenuazonic acid as the sodium salt inhibited the cytopathic effect of a wide spectrum of viruses. In addition to the original measles activity, the compound showed an antiviral effect in the range of 500 to 100 μg/ml. against enteroviruses (poliovirus *MEF*-1, *ECHO*-9, Coxsackie B_1), respiratory viruses (*para*influenza-3 (*HA*-1), Salisbury *HGP*), vaccinia, herpes simplex *HF*, and '*B*' virus. The compound was inactive in tissue culture against polyoma virus (Stewart and Eddy) and in the mouse against Asian influenza (*Jap.* 305), rabies (*CVS*), and Friend leukæmia. Sodium isotenuazonate (prepared by alkaline isomerization of the parent compound) was inactive against poliovirus but proved to be approximately as active as sodium tenuazonate against *ECHO*-9, *para*influenza, vaccinia, herpes simplex, and measles viruses.

Table 1. SODIUM TENUAZONATE VERSUS POLIOVIRUS *MEF*-1 IN AGAR DIFFUSION

Concentration (μg/ml.)	Zone diameter (mm) Activity	Zone diameter (mm) Toxicity
5,000	50	20
3,570	44	20
2,500	40	15
1,785	34	0
1,250	33	0
892	30	0
625	30	0
446	28	0
312	20	0
223	15	0
156	0	0

Table 1 shows the results of a polio agar diffusion test with sodium tenuazonate. The antibiotic was placed on filter disks on agar overlay and the zones were measured. A good dose response can be seen. Table 2 shows the concentration of sodium tenuazonate required to reduce the number of plaques formed by a representative group of viruses by at least 50 per cent.

Table 2. ABILITY OF SODIUM TENUAZONATE TO INHIBIT VIRUS PLAQUE PRODUCTION UNDER AGAR OVERLAY

Virus	μg/ml.* showing at least 50 per cent plaque reduction
Measles (Enders)	500
Vaccinia	500
Herpes simplex (*HF*)	160
ECHO-9	500
'B' virus	500

* Tested at half-log dilutions of 5–0·05 mg/ml.

Single doses of sodium tenuazonate, administered to dose-groups of 25 or 30 18–20-g *ICR* male mice, gave LD_{50} values of 125 mg/kg ± 10 (*S.E.*, Miller-Tainter) intravenously, 150 ± 10 intraperitoneally, 145 ± 20 subcutaneously, and 225 ± 20 orally. Cynomolgus monkeys tolerated a single oral dose of 50 mg/kg; doses of 100, 150, and 300 mg/kg *PO* gave survivors/treated ratios of 4/6, 2/6, and 1/6, respectively. Antiviral activity in monkeys has not been studied.

Sodium tenuazonate was found to be essentially inactive against bacteria (31 strains), yeasts (48 strains), and *Trichomonas vaginalis in vitro*. Against the 200-F strain of *Entamoeba histolytica* it proved to be markedly active *in vitro*, but only slightly active by mouth in cæcally infected rats.

We thank W. Russell Laster, jun., and Frank M. Schabel, jun. (both at Southern Research Institute), and

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FRANCIS A. MILLER
WILTON A. RIGHTSEL
BERNARD J. SLOAN
JOHN EHRLICH
JAMES C. FRENCH
QUENTIN R. BARTZ

Research Division,
Parke, Davis and Co.,
Detroit 32, Michigan.

GLEN J. DIXON

Chemotherapy Division,
Southern Research Institute,
Birmingham 5, Alabama.

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Inclusion Bodies in *Dahlia* infected with Dahlia Mosaic Virus

THE formation of inclusion bodies of various types in virus-infected plant tissue is not infrequent. Such inclusion bodies are located either in the cytoplasm or, less commonly, in the nucleus[1].

In a previous paper[2], it was noted that the inclusion bodies formed in leaf epidermal cells of *Dahlia variabilis* Cav. infected with dahlia mosaic virus were located in the cytoplasm. Recently, examination of epidermal tissue from immature leaves has shown the presence in the nuclei of inclusion bodies of a type exactly similar to that found in the cytoplasm (Fig. 1). Furthermore, stages have been observed which strongly suggest that the inclusion body, after formation in the nucleus, is extruded into the cytoplasm (Figs. 2 and 3).

The inclusion body at this stage is 2–7μ in diameter, spherical, and apparently composed of an outer membrane enclosing a matrix in which are many granular bodies. It is possible that the membranous portion is not confined to the outer surface but may take the form of a reticulum in which the granules are located.

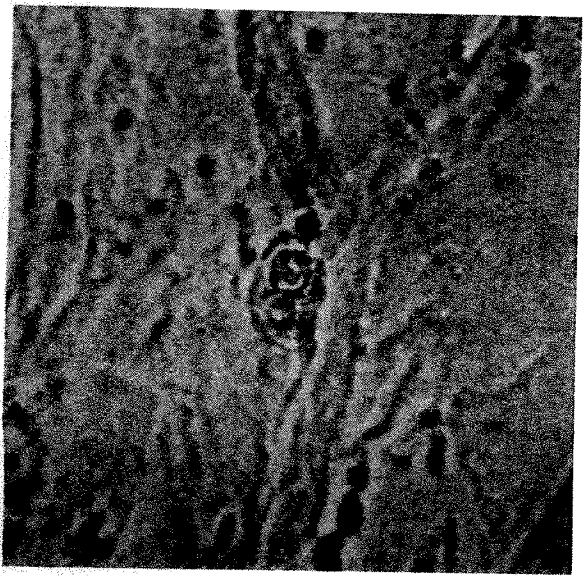

Fig. 1. Nucleus enclosing a large inclusion body and smaller nucleolus (× 1,000)

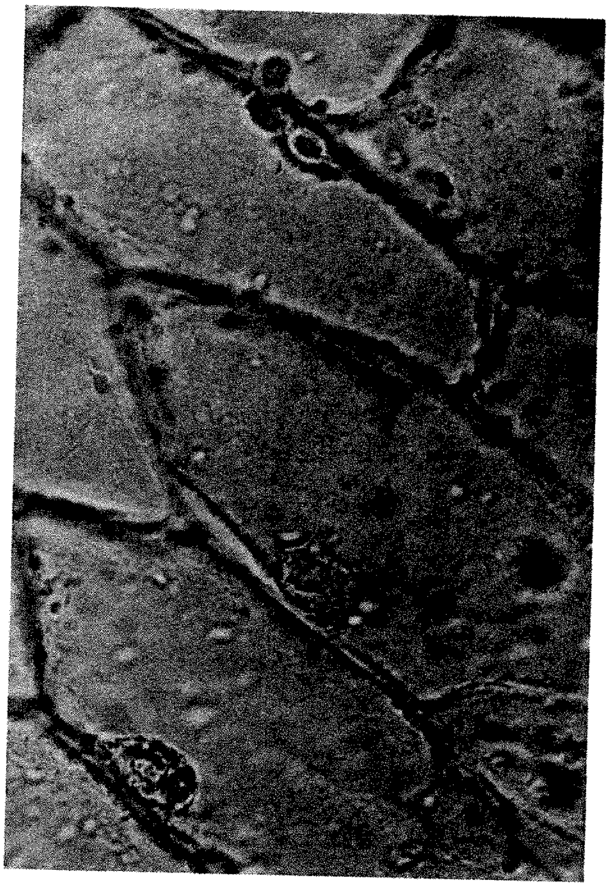

Fig. 2. Inclusion bodies in (bottom) or near (top) the nucleus (× 1,000)

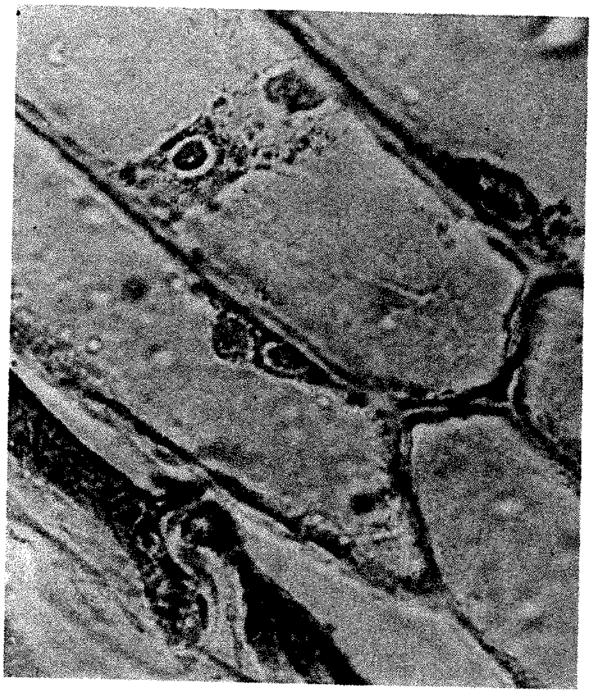

Fig. 3. Inclusion bodies apparently emerging from nuclei (× 1,000)

The granules stain positively for ribonucleic acid and protein, while the membrane stains with Nile blue and may therefore contain phospholipid. Present indications are that the virus aggregates or replicates at several sites in the nucleus, probably corresponding to the granules.

These early stages in development are at present under investigation and may provide a useful indication of the mechanism of virus replication in the plant.

SHEILA M. ROBB

Department of Botany,
University of Exeter.

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CYTOLOGY

Negatively Stained Lipoprotein Membranes

IN a recent communication Finean and Rumsby[1] have cast doubt on the reliability of the electron microscopic image obtained from negatively stained preparations of lipoprotein membrane structures (myelin, mitochondria, microsomes and red cell ghosts). Although we agree with their remarks about the need for caution in interpreting such electron micrographs, it is felt that further comment is necessary concerning some of the published literature[2,3] mentioned by them.

During the examination of sub-cellular components[2] a comparison was made between: (*a*) unfixed negatively stained preparations; (*b*) preparations fixed with either formaldehyde or osmium tetroxide, followed by negative staining; and (*c*) conventional thin sections[4,5]. Owing to the low osmolarity of the 1 and 2 per cent phosphotungstate used for negative staining, it was found to be necessary with structures sensitive to the osmotic pressure of the surrounding medium (for example, nerve ending particles, mitochondria) to fix the particles before negative staining in order to prevent obvious disruption. Liver mitochondria are particularly sensitive. With osmotically insensitive structures (for example, myelin fragments) similar results were obtained with fixed and unfixed material. However, exposure of unfixed material to the hypotonic phosphotungstate was kept as brief as possible. Under these conditions negatively stained preparations were found to possess structural features directly comparable with those of positively stained sectioned material as is clearly illustrated in the published micrographs[2].

We consider that inferences drawn from the X-ray diffraction study of air-dried material in the absence of a suitable negative staining agent do not necessarily apply to material dried in the presence of a negative stain. As with positive staining, the mechanism of negative staining is not fully understood, but it is probable that the electron dense material sets in a rigid 'glass' and dries more rapidly than does the biological material in the absence of the stain. This may well prevent some of the changes seen in material air-dried in the absence of negative staining from occurring.

Turning to the question of biological and artificial membranes studied by several authors[3,6], it has been suggested by Finean and Rumsby that the patterns observed after treatment with saponin are a result of changes occurring during the final stages of drying. As indirect evidence that this is not so is the observation that the birefringence of a lecithin–cholesterol dispersion in water is rapidly lost by the addition of a dilute (1 per cent) saponin solution, thus indicating a loss of the lamellar structure in the myelinics. Bangham and Horne reported at a recent meeting of the British Biophysical Society that the same basic structures could be seen in lecithin–cholesterol preparations treated with saponin after treatment with buffered osmium and then air-dried. It was suggested that although dehydration effects may still be present, the separation of lipid components to form the same basic patterns under these conditions was unlikely.

The observations of Lucy and Glauert[7], on the increase in length of the helical components with time in mixtures of lecithin, cholesterol and saponin, lend support to the view that these structures are produced in the liquid phase.

R. W. HORNE
A. D. BANGHAM
V. P. WHITTAKER

Agricultural Research Council,
Institute of Animal Physiology,
Babraham, Cambridge.

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X-RAY diffraction studies of the air drying of myelin have included experiments in which myelin fractions from brain were dried in the presence of phosphotungstate. It is clear that this material does contain layering which electron microscopy of thin sections shows to be similar to that of the normal preparation, but it also includes additional layered systems which are significantly different. The drying of such specimens was probably appreciably slower than that achieved in the preparation of negatively stained specimens for electron microscopy, but the possibility of the existence of more than one type of layering in the latter preparations is clearly indicated.

Our suggestion concerning the remarkably regular hexagonal system seen in negatively stained preparations of saponin-treated cell membranes, lecithin–cholesterol dispersions, and cholesterol monolayers was that this particular geometrical arrangement might have been formed during the final stages of dehydration. The observation that the birefringence of lipid droplets is decreased by the addition of saponin certainly indicates an interaction in the hydrated state but not necessarily a loss of lamellar structure nor the formation of hexagonal or helical systems. Comparisons of the swelling of brain lipid pellets in distilled water and in saponin solutions (up to 50 per cent saponin) have shown very similar sequences of changes in X-ray diffraction patterns, all patterns so far being readily interpretable in terms of a layered structure. Similar diffraction sequences have also been observed using sodium cholate, sodium deoxycholate, or sodium dodecyl sulphate, all of which will dramatically reduce the birefringence of lipid droplets in the way that saponin does, but which have not yet been shown to form hexagonal or helical systems. It would seem to us that the state of hydration at which such systems are formed remains unknown.

J. B. FINEAN
M. G. RUMSBY

Department of Medical Biochemistry and Pharmacology,
University of Birmingham.

GENETICS

Sex Ratios under Natural Selection

SEVERAL workers[1,2] have shown the numerical equality of two sexes (that is, a 1 : 1 sex ratio) to be selectively favoured in relation to the parental effort involved in the rearing of offsprings to maturity. More recently, Kalmus and Smith[3] have argued in support of the adaptive superiority of this ratio on the basis of such considerations as those of hybridity, recombination potential and the level of inbreeding as influenced by sex ratio variations. However, their arguments are not valid in general and at best seem to apply only to the monogamous species. In fact, widely deviant sex ratios are reported to occur in the natural populations of both polygamodiœcious and gynodiœcious species[4,5], and Lewis[5] has suggested that sex ratio adjustments frequently through a preponderance of females help ensure reproductive economy combined with the maintenance of certain hybridity optima in such species. In a few theoretical examples analysed by Shaw[6], such adaptive changes were examined in the presence of certain specific sex-ratio factors. In this note a model of differential selection for an autosomal recessive in the two sexes is utilized to show that maximum levels of heterozygosity (say 0·50 where gene frequencies $p, q = \frac{1}{2}$) could be attained with variable sex ratios, a result in disagreement with the view of Kalmus and Smith[3].

Consider, for example, a large random mating population with the following adaptive values at an autosomal locus:

	Genotype		
	AA	Aa	aa
in females	1	1	$1 + s$
in males	1	1	$1 - t\ (s, t > 0)$

Then, for $s > t > s/1 + 2s$, there is stable equilibrium with heterozygosity:

$$H = \frac{(s-t+2)\ (s-t)\ [s+t-\sqrt{(s-t+2)\ (s-t)}]}{\frac{1}{2}\ (s+t)^2}$$

and sex ratio (♀ : ♂) $= \frac{s}{t}$

Thus, for a constant sex ratio, say k, and $H_\infty \doteq 0{\cdot}50$, t is uniquely determined by putting $\frac{dH}{dt} = 0$, which gives:

$$t = \frac{8\ (k-1)}{(1+k)^2 - 4(k-1)^2}$$

Table 1 gives some numerical examples to show that as sex ratio departs from 1 : 1, selection has to become increasingly intense in order to give $H = 0{\cdot}50$ at equilibrium.

Table 1

Sex ratio (k)	1·0	1·1	1·2	1·3	1·4	1·5
t	0	0·18	0·33	0·49	0·62	0·76

A similar study at a sex-linked locus shows that differential selection can establish deviant sex ratios in a population with its hybridity levels even higher than those expected under no selection. Likewise with a model of cytoplasmic sex-ratio factor by Watson[7], it is easily shown that in the case of gynodiœcious that carry in effect male sterility governed by cytoplasmic factors, sex ratio variations do not affect the amount of heterozygosity.

The recombination potential of any population or species as a whole need not always be lower in case of unequal sex numbers although the effective population size is indeed maximum with a 1 : 1 sex ratio. In fact, a relative excess of homogametic sex, which is perhaps often true[5], should result in a greater recombination potential in species like *Drosophila* with no crossing-over in the males. As regards some amount of inbreeding resulting from an unequal sex ratio, it might be that an intermediate rather than maximum amount of outbreeding would be adaptively better in view of a compromise between the requirements of immediate fitness and long-term flexibility[5,8]. Thus, there appears to be no *a priori* reason to assume an adaptive value associated with a 1 : 1 sex ratio as a general rule, and natural selection of various forms can, in fact, regulate this important factor too, like many others such as breeding habit, population number, mode of dispersion or even karyotypic features of a genetic system[9].

S. K. Jain

Division of Botany,
Indian Agricultural Research Institute,
New Delhi, 12.

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Somatic Reduction of Chromosome Number in a *Ribes* Hybrid following Treatment with *para*-Fluorophenylalanine

The *Ribes* breeding programme at East Malling involves considerable use of widely different species as donor parents. From some of these species, genes of potential economic value are being transferred to cultivated varieties of currant and gooseberry (all of which are diploid, $2n = 16$). In many cases the inter-species F_1's are completely sterile so that, to regain fertility, it has been necessary to make allotetraploids by colchicine treatment. In transferring genes from gooseberry (*R. grossularia*; *G* genome) to black-currant (*R. nigrum*; *B* genome) and vice versa, a technique of back-crossing to commercial diploid varieties via the triploids *BBG* and *BGG* respectively has met with considerable success[1–3]. Nevertheless, with a 2–3-year cycle between seed sowing and flowering, any short cut to the production from alloploids of fertile or partially fertile diploid plants having a minimal genic content from the donor species would offer considerable advantages.

A number of substances have recently been shown to be capable of inducing chromosome loss in fungi[4–6] and in *Allium*[7–10]. Several of these chemicals are being tested in the hope that some of them may have a similar effect on *Ribes* hybrids and thus be capable of inducing the formation of diploid sectors, having a limited content of donor genome, in *BBGG* and similar alloploid plants. Results are reported here from the use of one of these substances, *para*-fluorophenylalanine (*p*FPA), in a pilot experiment.

Aqueous solutions of *p*FPA of 1/200, 1/500, 1/1,000 and 1/10,000 were used, the material being dissolved in 0·1 N sodium hydroxide before dilution; a trace of 'Triton *X*-100' was added as a wetter. Four methods of application were used: (1) injection into young succulent tissue; (2) absorption by means of a fine cotton wick threaded through young stems just below the growing point; (3) absorption by placing the growing point in the solution; (4) absorption by placing leafy decapitated shoots in polythene bags wetted with the solution.

In all, 47 growing points of *BBGG*, *BBBG*, *BGG* and *BGGG* plants were treated during the period July 4–12, 1962. Apart from the death of some shoot-tips and the production of necrotic areas in some others, no marked symptoms were evident in 1962. In September 1962, single-bud cuttings were propagated from a number of shoots that had developed near the points of treatment. In spring 1963, abnormal stunted growth was noted in three of the cuttings from a *BBGG* ($2n = 32$) plant that had been treated with 1/200 *p*FPA by method (4), and the foliage of one of these was markedly crumpled and mottled (Fig. 1).

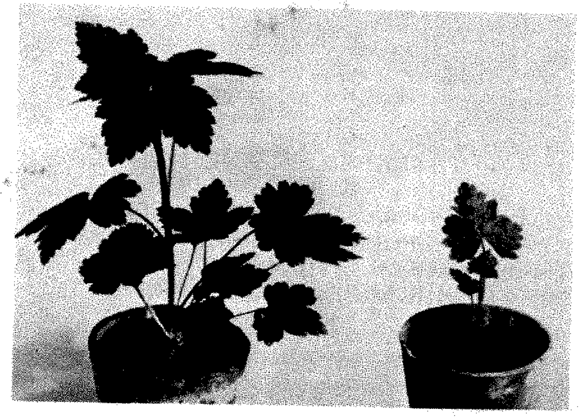

Fig. 1. Normal and abnormal growth of single-bud cuttings of a *BBGG Ribes* hybrid following treatment with *p*FPA

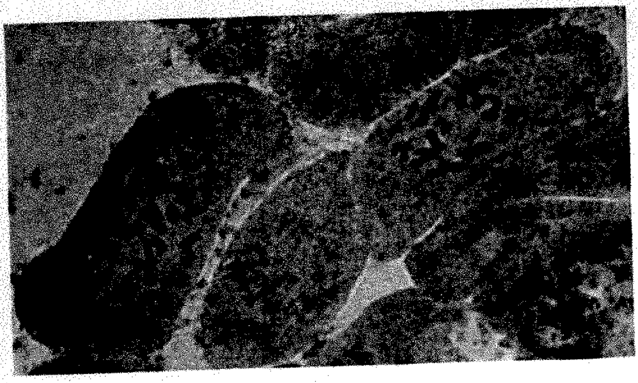

Fig. 2. Root tip cells, from the abnormal cutting shown in Fig. 1, with 20 and 32 chromosomes

Cytological examinations of root tip squashes from these three cuttings and from one that appeared normal were made in spring 1963. Cells having reduced chromosome numbers were found in all four. Of 30 root tips examined, 8 showed a low proportion of cells having chromosome numbers ranging from 8 to 24, including 3 cells having the diploid number $2n = 16$. Fig. 2 shows adjacent cells with 20 chromosomes and the normal number of 32 respectively. No cells with reduced chromosome numbers were seen in root tips of untreated cuttings from the same *BBGG* parent.

For plant breeding purposes, a technique capable of inducing the formation of homogeneous sectors of diploid tissue would be required. The extremely low proportion of diploid cells found in root tips following *p*FPA treatment, and the fact that all three abnormal shoots rapidly reverted to apparent normality, indicate that the technique described here will need considerable modification. Analogy with our experience of colchicine treatment of *Ribes* hybrids, where the growing points of very young seedlings are considerably more sensitive than are shoots of mature plants, suggests that sensitivity to *p*FPA will also be greater in young seedlings. The treatment of such seedlings with *p*FPA will be attempted next season.

R. L. KNIGHT
A. P. HAMILTON *
ELIZABETH KEEP

East Malling Research Station,
Maidstone,
Kent.

* Present address: Department of Botany, University of Durham.

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VETERINARY SCIENCE

Experimental Scrapie in Goats: a Modification of Incubation Period and Clinical Response following Pre-treatment with Normal Goat Brain

SCRAPIE is a naturally occurring, experimentally transmissible disease of sheep, the causal agent of which has not been identified. The disease has been produced experimentally in goats[1], in which species the incubation period ranges from 7 to 23 months[2]. Attention has been directed to similarities between the scrapie agent and the agent responsible for allergic encephalomyelitis, and although these two diseases are quite unlike pathologically, it has been suggested that similarities in pathogenesis may exist[3].

It was decided, therefore, to attempt the 'sensitization' of goats with a large intraperitoneal inoculation of normal goat brain, and later to expose the animals to an intracerebral test dose of scrapie goat brain.

The results of 2 experiments indicated that goats pre-treated with normal goat brain reacted more rapidly and more violently to the test inoculum than did untreated control animals.

The goats used were cross-bred animals from the Compton flock, reared in a scrapie-free environment. Normal brain inocula were prepared from a homogenate held at −18° C of 2 brains from clinically healthy goats of the Compton flock. In the first experiment, the normal brain inoculum was 100 ml. intraperitoneally of a 10 per cent suspension in physiological saline; 5 goats were inoculated at birth, and 3 at 15 days of age. In the second experiment, 3 newly born goats each received 100 ml. of a 10 per cent saline suspension of normal brain intraperitoneally, and 4 were given the same volume by the same route but in the form of 10 × 10 ml. inoculations at weekly intervals. Material for this series of inoculations was drawn each week from a pool of 10 per cent brain suspension held at 4° C.

The intracerebral test dose of 1·0 ml. of the supernatant of a lightly centrifuged 10 per cent suspension in physiological saline of scrapie goat brain of the scratching type[4] was given to all animals 10 weeks after the single 100-ml. preparatory intraperitoneal inoculation of normal brain, or (in the second experiment) 1 week after the final 10 ml. preparatory dose. The scrapie brain used in the first experiment was from a pool of 8 homogenized brains that had been held at −18° C for 3 months. In the second experiment, a pool of 3 different homogenized scrapie goat brains held at −18° C for 2 months was used.

The results of the 2 experiments are given in Figs. 1 and 2, from which it will be seen that, in both experiments, the incubation period for the occurrence of clinical scrapie was significantly longer in the 5 untreated goats than in the 15 pre-treated animals ($P = 0{\cdot}001$). In the first experiment there was no significant difference between the goats pre-treated at birth and those pre-treated

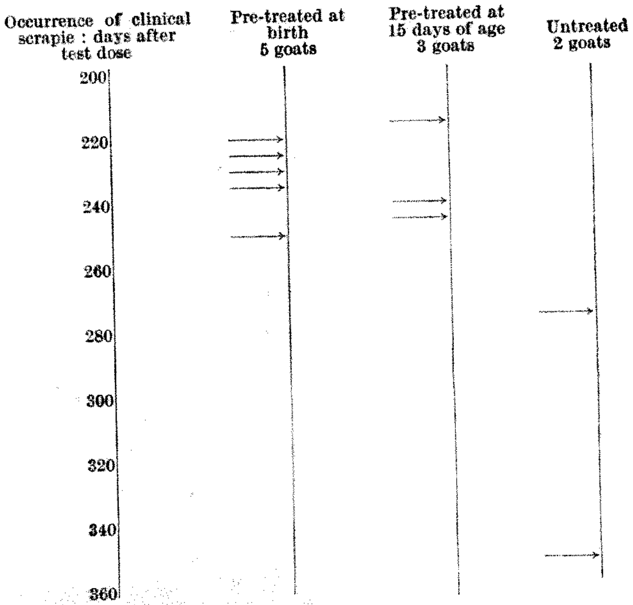

Fig. 1. First experiment. The occurrence of clinical scrapie in goats (*a*) pre-treated by intraperitoneal inoculation of 100 ml. of a 10 per cent suspension of normal goat brain at birth, or at 15 days of age (*b*) untreated. The intracerebral test dose was given ten weeks after pre-treatment

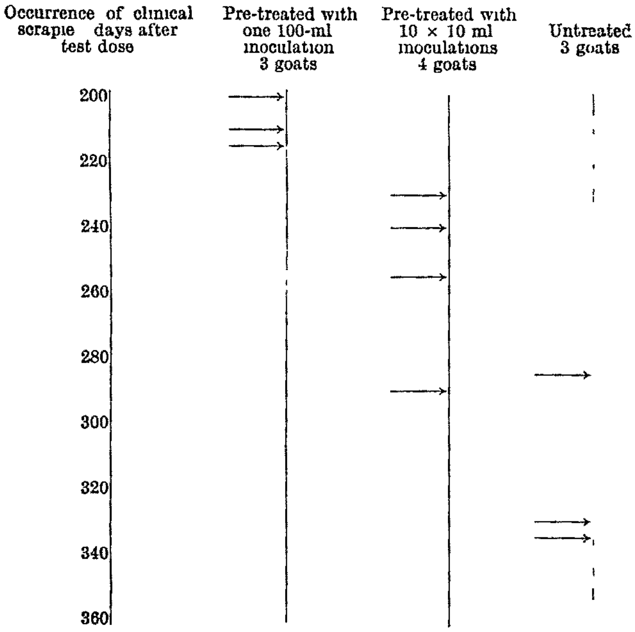

Fig 2 Second experiment The occurrence of clinical scrapie in goats (*a*) pre-treated by intraperitoneal inoculation of 100 ml of a 10 per cent suspension of normal goat brain at birth, or of 10 × 10 ml inoculations at weekly intervals from birth (*b*) untreated The intracerebral test dose was given ten weeks after the single 100-ml pre-treatment inoculation, or one week after the final 10-ml inoculation

at 15 days of age In the second experiment, the apparent slight difference between the group of 3 animals pre-treated with a single 100 ml dose at birth, and the group of 4 animals pre-treated from birth with 10 inoculations each of 10 ml at weekly intervals, was not significant ($P = 0\ 057$)

With one exception, the incubation periods for all animals in both experiments were within the 7–23 month range previously reported, the exceptional animal (Fig 2) developed clinical disease at 6 75 months, which is the shortest incubation period that we have noted in more than 350 cases of experimental scrapie in goats

In addition to the difference in incubation periods between untreated and pre-treated goats, there was a clearly marked difference in the severity of the clinical disease In the pre-treated animals the disease appeared suddenly, and scratching at the base of the neck was so severe that in 10 of the 15 animals there was bleeding followed by scab formation By contrast, in the 5 untreated animals the disease developed slowly, and scratching was much less violent

In both experiments, the intracerebral test dose was a suspension of scrapie goat brain It is not clear, therefore, whether the scrapie agent *per se* was affected by treatment, or whether the brain material in which the agent was held was the important element in the reaction Further experiments on this point are in progress

Zlotnik and Rennie[5,6] have recently considered the part that immunological tolerance may play in modifying the response of mice to a test inoculation of scrapie sheep brain In one series of observations they considered it possible that immunologically tolerant mice might have been rendered resistant to a scrapie test dose given by the subcutaneous route, but in later experiments they recorded that mice that were presumed to be tolerant to normal sheep tissue developed scrapie more or less at the same time as untreated control mice

The mechanism of the apparently enhanced clinical response of pre-treated goats is obscure Speculation on its nature would be unprofitable without further experimentation. However, from a practical point of view, pre-treatment with normal brain might be a useful technique for enhancing the clinical response of goats (and possibly, also, of other susceptible species) to experimental scrapie

I H PATTISON
K SMITH

Agricultural Research Council,
Institute for Research on Animal Diseases,
Compton, near Newbury, Berks

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SOIL SCIENCE

Interaction between Calcium Carbonates and Bentonite Suspensions

CALCIUM carbonate in soil is generally assumed to behave like pure calcite Equations relating calcium carbonate solubility to carbon dioxide pressure have been given, though their application to calcareous soils has not yet been tested thoroughly[1,2] Thus, usually only the system $CaCO_3$–CO_2–H_2O is considered, neglecting a possible interaction between the carbonates and other soil components[1–3]

Recently we found[4] that although the self-diffusion rate of calcium-45 into ground fractions of pure dolomite and calcite, as well as into unground calcium carbonate precipitate, was in accordance with expectations, the observed diffusion into comparable fractions of a commercial lime (*EMKAL*), consisting of ground marl, stopped after several hours As a possible explanation for this phenomenon might be an interaction between the calcite and, for example, silicate containing water in the marl deposit, it seemed warranted to investigate the behaviour of pure calcite in suspensions of soil clays

For this purpose suspensions of the fraction smaller than 5μ were prepared by grinding large-size crystals of dolomite and calcite The same fraction was separated from the commercial lime Also prepared was a suspension of calcium bentonite, consisting of particles smaller than 2μ of Wyoming bentonite saturated with calcium by repeated washing with 0 5 N calcium chloride and subsequent removal of excess salts All suspensions were made up so as to contain about 0 009 N calcium chloride.

Next the amount of calcium exchangeable against calcium-45 was determined as a function of time for all suspensions prepared and for mixtures of known composition of calcium bentonite with one of the foregoing carbonates Details of the procedure used are given in ref 4

If there were no interaction between the bentonite and the carbonates, obviously the total amount of calcium isotopically exchangeable against calcium-45 in a given time period in the mixed system should sum up to the calculated contribution of the two components, that is, clay and carbonate As is shown in Table 1, this is true for the mixture clay-*EMKAL*, but not for the mixtures of bentonite and the pure calcite In the latter case the self-diffusion of calcium-45 was impaired to a degree related to the ratio clay calcite

The reason for this phenomenon could be either the direct adsorption of clay on the carbonite particles (thus impeding the diffusion of calcium-45 into the crystals), or the interaction between the carbonate particles and molecular decomposition products of the clay—presumably silicates

In order to test the second possibility the foregoing experiments were repeated, this time replacing the bentonite by its dialysate or by a dilute solution of sodium silicate Although the exact composition of the dialysate was not determined, it is known that silicates were present.

Table 1

System			Isotopically exchangeable calcium microequivalents				
			Calculated contribution on basis of pure materials				
Carbonate	Time of shaking (h)	Bentonite/calcite (g/g)	Carbonate	Bentonite	Carbonate + bentonite	Experimental results of the mixture	Differences between calculated and experimental results
Calcite 1*	70	0.4	3.8	48.1	51.9	45.6	− 6.3
,,	192	0.4	7.3	55.2	62.5	55.1	− 7.4
Calcite 2*	98	0.06	225	16	241	202	− 39
,,	98	0.14	203	45	248	163	− 85
,,	98	0.27	181	106	287	141	− 146
EMKAL	70	0.29	9.4	48.7	58.1	59.8	+ 1.7

* Two degrees of grinding

As is shown in Table 2, the dialysate and the sodium silicate exhibited the same impeding action on the self-diffusion of calcium-45 into carbonates as the clay itself.

From this one can conclude that the observed decrease in the diffusion rate of calcium-45 into carbonates on contact with clays is due to the interaction between the surface of the calcium salts and dissolved compounds, presumably silicates, causing a surface coating of the carbonate particles with calcium silicate. With this explanation one has to assume that interaction with clays or other compounds has already occurred on the surface of the calcite particles of the commercial lime *EMKAL*, which therefore exhibited no further interaction with additional amounts of clay.

The question arises now as to the self-diffusion of calcium-45 into carbonates of calcareous soils. The nature of this process might be indicative with respect to the properties of the carbonate surface in these soils.

In a preliminary experiment, one Israeli calcareous soil containing 39 per cent of calcium carbonate was tested. Solutions labelled with calcium-45 were added to suspensions of the soil and the radioactivity of the supernatant was measured after different periods of shaking. The results show that there was a fast isotopic exchange in the first 2–3 h, after which there was no more exchange for more than a thousand hours, in contrast to the ever-continuing diffusion in similar experiments with pure calcite, dolomite and calcium carbonate precipitates[4].

This indicates that there was practically no self-diffusion of calcium-45 into the carbonates in the tested soil. On the basis of estimated surface area of the carbonates in the soil and assuming a diffusion constant of the order of magnitude of that of calcite[4], it could be shown that self-diffusion of calcium-45 would have been easily detected. One may suggest, therefore, that the carbonate particles in this soil were coated with compounds which did not allow or at least reduced considerably, the self-diffusion of calcium-45. This may also explain the observation[5] that calcium of the limestone did not interfere to any important degree with the isotopic exchange of calcium-45 in calcareous soils. It is interesting to note that the presence of some type of coating of carbonate particles has been suggested before by other workers[6].

Table 2

System		Radioisotopically exchangeable calcium (μequiv/g)		
Carbonate	Added solution	Time of shaking (h)	Calculated on basis of pure carbonate	Experimental results
Calcite 2	Dialysate 1*	98	> 580	44
	Dialysate 2†	98	> 200	25
Dolomite	Dialysate 2†	50	> 290	75
$CaCO_3$ (Merk Reagent)	Dialysate 2†	144	46	11
		462	> 80	35
	Sodium silicate	144	72	21
		462	> 120	21

* Dialysate of sodium bentonite in water

† Dialysate of calcium bentonite in equilibrium solution of calcium carbonate

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N. LAHAV
G. H. BOLT

Laboratory of Soils and Fertilizers,
Agricultural State University,
Wageningen, Netherlands.

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Evidence against Relationships between Root Cation Exchange Capacity and Cation Uptake by Plants

DICOTYLEDONOUS plants usually contain a larger proportion of divalent to monovalent cations than monocotyledons. The cation exchange capacity of the roots of dicotyledons is generally larger than that of monocotyledons[1] and many results, starting with Mattson's[2], have been published indicating a relationship between root cation exchange capacity and differential uptake of divalent and monovalent cations. Huffaker and Wallace[3] explained the good relationships they obtained between cation exchange capacity of roots and concentrations of potassium, calcium and magnesium of several plants, grown in soil and sand, on the basis of Donnan distribution. Other workers[4–7] have shown that the cation exchange capacity of plant roots grown in soil-less cultures is not constant but can be altered by changing the composition of the nutrient solutions, in particular, larger cation exchange capacity values have been associated with increased levels of nitrogen. Recently it was reported that the sum of the cations in plants on poor soils was positively correlated with the root cation exchange capacity of the plants[8].

An experiment we made with Italian ryegrass (*Lolium multiflorum*) did not support any of the foregoing relationships but showed that (1) the differential uptake of mono- and di-valent cations was not related to root cation exchange capacity, (2) root cation exchange capacity was constant and did not vary with increased nitrogen in the grass, (3) root cation exchange capacity was not correlated with the sum of the cations in the grass.

Italian ryegrass was grown from seed in a clay loam soil in the greenhouse for 9 weeks during October–December. Daylight was supplemented with 400-W *HPMV* fluorescent lamps to give approximately 200 cal cm^{-2} day^{-1} during an 18-h day. The soil was kept at three constant

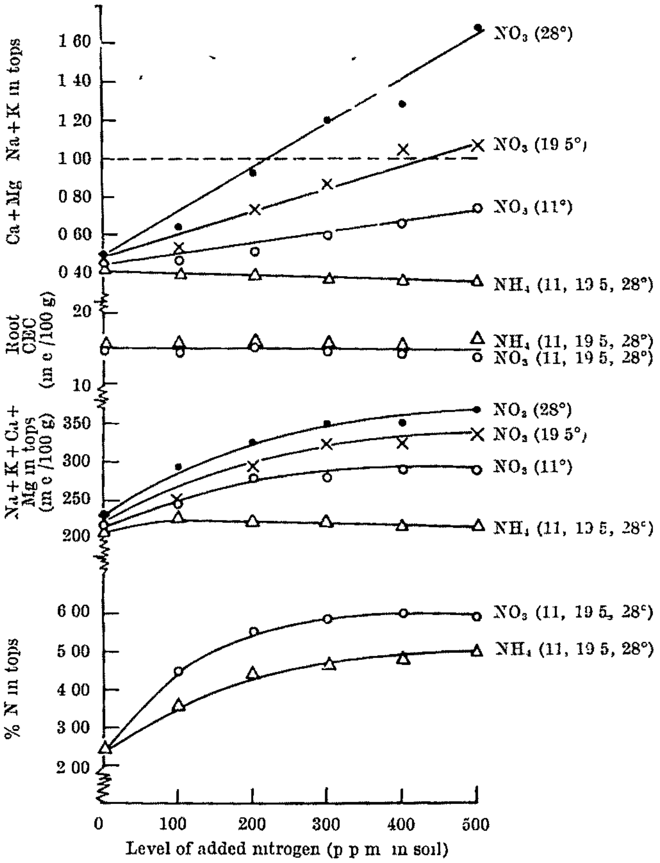

Fig 1 Effect of soil temperature, different levels of NO_3—N and NH_4—N on Ca + Mg Na + K ratio, sum of the cations, percentage nitrogen, and root cation exchange capacity (CEC) of Italian ryegrass

temperatures, 11°, 19 5°, 28°, in large water tanks, and uniform air temperatures were maintained in the foliage with circulating fans Six levels each of NO_3-N or NH_4-N (0-500 p p m) along with a uniform dressing of potassium, calcium, magnesium, phosphorus and sulphur were thoroughly mixed into the soil 'N-serve'[9] (2-chloro-6-(trichloromethyl pyridene)) was applied to the soil at 10 p p m to inhibit nitrification where NH_4-N was used

The figure gives measurements, on an oven-dry basis, of per cent nitrogen, sum of the cations (Na + K + Ca + Mg in m equiv /100 g) and the ratio of divalent to monovalent cations (Ca + Mg Na + K both in m equiv /100 g) in the tops of the plants and cation exchange capacity[10] (m equiv /100 g) of the roots The mineral contents of the roots were not measured, but it is doubtful whether including them with the mineral contents of the tops would have altered the results, root weights were always much less than those of the tops and at 28° and with much NO_3-N were only 10 per cent of total dry matter Averages were used when differences were small The results showed

(1) The ryegrass (a monocotyledon with small root cation exchange capacity), supplied with much NO_3-N and grown at high soil temperatures had a cation composition resembling that of a dicotyledon, that is, it absorbed more divalent than monovalent cations (Ca + Mg Na + K > 1) At low soil temperature and at all levels of NO_3-N the plants had more monovalent than divalent cations although the Ca + Mg Na + K ratio did increase with increasing NO_3-N Where NH_4-N was given, the divalent to monovalent cation ratio remained small at all soil temperatures and at all levels of NH_4-N

(2) The cation exchange capacity of the roots was not altered by soil temperature or by level or form of nitrogen, even though the percentage of nitrogen in the tops became greater with increasing levels of nitrogen, percentage nitrogen was always larger with NO_3-N than with NH_4-N

(3) The sum of the cations in the grass tops was not correlated with the cation exchange capacity of the roots, it did increase, however, with soil temperature and percentage nitrogen and was greater with NO_3-N than with NH_4-N, as found by Cunningham[11]

R K CUNNINGHAM
K F NIELSEN *

Rothamsted Experimental Station,
Harpenden, Herts

* Permanent address Experimental Farm, Swift Current, Saskatchewan, Canada

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PSYCHOLOGY

Evidence for a Physiological Explanation of the Waterfall Phenomenon and Figural After-effects

IN the rabbit's retina ganglion cells have recently been found which signal the direction of motion of objects moving in the visual field[1,2] These cells give brief responses at 'on' and 'off' to lights falling in their receptive fields and they give vigorous responses to moving spots, but only when these are moved in certain directions through their fields Motion in the 'preferred' direction gives the maximum discharge, while motion in the opposite, 'null', direction, causes no response The 'preferred' direction differs in different units thus which of a set of cells are active conveys information as to the direction of motion of objects in the visual field The frequency of the discharge depends greatly on speed and direction of motion, but is only slightly influenced by the intensity of the moving stimulus spot Furthermore, the discharge occurs for the same direction of motion with a black spot as with a white spot This behaviour is what one might expect in units of which the function is to abstract direction of motion from the pattern of light falling on the retina

While performing experiments on these units we made some observations on the effects of long-continued exposure to moving stimuli These observations are incomplete, but they are sufficient to suggest a physiological basis for the after-effects of sustained excitation by motion Since the observations will not be followed up in the near future they are briefly reported here

The activity of single units was recorded from electrodes inserted into the retina of the unopened rabbit's eye A directionally selective unit was found, its receptive field located, and the preferred direction determined To excite it, we rotated a large black disk carrying an irregular pattern of pieces of paper The disk was much larger than the receptive field, and the direction of motion of the part coinciding with the receptive field could be changed by shifting the axis of rotation of the disk Fig 1 shows what happens during and immediately after periods of exposure to motion in the preferred and null directions lasting 1 min The number of impulses in each second is plotted vertically, time horizontally For the first 15 sec the disk was stationary and the unit showed the normal, irregular, maintained discharge At the arrow, in the top graph, motion in the preferred direction was started The angular velocity at the eye was about 15°/sec The impulse frequency immediately rose to about 60/sec, but declined to about 25/sec during the first 15–20 sec. During the next 40 sec it fell only slightly further. Motion was stopped 57 sec after it had started, and the impulse frequency dropped abruptly to zero—below the maintained level preceding the stimulus. During the following 0·5 min it climbed slowly back to the level of the initial maintained discharge.

In the lower graph, the same sequence was repeated on the same unit, but this time the motion was in the null direction. There was no significant change in impulse frequency during the period of stimulation, nor during the period following it. (The peak just before the motion stopped should be disregarded, for it was caused by unintentional lateral movement of the disk while preparing to stop it spinning.) We were expecting to find some reduction in the maintained discharge during the motion in the null direction, and possibly some rebound excitation following it, for it is possible to inhibit the maintained discharge of such a unit by careful movements of single spots of light in the null direction. But in this experiment new spots were continually entering the field, and old ones leaving it: possibly the resulting 'on' and 'off' effects prevented a reduction in the maintained discharge. At all events we never recorded a clear-cut reduction in experiments of this type, nor did we ever observe definite rebound excitation in the period following stimulation.

We have seen similar changes in a number of units, and believe that the behaviour shown in Fig. 1 is typical for the units which signal direction of motion. In one case we maintained stimulation for 3 min without greatly changing the magnitude or duration of the after-effects. In another we stimulated for 0·5 min, and thought that the effects were less pronounced.

For reasons to be given in the following communication, it seems unlikely that similar effects occur at a retinal level in man. However, in the rabbit's retina we have a specimen movement analysing mechanism, and it is reasonable to ask if the observed changes fit in with the subjective after-effects of movement. Similar changes to those described here may well occur in lateral geniculate or cortex of man.

The initial adaptation of the discharge fits in well with the decline in the sensation of movement which is noticeable during the first 0·25 min of observation. A similar decline in vividness of inspection figures has been described in experiments on figural after-effects and Gibson-type illusions[3].

The after-effects themselves may be explicable by the reduction in maintained discharge in the period following excitation. In the figure itself, upper and lower graphs were obtained from a single unit by reversing the direction of motion, but one must remember that there is a large population of directionally selective units, some of which will have their null direction the same as the preferred direction of this particular cell. It is reasonable to presume that such a cell would behave as in the lower graph while the cell actually being examined behaves as in the upper graph. Considering these two cells, one sees that before the stimulus their maintained discharges would be roughly equal. During the stimulus the one, the preferred direction of which coincided with the direction of movement, would be more active than the other, while in the period immediately following the stimulus the other one, which normally signals motion in the reverse direction to that of the preceding stimulus, would be the more active and might give rise to the sensation of reverse movement. Thus the after-effects of motion may result from the temporary imbalance of the maintained discharges of cells responsive to motion in opposite directions. Our results suggest that the imbalance is caused by a reduction in discharge of the units that have been excited rather than by rebound excitation of other units.

The duration of the suppression of the maintained activity seems to coincide with the duration of the shorter-lived after-effects, such as the waterfall phenomenon itself, and the curved line illusion. Some figural after-effects are reported to be much longer-lasting, though in these cases the period of exposure to the stimulus has also been longer than we have used. Similarly the duration of exposure to movement required to cause suppression seems to agree with that required to cause the after-effect, though on this point our evidence is very incomplete.

The agreement of time-scales is encouraging, and there are two details of the subjective effects which seem to provide additional support. First, the magnitude of the after-effect is always limited, thus reverse motion is always rather slow. If the imbalance is caused by suppression of the maintained discharge this feature would be expected, for the rate of the suppressed unit cannot drop below zero, and the maintained level of discharge of the unstimulated unit is not high. Second, Wohlegemuth (1911) reported that exposure to motion first in one direction, then in the other, led to a state where neither direction of motion can induce an after-effect. This is readily explained if the initial exposure to alternating directions of motion suppresses the maintained discharge of both units, so that imbalance by suppression is no longer possible. Imbalance by increasing the discharge would still occur during exposure to moving stimuli, thus explaining why the subject is not rendered insensitive to motion by the treatment.

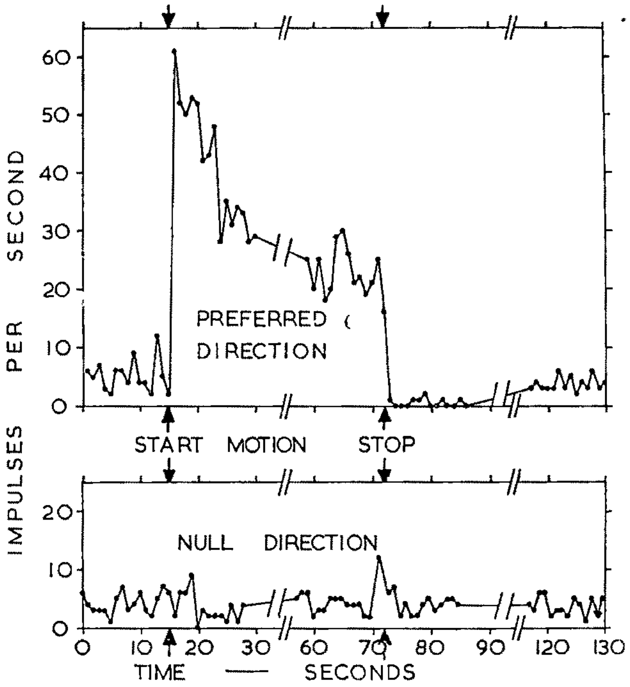

Fig. 1. Effects of stimulating a directionally selective unit for 1 min by motion in the preferred and null directions. Impulses/sec are plotted vertically, time horizontally. The maintained activity is suppressed following 1 min of excitation by motion in the preferred direction. Motion in the null direction has no significant effect, the peak at 70 sec being an artefact

Though the evidence is new, this explanation for after-effects is not, and is substantially the same as that advanced by Exner and modified by Wohlegemuth (1911). Originally it was framed in terms of 'tonus', 'drainage', and 'fatigue', but it does not depend on these notions and their idea can easily be translated as temporary suppression of the maintained discharge. Further, many of the after-effects of motion can be paralleled by the after-effects of exposure to contours, curved lines and tilted lines, as shown by Gibson[4], and Kohler and Wallach[5] (see also McEwen's review[3], and various authors[6]). It is plausible to suggest that suppression of the maintained discharge lies behind many of these effects, but notice that there are two requirements for such an explanation to hold up. First, the units concerned must have a maintained discharge; secondly, they must be arranged in reciprocally active pairs, so that the suppression of one unit can lead to subjective effects similar to those caused by increased activity in the other of the pair

Finally, it is interesting to ask if the argument can be used in reverse If after-effects are due to the imbalance of maintained activity of units specifically excited by features of the inspection figures, can one deduce the nature of the units which analyse sensory data from the nature of the after-effects which are readily produced ?

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H B BARLOW
R M HILL

School of Optometry,
University of California,
Berkeley, 4

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Inter-ocular Transfer of Movement After-effects during Pressure Blinding of the Stimulated Eye

THERE is evidence that pressure blinding abolishes all activity of the ganglion cells of the retina[1] It has been suggested[2] that the after-effect of exposing an eye to a moving stimulus (the waterfall illusion, see preceding communication) may be caused by the reduction of maintained discharge of the directionally sensitive retinal ganglion cells which were stimulated during the period of inspection, leaving the maintained activity of those which would signal the reverse motion unbalanced. If this is correct, then pressure blinding the eye during the period immediately following stimulation should abolish the effect Unfortunately, one is not clearly aware of the movement illusion if there are no objects that one can see as moving, and pressure blinding prevents seeing. One therefore has to perform the test on the illusion transferred to objects seen with the other eye Incidentally, it is interesting to note that the existence of this transfer has often been taken as convincing evidence for the central origin of the after-effects, but the transfer would be explicable on the hypothesis that it is due to suppression of maintained discharge even if this occurred only in the retina

The test was performed as follows A subject observed monocularly a rotating object which was either a spiral, or an irregular pattern of black marks on a white ground After 30 sec he applied pressure with his finger, trying not to disturb fixation, until complete fading had occurred (usually about 15 sec) The rotation was then stopped and the pressure-blinded eye covered, but the pressure was maintained The subject opened his other eye, observed the stationary disk, and reported on the appearance of movement

We ourselves and two other subjects were all able to get very clear transfer of the after-effect in spite of complete pressure blinding of the pre-exposed eye The completeness of pressure blinding was checked after the illusion had been observed No similar effects occurred when the eye was pressure blinded without the exposure to movement We compared the after-effect transferred from a pressure-blinded eye with that transferred from an eye for which the effect of pressure had been mimicked by dimming, and finally extinguishing, the light illuminating the rotating disk The after-effect from the pressure-blinded eye seemed as vivid, and lasted about as long as that from the control

The result shows that subjective movement after-effects are not caused by suppression of the maintained discharge of directionally selective ganglion cells in the retina of man The only escape from this conclusion would be if directionally selective units were unaffected by pressure blinding, or possibly if after-effects occurred both retinally and centrally. The observation does not decide which part or parts of the hypothesis are wrong It is not known whether human, primate, or monkey retinæ have directionally selective units, and it is certainly quite likely that in these species the analysis of direction of movement of objects in the visual field occurs at a higher level in the nervous pathway If that is so, then suppression of geniculate or cortical maintained activity remains a plausible explanation of the waterfall phenomenon.

H B BARLOW
G S BRINDLEY

Physiological Laboratory,
University of Cambridge

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Scaling and Personal Questionnaires

STEVENS'S[1] classification of types of scaling according to their group structure appears insufficient, since a higher ordered metric[2,3] and an interval scaling both belong to the same group, namely, the linear group (an interval scaling according to Stevens and a higher ordered metric scaling because the only function $f(x)$ such that for all a, b, c, d, if $|a-b| > |c-d|$ then $|f(a)-f(b)| > |f(c)-f(d)|$ is a linear function), yet they contain different amounts of information

The following alternative definition and classification are suggested A scaling of a set of n objects on a single dimension is the unique specification of a certain relationship (varying with the type of scaling) between pairs of elements, the elements being either ordinates of objects or functions thereof (depending on the type of scaling) (For example, an ordinal scaling specifies, for all pairs of objects, which has the greater ordinate an interval scaling specifies the ratio between all pairs of differences between the ordinates of objects, the ordinates themselves are in all cases generally not known) Scalings may consequently be classified according to relationship and according to elements this two-way scheme is illustrated in Table 1 with four of the commonest types of scaling

Table 1 TWO-WAY CLASSIFICATION OF FOUR TYPES OF SCALING

Elements	Relationship Direction of inequality	 Value of ratio	
Ordinates	$A >< B$? Ordinal	$A/B =$? Ratio	For all A, B
Differences between ordinates	$AB >< \overline{CD}$? h o m	$\overline{AB}/\overline{CD} =$? Interval	For all A, B, C, D where possibly $B = C$

A, B, C, D are ordinates of objects

A general direct method suggested by the foregoing definition (particular cases of which are well known) of obtaining a psycho-physical scaling, along a subjective dimension, of a set of objects by a single subject, is to present him with each of the possible appropriate subsets of objects, pairs (ordinal and ratio scalings) or pairs of pairs (higher ordered metric and interval) and ask him to specify the relationship, to make a judgement of greater or less (ordinal and higher ordered metric) or to estimate the ratio (interval and ratio) In the case of higher ordered metric and interval scalings, however, the number of pairs of pairs of objects, $3\binom{n+1}{4}$, rapidly becomes unmanageable with increasing n, and simpler techniques are available (ranking for higher ordered metric and interval estimation[4] for interval)

The general method gives more than enough information for assigning scale values to objects The case of ordinal scaling has been dealt with by Kendall and Babington Smith[5], higher ordered metric scaling by Coombs[2] and Siegel[3], Torgerson[6] gives a least squares estimation procedure for ratio scaling, and there is an obvious adaptation of this procedure to interval estimation The redundant information may be used to test reliability (internal consistency)

A common feature of the various internal consistency tests given in the literature is that they examine all appropriate sets of three responses to verify that certain necessary relationships hold between them Thus, if a subject estimates that $E/F = r$ and $F/G = s$, then he should also estimate that $E/G = rs$ (where E F, G are elements) although some allowance will be made for imperfect estimation, and, a special case of this, if he judges that $E > F$ and $F > G$, then he should also judge that $E > G$ (There are also certain 'internal' relationships that should hold for higher ordered metric and interval scaling for the latter, if the subject judges that $\bar{AB} = d$ and $\bar{BC} = e$ where $A < B < C$ then he should also judge that $\bar{AC} = d + e$, for the former similar but more complicated relationships should obtain)

These methods may be used, *inter alia*, to scale features of the symptomatology of an individual psychiatric patient (for example, sets of situations which a phobic finds frightening), the objects usually being represented by cards bearing descriptions of the features preferably in the patient's own words A particularly interesting case occurs when the objects are different degrees of intensity of a particular symptom (for example, *A*, 'I feel very depressed'; *B*. 'I feel rather depressed', *C*, 'I feel only a little depressed', *D* 'I do not feel depressed at all') After they have been scaled by the patient, that is, after he has specified all the relationships involving them, and if the scaling can be assumed to be sufficiently permanent, then the patient's position on the continuum can on different occasions be introduced as an extra object, and by asking him to specify all relationships involving it and the other objects a scale value can be assigned to it for each occasion these further specifications will thus constitute administrations of a personal questionnaire which will measure fluctuations in the intensity of the symptom For each administration, as a different conclusion of the initial scaling, the usual internal consistency checks will be available

To each type of scaling will correspond a type of personal questionnaire the first type to be described, and almost the only one which has so far been used at all extensively, is Shapiro's[7], which suggested the present generalization, and which is a partial higher order metric type with $n = 3$ (It has been found possible to generalize this to the case where $n \geq 4$) It is not always necessary that the initial scaling should be of the same type as the questionnaire scaling For example, Shapiro's type uses an initial ordinal scaling, and, surprisingly, the interval and ratio types do not need any initial scaling There are a number of such mixed types, of which those involving higher order metric scaling are the most intricate

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J P N PHILLIPS*

Psychology Department
Institute of Psychiatry,
The Maudsley Hospital, Denmark Hill, S E 5

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* Present address Department of Psychology, University of Hull

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MISCELLANEOUS

Detecting Fingerprints on Paper

DURING the continuation of an investigation[1] into the factors affecting the resistance of paper to attack by atmospheric pollution, we found that fingerprints were affecting our results Further investigation suggested that a method for detecting fingerprints on paper could be developed Fuller details are to be published shortly, but in essence the method consisted of exposing the sample of paper to radioactive sulphur dioxide at a concentration of about 10 p p m , followed by autoradiography Sulphur dioxide was picked up preferentially in the regions contaminated by the fingers, and this caused a greater darkening of the X-ray film An example of one of the prints obtained is shown in Fig 1 The specific activity of the sulphur dioxide was about 1 mc /m mole, the time of exposure about 12 h and the time for autoradiography one week

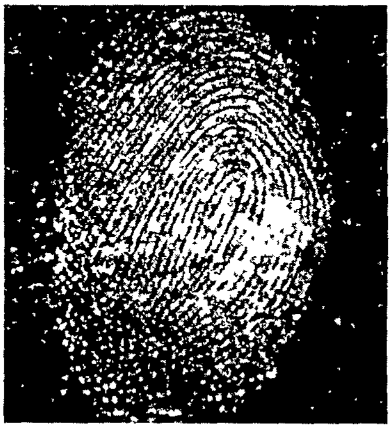

Fig 1

Since sulphur dioxide also combines with paper especially with the more impure grades, it did not appear to be ideal for this work and formaldehyde carbon-14 was therefore tried[3] The sample was exposed for 30 min to the vapour from ^{14}C-paraformaldehyde of specific activity 86 3 µc /mg at room temperature and left in contact with X-ray film for 15 h When developed the film showed a strong outline of the paper, but a weak and sketchy image of the print There was clearly too much general reaction between the formaldehyde and constituents of the paper to permit the fingerprint to develop

Both the methods at present used[2] for the detection of fingerprints on paper depend on the production of a colour, so that the method described here offers an advantage for use with coloured papers Research is called for to determine if there are other compounds more suitable for providing a convenient and sensitive method of detection using the same principle[3]

We thank the Directors of Wiggins Teape and Co Ltd for the grant of a research scholarship which made this work possible

R L GRANT
F LYTH HUDSON
J A HOCKEY

College of Science and Technology,
Manchester

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Wednesday, January 1

Royal Geographical Society (at 1 Kensington Gore, London S W 7) at 3 p m —Prof A R Clapham, F R S "Geography and Conservation"

Wednesday, January 1—Saturday, January 4

Institute of Physics and the Physical Society (at the H H Wills Physics Laboratory, The University, Bristol)—Conference on 'Solid State Physics'

Thursday, January 2

Institution of Mechanical Engineers, Nuclear Energy Group (at 1 Birdcage Walk, Westminster, London, S W 1), at 6 p m —Discussion on Fuel Element Temperature Limitations in Practice'

Institute of Metals (at 17 Belgrave Square, London, S W 1) at 7 p.m Dr C A Clark 'Some Recent Developments in Magnetic Alloys"

Friday, January 3

British Mycological Society (in the Department of Mechanical Engineering (E 1 Building), City and Guilds College, Exhibition Road, London, S W 7), at 10 45 a m —General Papers

Saturday, January 4

London County Council (at the Horniman Museum, Forest Hill, London, S E 23), at 3 30 p m —Dr Mary E Gillham "Wild Life in Australia' (Coloured Slides) *

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Librarian (with an honours degree, professional qualifications as a librarian, and responsible experience in a specialist library of high standing) —The Academic Registrar, Brunel College, Woodlands Avenue, London W 3 (January 8)

University Lecturer (preferably with qualifications in medicine and biochemistry) in Biochemistry—T D Kellaway, Secretary of the Appointments Committee, Department of Pathology, The University, Tennis Court Road, Cambridge (January 9)

Assistant Lecturers (2) in the Department of Chemistry—The Secretary, Battersea College of Technology, Battersea Park Road, London S W 11 (January 10)

Lecturer/Assistant Lecturer (preferably with qualifications in analysis) in the Department of Mathematics, University of Ghana—The Registrar, University of Ghana, P O Box 25, Legon, Accra, or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W C 1 (January 10)

Lecturer (preferably with an active interest in some aspect of experimental botany] in Botany—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1 (January 10)

Lecturer (with experience in high energy nuclear physics) in Physics and a Lecturer (with an interest and experience in magnetic resonance) in Physics—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1 (January 10)

Principal Scientific Officer (with a good honours degree and considerable postgraduate research, and preferably with practical experience of quantitative methods of population estimation, with experience of more than one taxonomic group, and an appreciation of statistics) in the Toxic Chemicals and Wildlife Section, Monk's Wood Experimental Station, Huntingdon, to direct work on insect populations—The Nature Conservancy (E), 19 Belgrave Square, London, S W 1 (January 10)

Research Fellow (with a first degree in biochemistry, chemistry or botany and research experience in biology) in Plant Biochemistry in the Department of Botany—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1 (January 10)

Lecturer (physicist or physical chemist, qualified in physics or physical chemistry and an interest in the application of physical techniques to biology and particularly macromolecules or electron microscopy) in the Department of Botany—The Secretary, University College, Gower Street, London, W C 1 (January 14)

Chair tenable in the School of Applied Science and Engineering Science—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (January 15)

Lecturer in Experimental Physics—The Registrar (Room 22, O R B) The University, Reading (January 15)

Lecturer or Assistant Lecturer in Applied Mathematics in the Department of Mathematics in the Faculty of Science—The Registrar The University, Manchester 13 (January 15)

Lecturer (with a degree in veterinary science or an appropriate degree in science) in Veterinary Anatomy at the University of Queensland, Australia—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London S W 1 (London and Brisbane, January 17)

Chair of Agricultural Chemistry and Head of the Department of Agricultural Chemistry and Soil Science at the University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C 1 (January 18)

Chair of Geology at Bedford College—The Academic Registrar, University of London, Senate House, London, W C 1 (January 30)

Associate Professor (with a higher degree and preferably a specialist in the field of power engineering) in the School of Electrical Engineering, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W C 2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 31)

Chair of Fluid Mechanics in the Department of Mechanical Engineering, University of the Witwatersrand, Johannesburg, South Africa—The Secretary Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (South Africa and London January 31)

Chairs of Mathematics—The Secretary, The University, Aberdeen (January 31)

Lecturer (dental, medical or science graduate, with bacteriological experience) in Dental Bacteriology, Turner Dental School—The Registrar, The University, Manchester 13 (January 31)

Lecturer (Grade II) in Pure Mathematics—The Registrar, The University, Senate House, Bristol 2 (January 31)

Turner and Newall Research Fellow for research in Engineering, Inorganic Chemistry or Physics (or possibly in an allied science)—The Registrar, University Registry, Clarendon Building, Oxford (February 8)

Assistant Professor in Anatomy—The Chairman, Department of Anatomy, University of Saskatchewan, Saskatoon, Sask , Canada

Assistant Professor of Theoretical Physics, to teach upper division physics courses—C E Wulfman, Chairman, Department of Physics, University of the Pacific, Stockton, California U S A

Grassland Investigator (single, between 25–35 years of age, with an honours degree in agriculture or an appropriate science and a minimum of three years experimental experience) with the Government of the Falkland Islands, to investigate, experiment and report on the best methods of developing and improving grasslands—The Director of Recruitment, Department of Technical Co-operation Eland House, Stag Place, London, S W 1, quoting Ref T/RC 213/48/01

Head (with a university degree, preferably in the physical sciences, a recognized diploma in librarianship, several years experience in a similar post, a very good knowledge of French and English, and preferably some knowledge of other European languages) of the Scientific Information Service and Librarian—The Head of Personnel, European Organization for Nuclear Research (CERN), Geneva 23, Switzerland, quoting Ref DD-SIS

Lecturer and an Assistant Professor in the Department of Philosophy—The Dean, Faculty of Arts and Science, University of Manitoba Winnipeg 19, Manitoba, Canada

Research Assistant (with a degree in biology and an interest in histochemistry), to work on problems of peripheral nerve disorders—Dr J B Cavanagh, Pathology Department, Guy's Hospital Medical School, London Bridge, London S E 1

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Senior Grade Biochemist in the Biochemistry Department—Dr D N Raine, Children's Hospital, Ladywood Road, Birmingham 16

Senior Lecturer (with experience in academic or industrial research and in teaching or direction of projects) in Physics—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N 7

Senior Zoologist (with an honours degree in zoology or equivalent and research or other experience in zoology) in the Veterinary Department Government of Kenya, for investigations into the treatment of trypanosomiasis research work in immunity both in the laboratory and in the field and to give advice on drug regimes in the various areas of the country—The Director of Recruitment, Department of Technical Co-operation, Eland House, Stag Place, London, S W 1, quoting Ref C1/RC 217/80/011

REPORTS and other PUBLICATIONS

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Year Book of the Royal Society of Edinburgh, 1963, (Session 1961–1962) Pp 144 (Edinburgh The Royal Society of Edinburgh, 1963) 20*s* , 3 50 dollars [3010

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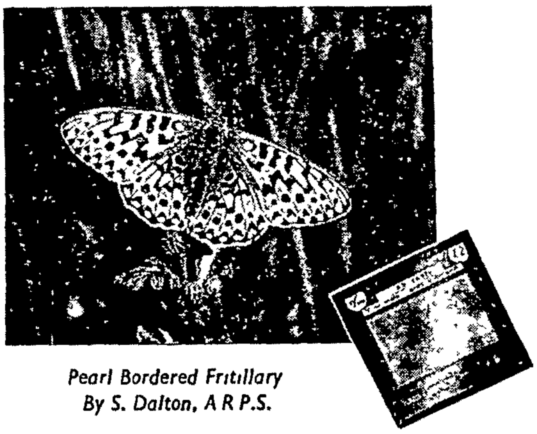

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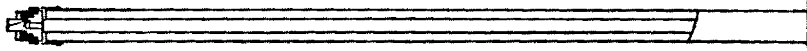

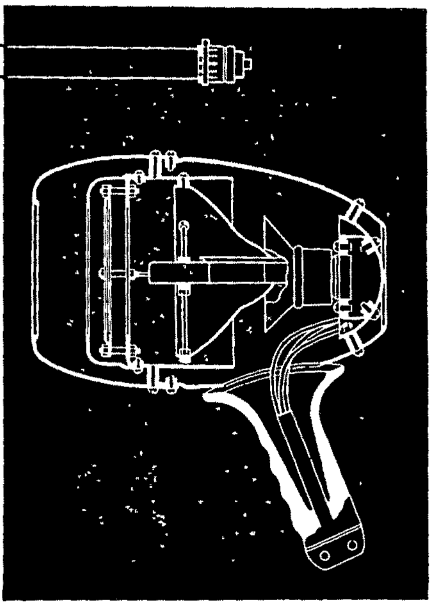

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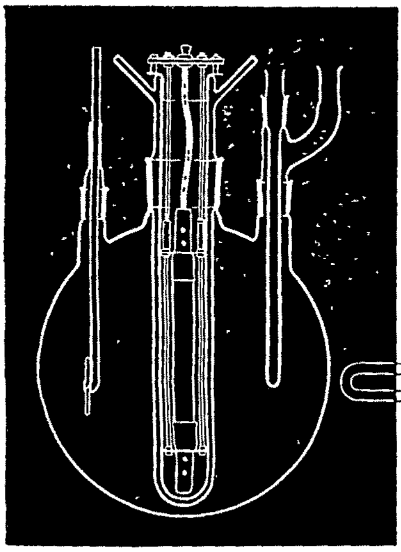

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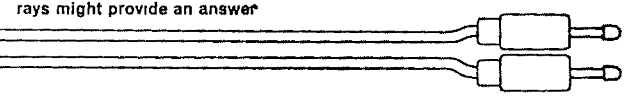

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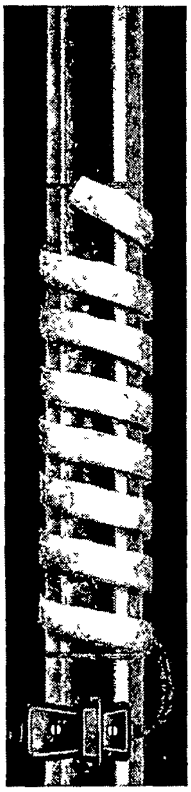

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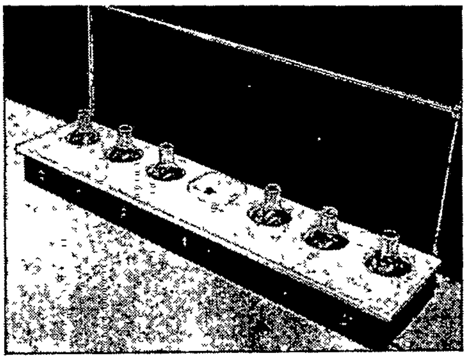

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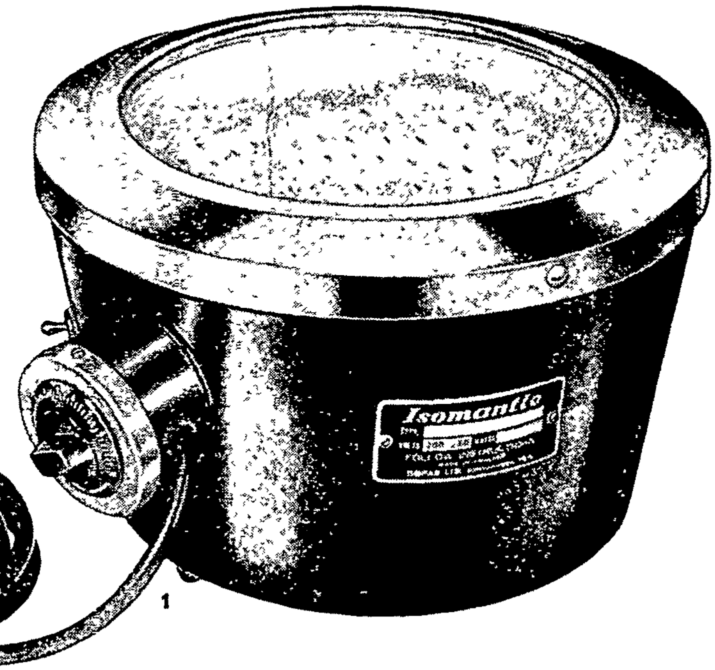

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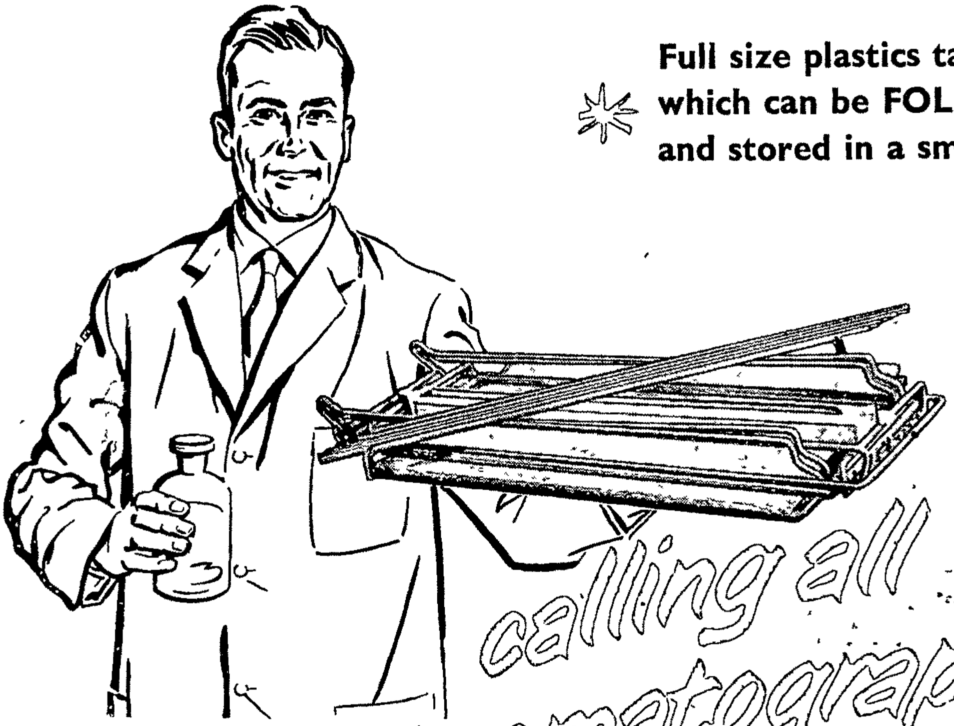

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with news of Chromatobags

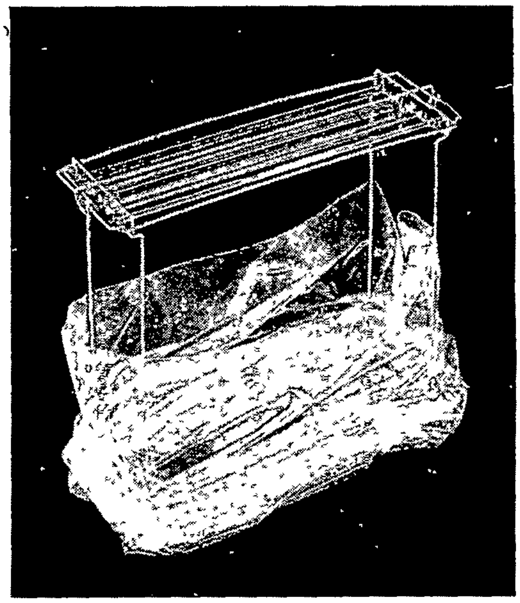
It is frequently necessary to exclude wood and metals from paper chromatography apparatus, and all-glass equipment is consequently widely used, but glass is fragile.

NOW CONSIDER THE ADVANTAGES OF CHROMATOBAGS

- Inexpensive to transport
- No cleaning problems—unbreakable transparent containers are disposable when contaminated
- Quick-strip framework Convenient for folding up and storing in small space
- Airtight—resistant to all common solvents
- Complete accessibility to chromatograms
- Specially suitable for work with radioactive isotopes or highly toxic chemicals

HOW CHROMATOBAGS ARE USED

A Gallenkamp 'Chromatobag' consists of a readily demountable plastics coated framework which supports the solvent trough and the guide rods for the paper strips or sheets A tough flexible plastics bag draws up over this frame to form a 'tank' and the enclosure is rendered airtight by clipping the neck of the bag To replenish the trough or remove the chromatograms, the bag is opened and, if necessary, slipped down over the framework The bags are transparent so that the strips can be inspected when the bag is closed The design does not necessitate any special chromatography techniques, but rather simplifies procedure by enabling the user to reach wet chromatograms from the side instead of from the top 'Chromatobags' can be used equally satisfactorily for descending, ascending or two-dimensional work using paper strips or sheets. When the bags become old, soiled or contaminated they can be replaced very easily and their destruction presents no problem



Ask for News and Review for full details of Chromatobags and many other new items

Technico House, Christopher Street, London, E.C 2 Tel BIShopsgate 3211

Gallenkamp

WIDNES
Victoria House, Widnes, Lancs
Tel : Widnes 2040

STOCKTON-ON-TEES
Portrack Lane, Stockton
Tel: Stockton 63441

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